

Effect of phenolic compounds present in Argentinian plant extracts on mycelial growth of the plant pathogen *Botrytis cinerea* Pers.

Efecto de compuestos fenólicos presentes en extractos de plantas argentinas en el crecimiento micelial del fitopatógeno *Botrytis cinerea* Pers.

Hapon MV^{1,2}, JJ Boiteux^{1,2}, MA Fernández¹, G Lucero^{1,2}, MF Silva¹, PH Pizzuolo^{1,2}

Abstract. The polyphagous plant pathogen *Botrytis cinerea* has the ability of growing fast under several conditions, and producing grey moulds in different plant tissues. Because of its adaptive plasticity, resistant strains have made difficult their chemical control. Therefore, new strategies of management and the use of natural compounds could be important control alternatives. The aim of this work was to study the potential value of four Argentine native plant extracts and some of its phenolic compounds as biodrugs on *B. cinerea*. Aqueous plant extracts of *Larrea divaricata*, *Prosopis strombulifera*, *Tessaria absinthioides* and *Schinus molle* var. *areira*, were used. The capability to inhibit the mycelial growth was studied using either the crude plant extract or same its most important, detected phenolic compounds. Phenolic compounds were analyzed by Capillary Zone Electrophoresis (CZE) with UV detection, and then statistically analyzed by Principal Component Analysis to correlate them and their biological activity against the fungus. *Prosopis strombulifera* and *T. absinthioides* extracts did not inhibit mycelial growth while *S. molle* stimulated it. *Larrea divaricata* demonstrated inhibition above 50% at plant extracts concentrations of 100 mg/mL.

Keywords: *Larrea divaricata*; Luteolin; Naringenin; Cinnamic acid, Capillary Zone Electrophoresis; Antifungal.

Resumen. El polífago fitopatógeno *Botrytis cinerea* tiene la habilidad de crecer rápidamente en diferentes condiciones y producir podredumbre gris en diferentes tejidos de plantas. Debido a su plasticidad adaptativa, la existencia de cepas resistentes ha dificultado su control químico. Es por esto que nuevas estrategias de manejo y el uso de compuestos naturales pueden ser importantes alternativas de control. El objetivo de este trabajo fue estudiar el valor potencial de cuatro extractos de plantas nativas de Argentina y alguno de sus compuestos fenólicos como biofármacos sobre *B. cinerea*. Se utilizaron extractos acuosos de cuatro especies nativas argentinas, *Larrea divaricata*, *Prosopis strombulifera*, *Tessaria absinthioides* y *Schinus molle* var. *areira*. La capacidad de inhibir el crecimiento micelial fue estudiado usando tanto los extractos crudos de plantas como así también sus compuestos fenólicos detectados más importantes. Los compuestos fenólicos estudiados fueron analizados por Electroforesis Capilar de Zona con detección UV, y después analizados estadísticamente por Análisis de Componentes Principales para correlacionar su actividad biológica contra el hongo. Los extractos de *P. strombulifera* y *T. absinthioides* no inhibieron el crecimiento micelial mientras que *S. molle* lo estimuló. *Larrea divaricata* demostró una inhibición superior al 50% con concentraciones de 100 mg/mL de su extracto vegetal.

Palabras clave: *Larrea divaricata*; Luteolina; Naringenina; Ácido cinámico, Electroforesis Capilar de Zona; Antifúngico.

¹ Instituto de Biología Agrícola de Mendoza, Conicet. Mendoza, Argentina C.P. M5528AHB.

² Cátedra de Fitopatología, Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Mendoza, Argentina. C.P. M5528AHB.

Address correspondence to: M. Vanda Hapon, Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Almirante Brown 500, Luján de Cuyo, Mendoza, Argentina. CP: M5528AHB. Tel +54 261 4135000 int. 1235, e-mail: mavahapon@hotmail.com

Received 27.I.2016. Accepted 23.VIII.2016.

INTRODUCTION

Downy mildew, powdery mildew and bunch rots are among the main grapevine canopy diseases. Bunch rots could produce yield losses between 30 to 70%; *Botrytis cinerea* Pers. Fr. is the main microorganism involved in bunch rots (Lucero et al., 2009a). Typical symptoms of diseases produced by *B. cinerea* are soft rots on leaves and fruits followed by a grey mass of mycelia and conidia (Williamson et al., 2007). Factors that influence the occurrence of grey mould are high humidity, reduced light and moderate temperature (Williamson et al., 2007).

Botrytis cinerea has the ability of surviving saprophytically or endophytically, and rapidly causes grey mould and other diseases (Lucero et al., 2009b). It has a wide range of hosts, and could colonize different plant parts; this is because of their action against a broad range of mono and dicotyledonous plants (Tenberge, 2007). This polyphagous pathogen has several modes of attacking hosts, and different sources of inoculum surviving in crop debris as mycelia and/or conidia and sclerotia (Williamson et al., 2007).

The control of this pathogen is mainly chemical and preventive. Despite existing various site-specific fungicides against gray mold (e.g., anilinopyrimidines, benzimidazoles, dicarboximides, hydroxyanilides, quinone outside inhibitors, phenylpyrroles, and succinate dehydrogenase inhibitors), its effective control depends on fungicide resistance emergency (Grabke et al., 2012). The *B. cinerea* adaptive plasticity (Kumari et al., 2014) enables selection of resistant strains when mono or oligosite fungicides are excessively used. Moreover, inadequate use of agrochemicals results harmful to the environment and humans (Maia et al., 2014).

Worldwide cases of fungicide resistance have become a major problem, and *B. cinerea* is not the exception because it presents high both resistance factors and frequencies of mutant phenotypes in its population (Leroux et al., 2010). Some resistance cases in commercial strawberry fields have been disclosed with the use of fenhexamid (Grabke et al., 2012). *Botrytis cinerea* phenotypes have been found with multifungicide resistance of up to four chemical groups in the U.S.A. (Amiri et al., 2012), and five in Germany (Weber, 2011).

Management strategies such as rotating chemical classes and/or mixing products from different chemical classes should be taken into account to avoid this resistance phenomenon (Grabke et al., 2012). Anyhow, consumers tend to avoid chemical additives and demand for natural and more socially acceptable choices (Stević et al., 2014). There are several alternatives to chemical control like biocontrol agents, essential oils and extracts or infusions of different plants (Sivakumar & Bautista-Baños, 2014). In industrialized countries, botanical fungicides are used because of the pesticides regulations or in cases of food organic production (Dal Bello & Sisterna, 2010).

In Argentine, there are a wide variety of native plants known for their medicinal use in humans and animals. The bioactivity could be associated with the presence of several substances, like secondary plant metabolites (e.g., flavonoids, phenolic acids, tannins, stilbene, lignans and alkaloids: Ignat et al., 2011; Villa-Martínez et al., 2014). Some of these substances usually have antimicrobial activity (Orhan et al., 2010; Hassanein et al., 2014), specifically against plant pathogens (Lehmann et al., 2015). This is why the aim of this work was to study the potential value of four Argentine native plant extracts and some of their phenolic compounds as biodrugs on *B. cinerea*. To achieve this objective, the phenolic composition of aqueous extracts was studied on *Larrea divaricata* Cav. (jarilla), *Prosopis strombulifera* (Lam.) Benth (retortuño), *Tessaria absinthioides* (Hook. & Arn.) DC. (pájaro bobo) and *Schinus molle* var *areira* (L.) (aguaribay). Mycelial growth of *B. cinerea* treated with the crude plant extracts was also evaluated.

MATERIALS AND METHODS

Fungal isolate and culture conditions. The *B. cinerea* strain used in the assays was isolated from naturally infected grape (*Vitis vinifera* L., cv Chardonnay) from Mendoza, Argentina. A monosporic colony obtained from a pure fungus culture was maintained on PDA (Potato Dextrose Agar) slant tube at 4 ± 1 °C, for long term conservation. When needed, colonies of the pathogen were subcultured on PDA at 22 ± 2 °C.

Chemicals and standards. Phenolic compounds (catechin, naringenin, cinnamic acid, syringic acid, chlorogenic acid, vanillic acid, luteolin, quercetin and caffeic acid), constituents of BGE (30 mM boric acid buffer) and organic modifiers [acetonitrile (ACN) and methanol (MeOH)] were purchased from Sigma (St. Louis, MO). Ultrapure water (resistivity 18.3 M Ω cm) obtained from Barnstead EASY pure® RF water system (Iowa) was used to prepare solutions, including the electrolyte solutions. Millex- HV Syringe Filters (0.22 μ m pore size), Millipore® were also used.

Solvents and reagents. Standard stock solutions of the analytes were prepared by dissolving an appropriate amount of each pure substance in 10 mL of a HPLC-grade methanol to obtain a final concentration of 1000 mg/L. The resulting solutions were stored at 4 °C in amber glasses. Working standard solutions at a 5 mg/L concentration were prepared on a daily basis by diluting appropriate aliquots of the previous standard stock solutions in methanol. Before use, all solutions were degassed by sonication for 5 min and filtered through 0.22 μ m nylon filters.

Plant extracts and purification. Plant material of Argentine native botanical species [i.e., *L. divaricata* (jarilla),

P. strombulifera, *S. molle* var. *areira* (aguaribay) and *T. absinthioides* (pájaro bobo)] was harvested in the countryside area in the North of Mendoza province, Argentina. The harvest period was coincident with the flowering time of plants.

Crude plant extracts were obtained as proposed by Widmer & Laurent (2006), with some modifications. Briefly, 20 g of *L. divaricata*, *P. strombulifera*, *T. absinthioides* and *S. molle* var. *areira* leaves were taken and autoclaved on 1L Erlenmeyer flasks containing 200 mL of distilled water for 45 min at 121 °C. The supernatant was filtered and reduced by boiling in laminar flow-hood to 20 mL. The extract was then centrifuged at 2000g for 15 min to remove solids. For sterilization, flasks were autoclaved for 20 min at 121 °C.

For the phenolic phase extraction, crude plant extract samples (1 g/mL) were thoroughly mixed with five parts (1:5, w/v) of acidic water until complete homogenization. An aliquot of 250 µL of each extract was used for extraction by the Solid Phase Extraction (SPE) method. Phenolic compounds extractions were performed by a miniaturized SPE using a home-made column packed with suitable filtering material. C18 cartridges (50 mg) were made in 1 mL syringes using 25 mg of glass wool as frits. These cartridges were placed in a vacuum elution apparatus (Varian VacElut 20 manifold and a Vacuubrand vacuum pump ME 2C) and preconditioned using 5 mL of MeOH and 5 mL of acid water (water pH 2 with HCl). The samples were loaded onto the preconditioned column, leaving the sample on the solid phase under vacuum. Then, the column was washed with 1 mL of acid water (water pH 2, with HCl). The phenolic fraction was eluted with MeOH (500 µL). The eluent was directly injected and analyzed by Capillary Zone Electrophoresis (CZE).

Phenolic compounds in plant extracts by CZE. Phenolic compounds were determined according with Boiteux et al. (2014). CZE separations were carried out using a Capel™ 105M apparatus equipped with a 67 cm full length, 50 cm effective length, 75 µm ID and 375 µm OD fused silica capillary. The running buffer was boric acid 30 mM; pH of the buffer was 9.5. The separation voltage was 25 kV and the capillary temperature was 25 °C. Samples were injected by hydrodynamic injection at 30 mbar for 2 s. Electropherograms were recorded at 290 nm.

Effect of plant extracts on mycelial growth of *B. cinerea*. Toxicity of crude plant extracts against the fungus was assayed using the amended media test (Soliman & Badeaa, 2002) on Potato Dextrose Agar (PDA). Extracts of *L. divaricata*, *S. molle* var. *areira*, *P. strombulifera* and *T. absinthioides* were added to the culture medium at different concentrations (0.1 to 200 mg/mL). Sterile distilled water was used as a control. The culture medium with or without extracts was poured into 5.5 cm diameter Petri dishes. Subsequently, a 4 mm disk of solid culture medium, colonized with *B. cinerea* mycelia obtained

from the edge of 15 day-old culture, was placed in the center of Petri dishes. The fungus colony area was daily measured using the software Axio Vision 4.8. The results were expressed as mycelial growth inhibition percentage (MGI) using the formula $[(C - T)/C] * 100$, where C and T corresponded to the fungus colony area (cm²) of control and treatments, respectively.

Individual effects of selected phenolic compounds on mycelial growth. The effect of the pure phenolic compounds showing positive correlation with the antimicrobial activity against *B. cinerea* was evaluated. For this purpose, the solid agar bioassay described by Boiteux et al. (2014) was performed. In this case, a 4 mm disk of culture medium, colonized with *B. cinerea* mycelia was placed in the center of Petri dishes containing PDA amended with phenolic compounds at a concentration equivalent to that considered as bioactive in crude extract assays (9.5 mg/L of naringenin, 3.2 mg/L of cinnamic acid and 6.9 mg/L of luteolin). Phenolics compound were dissolved in MeOH. Control plates were run simultaneously, using the same growth medium plus the solvent and without phenolic compounds. Three replicate plates containing each phenolic compound including the control treatment were prepared. Petri dishes prepared as above were incubated at 22 ± 2 °C for 4 days, and colony growth was daily recorded. Mycelial growth inhibition was determined as described above.

Statistical analysis. Statistical analysis was performed by ANOVA, and means were compared using Tukey's test. All the analyses were done in triplicate. The results were significant at P ≤ 0.05 unless specified otherwise. The principal component analysis (PCA) was used to find the relationships between phenolic compounds and percentage of *B. cinerea* mycelial growth inhibition. Statistical analyses were carried out using InfoStat-Statistical Software and Statgraphics Centurion XVI.II.

RESULTS AND DISCUSSION

Phenolic compounds profile of vegetal extracts. All vegetal extracts showed a distinctive profile when analyzed by CZE (Table 1). Some phenolic compounds were detected only in one or a few extracts as rutin in *P. strombulifera*, naringenin in *L. divaricata* or syringic acid in *S. molle* and *P. strombulifera*. Only quercetin was found in all four extracts differing statistically on its concentration, except on *S. molle* and *T. absinthioides*. Moreover, *P. strombulifera* extracts have exhibited the greatest variety and the highest concentration of total phenolic compounds (635.8 mg/L). Meanwhile, *L. divaricata* showed the lowest amount of the studied phenolic compounds (231.0 mg/L). *Larrea divaricata* did not differ statistically from *T. absinthioides*.

Table 1. Phenolic compounds (mg/L) found in plant extracts analyzed by Capillary Zone Electrophoresis.
Tabla 1. Compuestos fenólicos (mg/L) presentes en extractos de plantas analizados por Electroforesis capilar de zona.

| Phenolic compound (mg/L) | Plant extract | | | |
|--------------------------|-----------------|----------------------|-------------------------|-------------------------|
| | <i>S. molle</i> | <i>L. divaricata</i> | <i>T. absinthioides</i> | <i>P. strombulifera</i> |
| Rutin | nd a | nd a | nd a | 125.40 ± 2.60 b |
| Catechin | 97.37 ± 2.92 b | nd a | 69.87 ± 1.82 b | 489.00 ± 17.61 c |
| Naringenin | nd a | 63.00 ± 0.12 b | nd a | nd a |
| Cinnamic acid | 5.10 ± 0.23 c | 21.10 ± 0.06 d | nd a | 2.07 ± 0.03 b |
| Chlorogenic acid | 187.30 ± 4.16 c | nd a | 81.93 ± 10.77 b | 2.20 ± 0.06 a |
| Syringic acid | 4.27 ± 0.20 b | nd a | nd a | 5.50 ± 0.75 b |
| Luteolin | nd a | 46.07 ± 1.53 b | 3.33 ± 0.43 a | 2.10 ± 0.23 a |
| Vanillic acid | nd a | 16.90 ± 0.06 b | 75.73 ± 6.15 c | 4.03 ± 1.59 ab |
| Caffeic acid | 56.00 ± 1.21 d | 41.37 ± 0.03 c | nd a | 4.07 ± 0.03 b |
| TPC | 361.73 ± 8.28 b | 231.03 ± 1.47 a | 240.07 ± 16.95 a | 635.83 ± 15.56 c |

Data represent the mean values of three technical replicates ± standard error. “nd” (not detected). Mean values with different letters within the same row differs statistically according to Tukey’s test ($P \leq 0.05$).

Los datos representan la media de tres repeticiones técnicas ± error estándar. “nd” (no detectado). Los valores de media con letras diferentes en una misma fila difieren estadísticamente según el test de Tukey ($P \leq 0,05$).

Biological activity of plant extracts against *B. cinerea*.

The biological activity of the different plant extracts on mycelia of *B. cinerea* is shown in Figure 1. All extracts showed a diverse activity. Some of them exhibited a mycelia growth stimulation compared with the control like extracts belonging to *T. absinthioides*, and *S. molle* var. *areira*. Conversely, *P. strombulifera* and *L. divaricata* extracts have exhibited inhibition of mycelial growth.

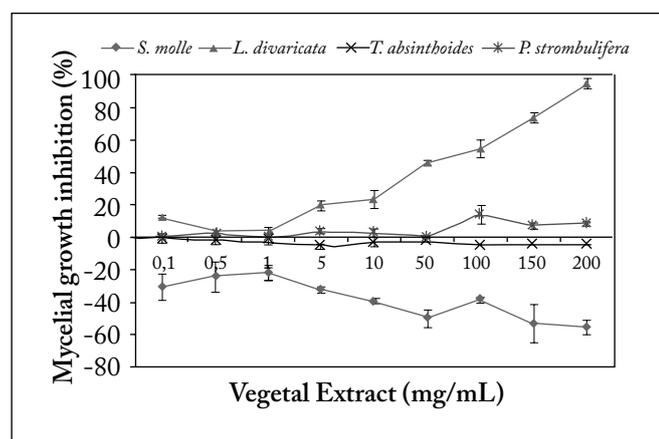


Fig. 1. Mycelial growth inhibition (%) of *B. cinerea* cultured on PDA amended with increased concentrations of plant extract (mg/mL) during an incubation period of 4 days at 22 ± 2 °C. Bars “I” represent standard error.

Fig. 1. Inhibición del crecimiento micelial (%) de *B. cinerea* cultivado en PDA adicionado con concentraciones crecientes de extracto vegetal (mg/mL) durante un período de incubación de 4 días a 22 ± 2 °C. Barras “I” representan error estándar.

Treatments with either *T. absinthioides* or *P. strombulifera* plant extract did not show statistical differences between their biological activities against the fungus at any of the concentrations analyzed.

The lowest stimulatory or inhibitory effects were observed between concentrations of 0.1 and 1.0 mg/L of plant extract. A dose-dependent mode of action was only observed in the inhibitory effect of the *L. divaricata* extract and in the stimulatory effect of the *S. molle* var. *areira* extract.

The most effective and interesting aqueous extract was that of *L. divaricata*, which produced the highest inhibition percentage (about 94%) at a concentration of 200 mg/mL. It was also able to hinder mycelial growth at the lowest concentration, i.e. 5 mg/mL. This extract did not stimulate the pathogen ever.

The variability of the biological activity could be related to the capability of *B. cinerea* of detoxifying compounds presents in plants (Pedras et al., 2011).

Even though an *in vitro* antifungal activity of *S. molle* was reported by Dikshit et al. (1986) against *Aspergillus* and *Penicillium* species, in our work *S. molle* plant extract showed a stimulatory effect against *B. cinerea*. It might be that they used an essential oil obtained from leaves instead of an aqueous extract. Nonetheless, Davicino et al. (2007) demonstrated that the aqueous extract had no activity against *P. notatum* and *A. niger*. Conversely, Rhouma et al. (2009) showed that leaf ethanolic, methanolic and aqueous extracts inhibited *F. solani* growth within values from 60% to 66%.

It is quite difficult to compare these results with those of other authors because of the composition of the plant extracts can vary depending upon the geographical region, the plant

age or variety, and the extraction method (Al-Reza et al., 2010). Saks and Barkai (1995) demonstrated that *Aloe vera* L. aqueous extract inhibited the *B. cinerea* mycelial growth in a 68% at a concentration of 1 $\mu\text{L/L}$. Other plants extracts like those of *Quillaja saponaria* M. at 15% or *Pithecellobium dulce* at 25% inhibited the mycelial growth of *B. cinerea* in an either 45% or 60%, respectively (Baños et al., 2004; Fonseca Rivera, 2007).

This work, as well as those of Davicino et al. (2007) and Vogt et al. (2007) showed that *L. divaricata* extracts had an inhibitory activity against fungus. Davicino et al. (2007), however, found that ethanolic extracts of *L. divaricata* had a better inhibitory activity than aqueous extracts. In our work, the aqueous extract exhibited a good inhibitory effect against *B. cinerea*.

Correlation between the analytical composition of the extracts and the *in vitro* antimicrobial activity. Relationships between phenolic compounds and inhibition of *B. cinerea* mycelial growth were analyzed by PCA. From this study three principal components were obtained and contributed to 99.3% of the total variance (CP1 50.0; CP2 27.6 and CP3 21.7%) (Fig. 2). *Larrea divaricata* was characterized by a high concentration of naringenin, luteolin and cinnamic acid. These compounds could be associated with the *B. cinerea* mycelial growth inhibition. These results are in accordance with those of Boiteux et al. (2014). They reported their association with *Phytophthora* spp. mycelial growth inhibition. Conversely, the high concentration of chlorogenic acid present on *S. molle* var. *areira* extract could be associated with its low mycelial growth inhibition. Similar results were observed for such phenolic

compound on different pathogen species such as *Acremonium* sp., *Beauveria* sp., *Fusarium solani*, *Microsporium canis* sp., *Scopulariopsis brevicaulis*, *Trichoderma* sp. (Sisti et al., 2008), and *Phytophthora* spp. (Boiteux et al., 2014). Other phenolic compounds like vanillic acid and syringic acid have been reported to have a low antifungal activity against *B. cinerea* (Mendoza et al., 2013).

Between the phenolic compounds that could be associated with *B. cinerea* MGI (Fig. 3), the most effective was luteolin (84.3%) followed by naringenin (75.7%) and cinnamic acid (67.8%). When the three selected phenolic compound were tested together, they showed an additive inhibitory activity (99.0%) (Fig. 4). It should be noted that the MGI of the *B. cinerea* strain studied was lower when it was treated with the *L. divaricata* crude extract than when it was treated with the pure phenolic compounds at the same quantities detected in the extract. This could be a consequence of the combined effects of different compounds present in a high complexity matrix like this plant extract. This bioassay provided sufficient evidence to confirm that the three compounds that the PCA analysis identified as those explaining the major growth inhibitory activity were effectively those.

The bioactivity of these compounds is probably interfering with the permeability of the fungus cell membrane altering its functionality. Some studies have reported that phenolic compounds could (1) dissipate the pH gradient and electrical potential components of the proton motive force, (2) interfere with the energy (ATP) generating and conservation system of the cell, (3) inhibit membrane-bound enzymes, and (4) prevent the substrate utilization for energy production (De

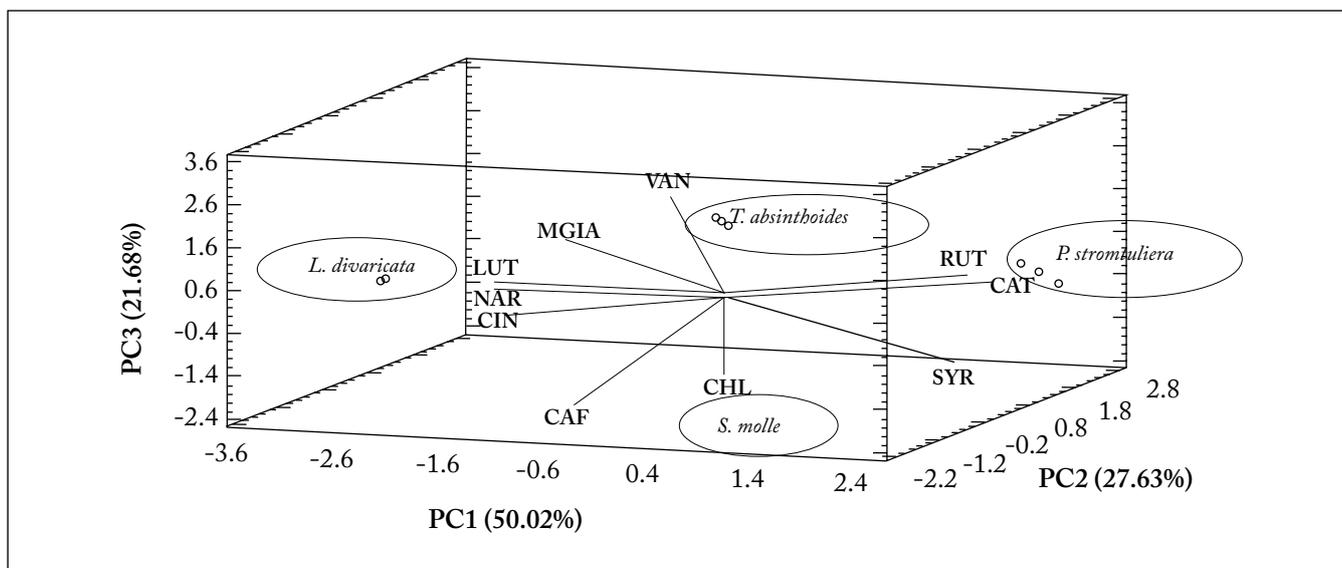


Fig. 2. Principal Components Analysis (PCA) of four plant extracts *L. divaricata*, *T. absinthoides*, *P. strombulifera*, and *S. molle* based on the studied phenolic compounds and *B. cinerea* Mycelial Growth Inhibition Area (MGIA).

Fig. 2. Análisis de Componentes Principales (PCA) de cuatro extractos vegetales *L. divaricata*, *T. absinthoides*, *P. strombulifera*, y *S. molle* basado en el estudio de compuestos fenólicos y la Inhibición del Área del Crecimiento Micelial de *B. cinerea* (MGIA).

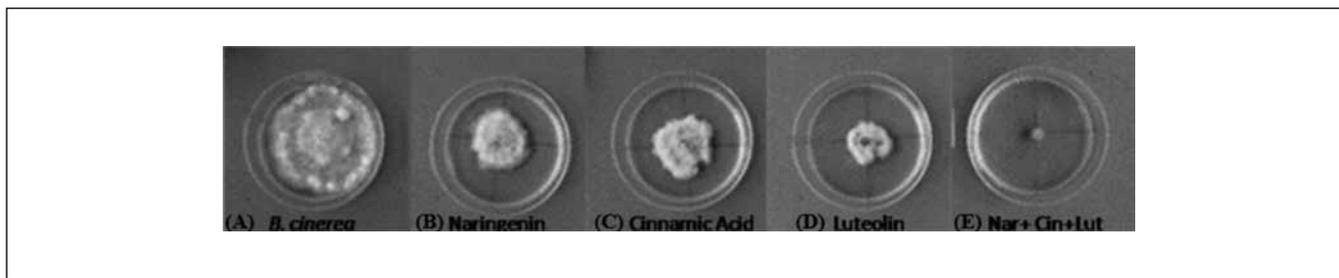


Fig. 3. Inhibition of mycelial growth of *B. cinerea* grown in PDA amended with selected phenolic compounds for 4 days at 22 ± 2 °C. (A) Control. (B) PDA amended with 9.5 mg/L of Naringenin; (C) PDA amended with 3.2 mg/L of Cinnamic Acid; (D) PDA amended with 6.9 mg/L of Luteolin, and (E) PDA amended with a mixture of 9.5 mg/L of Naringenin, 3.2 mg/L of Cinnamic Acid and 6.9 mg/L of Luteolin.

Fig. 3. Inhibición del crecimiento micelial de *B. cinerea* cultivado en PDA adicionado con compuestos fenólicos seleccionados por 4 días a 22 ± 2 °C. (A) Control. (B) PDA adicionado con 9,5 mg/L de Naringenina; (C) PDA adicionado con 3,2 mg/L de Ácido Cinámico; (D) PDA adicionado con 6,9 mg/L de Luteolina y (E) PDA adicionado con una mezcla de 9,5 mg/L de Naringenina más 3,2 mg/L de Ácido Cinámico más 6,9 mg/L de Luteolina.

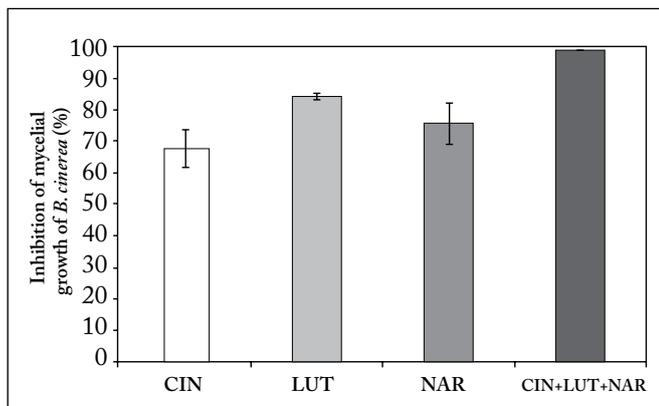


Fig. 4. Inhibition of mycelial growth of *B. cinerea* (%) grown in PDA amended with selected phenolic compound for 4 days at 22 ± 2 °C. Treatments: NAR: 9.5 mg/L of Naringenin; CIN: 3.2 mg/L of Cinnamic acid; LUT: 6.9 mg/L of Luteolin and CIN+LUT+NAR: 9.5 mg/L of Naringenin; 3.2 mg/L of Cinnamic acid and 6.9 mg/L of Luteolin.

Fig. 4. Inhibición del crecimiento micelial de *B. cinerea* (%) cultivado en PDA adicionado con compuestos fenólicos seleccionados por 4 días a 22 ± 2 °C. Tratamientos: NAR: 9,5 mg/L de Naringenina; CIN: 3,2 mg/L de Ácido Cinámico; LUT: 6,9 mg/L de Luteolina y CIN+LUT+NAR: 9,5 mg/L de Naringenina; 3,2 mg/L de Ácido Cinámico y 6,9 mg/L de Luteolina.

Oliveira et al., 2011; El-Mogy and Alsanian, 2012; Pusztahelyi et al., 2015).

These are known to alter microbial cell permeability and interact with membrane proteins, causing deformation in the structure and functionality of these proteins. These disadvantageous changes may lead to dysfunction and subsequent disruption of membranes.

As we noticed for the *L. divaricata* extract, other *Larrea* species like *L. tridentata* have demonstrated inhibition activity against microorganisms (Martins et al., 2013). Other compounds present on some extracts obtained from *Larrea*

species [e.g., nordihydroguaiaretic acid (NDGA)] have shown inhibitory activity against pathogenic fungi like *A. flavus* and *A. parasiticus* (Vargas-Arispuro et al., 2005). Regardless that NDGA was detected in some *L. divaricata* extracts, Davicino et al. (2011) noticed that its concentration was higher in ethanolic than aqueous extraction. The same consideration could be made for the chloroformic extract, which had a good antifungal activity, containing a considerable amount of NDGA (Vogt et al., 2007; Vogt et al., 2013). This is an important point because of NDGA is a lignan with nephrotoxic and hepatotoxic effects. Consequently, extracts without NDGA or with a low concentration of it could be more acceptable and considered safer (Steger et al., 2011).

This work indicated that other phenolic compounds, like naringenin, cinnamic acid and luteolin, might also have inhibitory activity against the plant pathogen *B. cinerea*.

CONCLUSIONS

Results support that *L. divaricata* aqueous extracts are potent inhibitors of *B. cinerea* growth. A large part of this bioactivity was explained by the presence of phenolic substances such as naringenin, cinnamic acid and luteolin. Although the other studied plant species have, between their constituents, some of the previous mentioned bioactive substances, their concentration was likely not high enough to inhibit the pathogen. Therefore, the aqueous extracts of *L. divaricata* could represent a potentially valid control alternative. This will be known after verifying its effectiveness *in vivo*, and adjusting the most suitable concentration and formulation for its use.

Our findings are not only of fundamental interest, but also have practical implications for crop protection, food safety, and animal and human health. Further toxicological and pharmacological studies will be useful to confirm the use of bioagrochemicals, using phytochemicals like those from *L. divaricata* leaves.

ACKNOWLEDGEMENTS

The authors thank financial support from to SECTyP Universidad Nacional de Cuyo, Mendoza, Argentina, and Agencia Nacional de Promoción Científica y Tecnológica (PICT 2189).

REFERENCES

- Al-Reza, S.M., A. Rahman, Y. Ahmed & S.C. Kang (2010). Inhibition of plant pathogens *in vitro* and *in vivo* with essential oil and organic extracts of *Cestrum nocturnum* L. *Pesticide Biochemistry and Physiology* 96: 86-92.
- Amiri, A., S.M. Heath & N.A. Peres (2012). Phenotypic Characterization of Multifungicide Resistance in *Botrytis cinerea* Isolates from Strawberry Fields in Florida. *Plant Disease* 97: 393-401.
- Baños, P.E., E.M. Zavaleta, M.T. Colinas, I.R. Luna & J.A. Gutiérrez (2004). Control biológico de *Colletotrichum gloeosporoides* en papaya Marado Roja (*Carioca papaya* L.) y fisiología postcosecha de frutos infectados. *Revista Mexicana de Fitopatología* 22: 198-205.
- Boiteux, J., C.S. Vargas, P. Pizzuolo, G. Lucero & M.F. Silva (2014). Phenolic characterization and antimicrobial activity of folk medicinal plant extracts for their applications in olive production. *Electrophoresis* 35: 1709-1718.
- Dal Bello, G. & M. Sisterna (2010). Use of plant extracts as natural fungicides in the management of seedborne diseases. In: A. Arya and A.E. Perelló (eds.), pp. 51-66. Management of Fungal Plant Pathogens. CAB International, UK.
- Davicino, R., R. Martino & C. Anesini (2011). *Larrea divaricata* Cav.: Scientific evidence showing its beneficial effects and its wide potential application. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* 10: 92-103.
- Davicino, R., M.A. Mattar, Y.A. Casali, S.G. Correa & E.M. Pette-nati (2007). Actividad antifúngica de extractos de plantas usadas en medicina popular en Argentina. *Rev. Perú. Biol* 14: 247-251.
- De Oliveira, T.L.C., R. de Araújo Soares, E.M. Ramos, M. das Graças Cardoso, E. Alves & R.H. Piccoli (2011). Antimicrobial activity of *Satureja montana* L. essential oil against *Clostridium perfringens* type A inoculated in mortadella-type sausages formulated with different levels of sodium nitrite. *International Journal of Food Microbiology* 144: 546-555. doi: 10.1016/j.ijfoodmicro.2010.11.022
- Dikshit, A., A.A. Naqvi & A. Husain (1986). *Schinus molle*: a new source of natural fungitoxicant. *Applied and Environmental Microbiology* 51: 1085-1088.
- Fonseca-Rivera, A. (2007). Evaluación y caracterización de la actividad antifúngica de la especie *Quillaja saponaria* Mol. cultivada *in vitro* en *Botrytis cinerea* Pers. Facultad de Ingeniería, Ciencias y Administración. Temuco, Chile.
- Grabke, A., D. Fernández-Ortuño & G. Schnabel (2012). Fehexamid Resistance in *Botrytis cinerea* from Strawberry Fields in the Carolinas Is Associated with Four Target Gene Mutations. *Plant Disease* 97: 271-276.
- El-Mogy, M.M. & B.W. Alsanius (2012). Cassia oil for controlling plant and human pathogens on fresh strawberries. *Food Control* 28: 157-162. doi:10.1016/j.foodcont.2012.04.036
- Hassanein, H.D., S.A. Ahl Hah & M.M. Abdelmohsen (2014). Antioxidant polyphenolic constituents of *Satureja montana* L. growing in Egypt. *International Journal of Pharmacy and Pharmaceutical Sciences* 6: 578-581. ISSN- 0975-1491.
- Ignat Ioana, I. Volf & V.I. Popa (2011). A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chemistry* 126: 1821-1835.
- Kumari, S., P.Tayal, E. Sharma & R. Kapoor (2014). Analyses of genetic and pathogenic variability among *Botrytis cinerea* isolates. *Microbiological Research* 169: 862-872.
- Lehmann, S., M. Serrano, F. L'Haridon, S.E. Tjamos & J.P. Metraux (2015). Reactive oxygen species and plant resistance to fungal pathogens. *Phytochemistry* 112: 54-62.
- Leroux, P., M. Gredt, M. Leroch & A.S.Walker (2010). Exploring Mechanisms of Resistance to Respiratory Inhibitors in Field Strains of *Botrytis cinerea*, the Causal Agent of Gray Mold. *Applied and Environmental Microbiology* 76: 6615-6630.
- Lucero, G., N. Cucchi & P.H. Pizzuolo (2009a). Enfermedades (hongos y bacterias). In: N. Cucchi and V. Becerra (eds.), pp. 150-192. Manual de Tratamientos Fitosanitarios para cultivos de clima templado bajo riego Sección III: Vid, vol. 1. Inta publicaciones, Buenos Aires.
- Lucero, G., P.H. Pizzuolo & H. Lucero (2009b). Principales enfermedades de origen criptogámico de la vid en la región de Cuyo. In: A. Cavallo, E. Pasqualini and C. Passera (eds.), pp. 43-56. II Encuentro Italo-Argentino sobre la producción integrada de los cultivos: vides y vinos. Báez impresiones, Córdoba- Argentina.
- Maia, A.J., J.S.B. Oliveira, K.R.F. Schwan-Estrada, C.M.R. Faria, A.F. Batista, W. F. Costa & B.N. Batista (2014). The control of isariopsis leaf spot and downy mildew in grapevine cv. Isabel with the essential oil of lemon grass and the activity of defensive enzymes in response to the essential oil. *Crop Protection* 63: 57-67.
- Martins, S., E.L.C. Amorim, T.J.S.P. Sobrinho, A.M. Saraiva, M.N.C. Pisciotano, C.N. Aguilar, J.A. Teixeira & S.I. Mussatto (2013). Antibacterial activity of crude methanolic extract and fractions obtained from *Larrea tridentata* leaves. *Industrial Crops and Products* 41: 306-311.
- Mendoza, L., K. Yañez, M. Vivanco, R. Melo & M. Cotoras (2013). Characterization of extracts from winery by-products with antifungal activity against *Botrytis cinerea*. *Industrial Crops and Products* 43: 360-364.
- Orhan, D.D., B. Özçelik, S. Özgen & F. Ergun (2010). Antibacterial, antifungal, and antiviral activities of some flavonoids. *Microbiological Research* 165: 496-504.
- Pedras, M.S.C., S. Hossain & R.B. Snitynsky (2011). Detoxification of cruciferous phytoalexins in *Botrytis cinerea*: Spontaneous dimerization of a camalexin metabolite. *Phytochemistry* 72: 199-206.
- Pusztahelyi T., I.J. Holb & I. Pócsi (2015). Secondary metabolites in fungus-plant interactions. *Frontiers in Plant Science* 6: 573, 1-23. doi: 10.3389/fpls.2015.00573
- Rhouma, A., H. Ben Daoud, S. Ghanni, H. ben Salah, M. Romdhane & M. Demak (2009). Antimicrobial activities of leaf extracts of *Pistacia* and *Schinus* species against some plant pathogenic fungi and bacteria. *Journal of Plant Pathology* 91: 339-345.
- Saks Y. & G. Barkai (1995). *Aloe vera* gel activity against plant pathogenic fungi. *Postharvest Biology and Technology* 6: 159-165.
- Sisti, M., M. De Santi, D. Fraternali, P. Ninfali, V. Scoccianti & G. Brandi (2008). Antifungal activity of *Rubus ulmifolius* Schott standardized *in vitro* culture. *LWT - Food Science and Technology* 41: 946-950.
- Sivakumar, D. & S.Bautista-Baños (2014). A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. *Crop Protection* 64: 27-37.

- Soliman, K.M. & R.I. Badeaa (2002). Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. *Food and Chemical Toxicology* 40: 1669-1675.
- Stege, P.W., L.L. Sombra, R.C. Davicino & R.A. Olsina (2011). Analysis of nordihydroguaiaretic acid in *Larrea divaricata* Cav. extracts by micellar electrokinetic chromatography. *Phytochemical Analysis* 22: 74-79.
- Stević, T., T. Berić, K. Šavikin, M. Soković, D. Godevac, I. Dimkić & S. Stanković (2014). Antifungal activity of selected essential oils against fungi isolated from medicinal plant. *Industrial Crops and Products* 55: 116-122.
- Tenberge, K.B. (2007). Morphology and cellular organisation in *Botrytis* interactions with plants. *Botrytis: Biology, Pathology and Control*, pp. 67-84.
- Vargas-Arispuro, I., R. Reyes-Báez, G. Rivera-Castañeda, M.A. Martínez-Téllez & I. Rivero-Espejel (2005). Antifungal lignans from the creosotebush (*Larrea tridentata*). *Industrial Crops and Products* 22: 101-107.
- Villa-Martínez, A., R. Pérez-Leal, H.A. Morales-Morales, M. Bar-surto-Sotelo, J.M. Soto-Parra & E. Martínez-Escudero (2015). Situación actual en el control de *Fusarium* spp. y evaluación de la actividad antifúngica de extractos vegetales. *Acta Agronómica* 64: 194-205.
- Vogt, V., D. Cifuentes, C. Tonn, L. Sabini & S. Rosas (2013). Antifungal activity *in vitro* and *in vivo* of extracts and lignans isolated from *Larrea divaricata* Cav. against phytopathogenic fungus. *Industrial Crops and Products* 42: 583-586.
- Vogt, V., M. Rovera, C. Tonn, L. Sabini & S. Rosas (2007). Valoración preliminar del efecto fungitóxico y alelopático del extracto clorofórmico de *Larrea divaricata*. *Bol. Latinoam. Caribe Plant. Med. Aromaticas* 6: 369-370.
- Weber, R.W.S. (2011). Resistance of *Botrytis cinerea* to Multiple Fungicides in Northern German Small-Fruit Production. *Plant Disease* 95: 1263-1269.
- Widmer, T. & N. Laurent (2006). Plant extracts containing caffeic acid and rosmarinic acid inhibit zoospore germination of *Phytophthora* spp. pathogenic to *Theobroma cacao*. *European Journal of Plant Pathology* 115: 377-388.
- Williamson, B., B. Tudzynski, P. Tudzynski & J.A.L. Van Kan (2007). *Botrytis cinerea*: the cause of grey mould disease. *Molecular Plant Pathology* 8: 561-580.