Colegio de Postgraduados

Phytopathogenic fungi associated with blueberry dieback (*Vaccinium corymbosum* L.) pruning and sealing management

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ABSTRACT

Objective: To identify the phytopathogenic fungi related to blueberry dieback, verify their pathogenicity, incidence after pruning, and the type of sealing.

Design/methodology/approach: For this, symptomatic stem and branch samples were collected in eight commercial blueberry lots at the Ahome, El Fuerte, and Guasave municipalities, state of Sinaloa, from which 196 fungal isolates were obtained. These were morphologically identified to subsequently perform detached leaf and twig pathogenicity tests, on the Biloxi variety; likewise, two pruning angles and three sealants were evaluated and compared to an absolute control in a completely random arrangement.

Results: Based on morphological analysis the *Alternaria*, *Fusarium*, *Lasiodiplodia*, *Pestalotia*, and *Curvularia* genera were detected. However, *Lasiodiplodia* isolates were pathogenic in leaves and twigs, while the best result is achieved with the angle 45° pruning sealing with washable plastic-type white vinyl paint plus copper oxychloride.

Limitations on study/implications: None.

Findings/conclusions: The results open new research lines related to molecular identification and disease impact on performance.

Keywords: blueberry, dieback, *Lasiodiplodia*.

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INTRODUCTION

Blueberry (*Vaccinium corymbosum* L.) is a fruit plant, recently grown in at least 30 countries. Peru (261,450 t), Chile (185,300 t), Mexico (85,100 t), United States (328,210 t), South Africa (26,000 t), Poland (55,500 t) and Canada (80,420 t) stand out as suppliers (USDA-FAS, 2021). In Mexico, job creation during its harvesting season is highlighted, since it is a 100% manual labor process with a significant economic impact (Pérez-Cruz, 2018). From planting to harvest, blueberries require technical support, constant care, and different conditions, coupled with intensive agricultural practices. This has induced emerging phytopathogens that impact production. Worldwide, various fungi have been

reported to induce damage in different producing areas, among which *Alternaria* sp., *Curvularia* sp., *Microsphaera vaccinii*, *Phomopsis vaccinii*, *Stemphyllium* sp. (Cline and Schilder, 2006), *Bipolaris cynodontis* (Sisterna *et al.*, 2009), *Botrytis cinerea* (Bristow and Milholland, 1995) stand out. One of the most common diseases is the so-called "descending death blueberry dieback", caused by members of the Botryosphaericeae family, particularly *Lasiodiplodia theobromae*, *Botryosphaeria dothidea*, and *Neofusicoccum parvum*. These, without exception, colonize all stem tissues, inducing leaf and stem necrosis due to a lack of water and nutrients. The above makes knowing the pathogens present in commercial plantations necessary to establish management strategies, and these phytopathogens' correct identification and characterization essential. Therefore, this research objectives were a) to identify the fungi related to blueberry dieback through morphological studies; b) *in vitro*, determine the pathogenicity of fungi associated with blueberry dieback; c) to evaluate *in planta* two pruning and sealing techniques used in the region, and their correlation to blueberry dieback incidence.

MATERIALS AND METHODS

Seven commercial orchards of between two and four hectares were sampled at the Guasave, El Fuerte, and Ahome municipalities (state of Sinaloa) from October 2020 to July 2021; sampling in zigzag, 28 symptomatic stems were taken per orchard. The collected samples were transported to a laboratory in humid chambers at 4 °C. Isolation was performed in water-agar (AA; Bioxon; Cuautitlán Izcalli, State of Mexico, Mexico) following the procedure by Maraite *et al.* (1997) with a modification. To purify the isolates, hyphal tips were transferred to potato-dextrose-agar (PDA; Bioxon; Cuautitlán Izcalli, State of Mexico, Mexico). The pure culture media were preserved in filter paper, 10% glycerol, and three-times-distilled sterile water (TDSW), for later use.

The morphological characterization was done by taking macroscopic variables such as the colony color, mycelium type, acervuli, pycnidia, sporodochia, ascostromas, free conidiophores, and conidia presence; in addition to their radial growth rate (Granados-Montero, 2018). The pure isolates were placed in Petri dishes with PDA medium and incubated for 12 days at 25±2 °C in a 12 h light by 12 h dark regime, to determine their phenotypic characteristics and assess the mycelial growth rate (French and Hebert, 1980). The microscopic characterization was done with an Olympus Lx compound microscope with a micrometer. The shape of the conidia, the number of cells, coloration, length, width, and ornamentations were also considered. The production of pigments, type of margin, texture, and density were determined by reports in the literature, and the colony color, on the front and back, based on the color scale by Kelly and Judd (1976). The detached leaf pathogenicity occurred in the Biloxi varieties, on the first run, and in the Atlanthis during the second. Well-developed leaves were taken from the middle part of the plants. Inoculum production was done following the methodology by Foolad *et al.* (2000) with some modifications. Pathogenicity tests on detached leaves were carried out following the methodology by Peever *et al.* (2000) with some modifications. The experiment was carried out on two occasions, their treatments were distributed in a completely random arrangement with four repetitions and an absolute control.

Pathogenicity was evaluated seven days later by observing and measuring the affected leaf area (ALA). The pathogenicity tests on twigs were assessed in healthy 12 cm twigs with no leaves and disinfected with a 1.25% sodium hypochlorite solution. For this, 5 mm diameter mycelium discs from the colony's growth margin were placed in the center of pre-punctured twigs, then, incubating four twigs per tray in a humid chamber. The experiment was run twice, under normal light and temperature conditions for nine days, with a random distribution; Affected length area was the evaluated parameter (De la Mora-Castañeda *et al.*, 2014). The *in planta* tests were done on two-year-old Atlanthis variety plants, evaluating two pruning angles, 180° and 45° in a three sealants combination (Berel brand, washable plastic-type white vinyl paint, washable plastic-type white vinyl paint brand Berel plus copper oxychloride at a rate of 100 g per L of paint, and hydrogen peroxide), compared to plants with no sealing, in four repetitions; The experiment ran for 60 days in a random arrangement, under natural conditions of light, humidity, and temperature.

The affected leaf area percentages were statistically analyzed using the Kormogorof-Smirnov normality test and applying the Lilliefors correction, before an ANOVA using the SPSS Statistics 26 software. Given that the experiments were run on two occasions, and these showed an interaction between isolates and experiments, both results are expressed together.

RESULTS AND DISCUSSION

The colony and conidia phenotypic characteristics were determined in PDA after the corresponding incubation period. This allowed the identification of isolates (Figure 1) in the *Alternaria* spp, *Fusarium* spp, *Lasiodiplodia* spp, *Pestalotia* spp., and *Curvularia* spp. genus (Barnett and Hunter, 1972 and Phillips *et al.*, 2013).

From the total obtained isolates, characteristics from several genera were observed in different percentages (Figure 2), *Alternaria* spp. the most frequent.

In the detached leaves, not all the collected isolates were pathogenic. There are significant differences between isolates, $\alpha=0.05$, $F=2.344$, and 95% CI. The most pathogenic

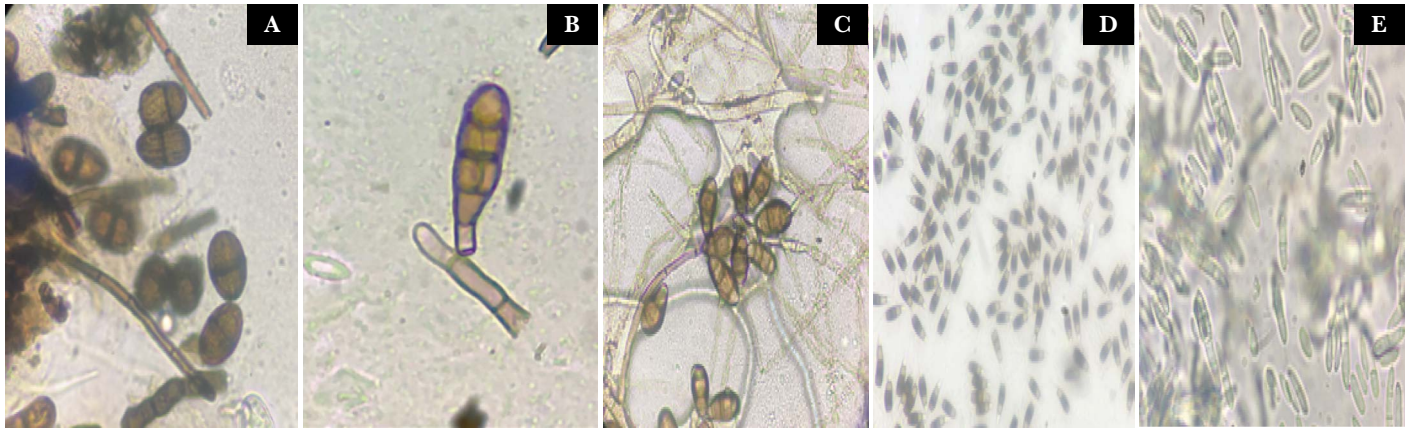

Figure 1. Conidia from PDA culture medium. A) *Lasiodiplodia* spp; B) *Alternaria* spp; C) *Curvulary*; D) *Pestalotia* spp, and E) *Fusarium* spp.

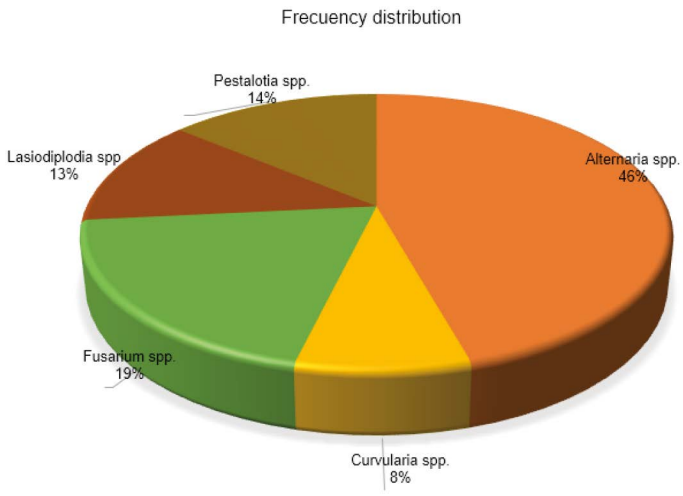

Figure 2. Pathogens percentage by gender.

being *Lasiodiplodia* spp. and *Pestalotia* spp. (Figure 3a) in their tests. Meanwhile, in the pathogenicity tests on vareta, only the isolates corresponding to the genus *Lasiodiplodia* spp. were pathogenic (Figure 3b).

The first in planta symptoms in the tests occurred between days 45 to 60 and appeared from the cutting area. There are significant differences between the tested treatments, vinyl paint plus copper oxychloride treatment the one with the best results.

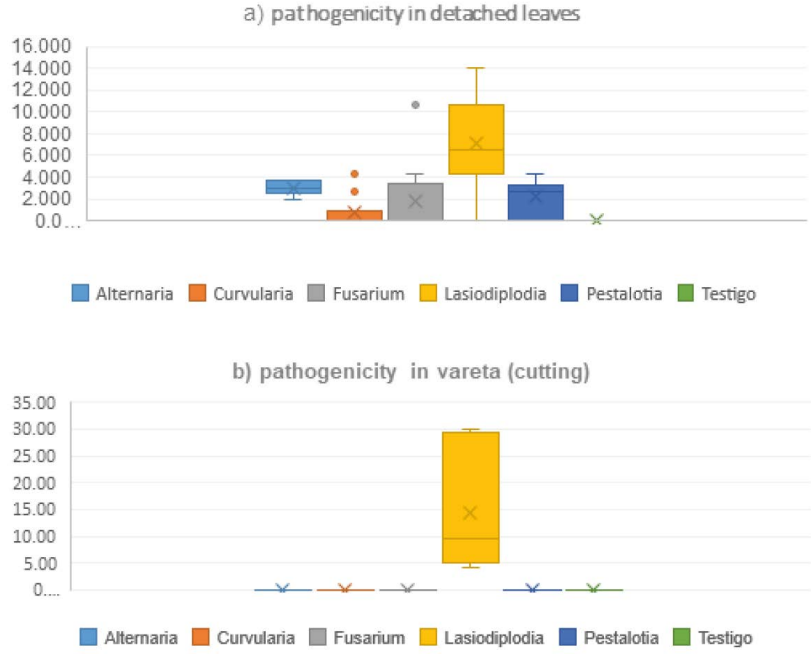

Figure 3. Pathogenicity tests. a) pathogenic isolates in detached leaves; b) pathogenicity in twigs (*Lasiodiplodia* spp.).

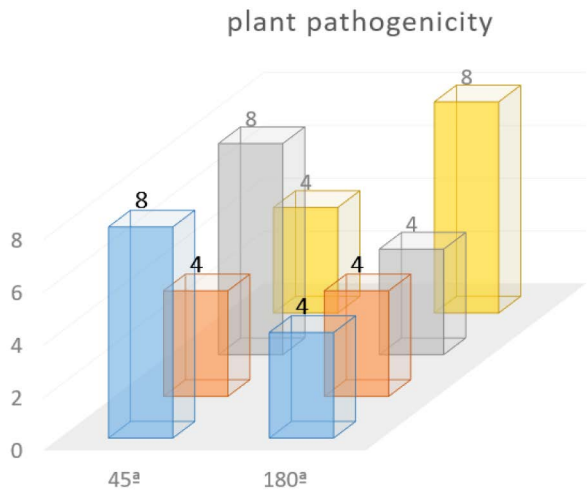

Figure 4. Plants with initial symptoms of blueberry dieback 60 days after pruning: blue bar: vinyl Paint; red bar: vinyl Paint + copper; yellow bar: unsealed

CONCLUSIONS

The pathogens of *Alternaria* spp., *Fusarium* spp., *Lasiodiplodia* spp., *Pestalotia* spp. *Curvularia* spp. and *Botryosphaeria* spp. genera were found associated with blueberry dieback, which occurs due to the different pruning on the crop. The identity of the species was done through morphological characterization. From the total tested isolates, only those from the *Lasiodiplodia* spp. genus correlate with the leaf and rod pathogenicity tests. Likewise, the best treatment to seal, with any pruning angle, is vinyl paint plus copper oxychloride at a 100g/L rate.

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