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Occurrence of *Alternaria alternata* causing leaf blight of blueberry in Mexico

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Abstract

From November of 2023 to February 2024, severe leaf blight symptoms were found on blueberry (*Vaccinium corymbosum* L.) plants in a commercial field in Navolato, Sinaloa, Mexico. Three single-spore isolates were obtained and identified as *Alternaria alternata* by phylogenetic analyses of concatenated sequences of the *gapdh, rpb2*, and *tef1-α* genes. The pathogenicity of the isolates was confirmed by inoculating conidial suspensions on healthy blueberry leaves. This represents the first report that *A. alternata* causes leaf blight of blueberry in Mexico.

Keywords: Vaccinium corymbosum, Alternaria alternata, morphology, phylogeny, pathogenicity

Mexico has established itself as one of the world's leading blueberry producers, producing 80,133 tons during the 2023 season, positioning itself as the 6th largest global producer. This positively impacted the Mexican economy, generating an income of $649 million USD. The leading blueberry-producing states in Mexico are Jalisco (28,121 tons), Sinaloa (26,634 tons), and Michoacán (12,110 tons). During the 2022 and 2023 agricultural seasons, Sinaloa stood out as a key region for blueberry production in Mexico, with 1,993 hectares dedicated to blueberry cultivation (SIAP 2024).

In the spring of 2024, severe leaf blight symptoms were observed on approximately 85% of blueberry plants in a 10-hectare commercial field in Navolato, Sinaloa, Mexico.

The symptom on blueberry leaves initially appeared as dark brown circular lesions, which subsequently expanded and coalesced to form blights on the leaves, accompanied by abundant dark brown to black sporulation of the fungus on the advanced lesions on both sides of the leaves. Foliar blight often began from the distal part of the leaves. Under conditions of high disease severity, premature defoliation of the plants was observed (Fig. 1).

The aim of this study was to identify the causal agent of leaf blight of blueberry, based on morphological characterization, phylogeny, and pathogenicity tests.

For fungal isolation, small fragments (5 mm^2) from the periphery of leaf lesions were surface sterilized with 2% sodium hypochlorite solution for 1 min, rinsed with sterilized distilled water twice, and blotted dry on sterile filter paper. Leaf fragments were placed onto potato dextrose agar (PDA, Difco, France) medium and incubated in darkness at 25ºC for 5 days. *Alternaria*-like colonies were consistently isolated, and 12 monosporic cultures were obtained. Three isolates were selected as representative for morphological characterization, multilocus phylogenetic analysis, and pathogenicity tests. Isolates were deposited as

CCLF433–CCLF435 in the Culture Collection of Phytopathogenic Fungi at the Research Center for Food and Development located in Culiacán, Sinaloa (Mexico).

The morphology of 8-day-old fungal cultures grown on PDA medium and incubated at 25°C under dark conditions was examined. Slide preparations were made in clear lactic acid, and microscopic examinations of 50 conidia and 30 conidiophores were performed using an Axio Imager M2 microscope (Zeiss, Germany). Micrographs were taken using an Axiocam 305 (Zeiss) and processed using ZEN 2.3 SP1 imaging software (Zeiss).

To determine the phylogenetic identity of three isolates, genomic DNA was extracted according to the CTAB method (Doyle and Doyle 1990), and the partial sequences of the glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), the second largest subunit of RNA polymerase II (*rpb2*), and the translation elongation factor 1-alpha (*tef1-α*) genes were amplified using the primers pairs GPD1/GPD2 (Berbee et al. 1999), 980F/7cR (Liu et al. 1999) and EF1-688F/EF1-1251R (Alves et al. 2008), respectively. The PCR conditions were as follows: an initial denaturation step at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 s; annealing for 30 s at 57°C for GPD1/GPD2, 52°C for 980F/7cR, and 54°C for EF1-688F/EF1-1251R; extension at 72°C for 1 min; and a final extension step at 72°C for 5 min. The PCR assays were conducted in a Bio-Rad C1000 thermocycler (Hercules, CA, USA). The PCR products were separated by electrophoresis in 1% agarose gel stained with ethidium bromide and viewed under ultraviolet light. The amplicons were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and sequenced by Macrogen (Seoul, South Korea) in both directions with the same primers that were used for the PCR reactions. The sequences were deposited in GenBank under the accession numbers PQ519964–PQ519966 for *gapdh,* PQ519967–PQ519969 for *rpb2,* and PQ519970–PQ519972 for *tef1-α*.

The consensus sequences of each isolate and the sequences from related species retrieved from GenBank were aligned in MAFFT version 7 (Katoh et al. 2017) and trimmed in MEGA 7 (Kumar et al., 2016). For phylogenetic reconstruction, a Bayesian Inference (BI) analysis was performed in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) based on the Akaike Information Criterion (AIC) determined in jModelTest 2.1.10. for each partition (Darriba et al. 2012). Markov Chain Monte Carlo (MCMC) with 2×10^6 generations was run to determine posterior probabilities. Trees were sampled every 1000 generations and 25% of the trees displayed in the burn-in phase were discarded. Additionally, a Maximum Likelihood (ML) analysis was performed in RaxmlGUI 2.0 (Edler et al. 2021) using the GTR + G model. The ML phylogenetic tree was inferred with a Bootstrap analysis of 1,000 replicates for branch support.

Pathogenicity of the three isolates was verified on healthy blueberry leaves. For each isolate, 20 leaves were inoculated by spraying a conidial suspension (1×10^6 spores/mL). The controls consisted of leaves sprayed only with sterile distilled water. The plants were covered with a plastic bag for 48 hours to maintain humidity and were managed agronomically for 15 days in a greenhouse at temperatures of 23 to 35ºC.

The colonies of the three isolates morphologically characterized on PDA exhibited abundant mycelial growth, initially gray in color, later becoming dark gray (Fig. 2). Microscopic examination showed conidiophores that were short, straight or curved, septate, brown, unbranched or sometimes branched. Conidia were obclavate or obpyriform, brown, measuring 22.2 to 45.9 × 8.1 to 15.5 µm, with 2 to 6 transverse septa and 0 to 3 longisepta (Fig. 2). Conidia were produced in long chains. Based on morphological features, the fungal isolates were identified as *Alternaria alternata* (Simmons 2007).

The phylogenetic analyses grouped the three isolates CCLF433–CCLF435 in the same clade as *Alternaria alternata* (Fig. 3). The clade was supported (1 posterior probability) in the multilocus analysis, being the isolates confidently assigned to the species *A. alternata*.

All inoculated leaves developed irregular and necrotic lesions 8 days after inoculation. No symptoms were observed on the control leaves. The fungi were consistently re-isolated from the diseased leaves and presented morphology similar to the original isolates, fulfilling Koch´s postulates. The experiment was repeated twice with similar results.

In this research, a multilocus phylogenetic approach combined with morphological data identified three fungal isolates as *Alternaria alternata*. To our knowledge, this is the first report of *A. alternata* causing leaf blight of blueberry in Mexico. Previously, in South Korea, the presence of *Alternaria* sp. inducing leaf spot was recorded (Kwon et al. 2014). Meanwhile, in California (USA), five species of *Alternaria* were reported, including *Alternaria alternata, A. tenuissima, A. arborescens, A. infectoria*, and *A. rosae* as causal agents of blueberry fruit rot (Zhu and Xiao 2015). Additionally, isolates of *A. alternata* causing postharvest fruit rot in California have been reported with resistance to QoI fungicides (Wang et al. 2022).

The leaf blight of blueberry is a common disease in blueberry fields in Sinaloa, Mexico; therefore, additional studies are needed to establish the most effective strategies for managing this disease.

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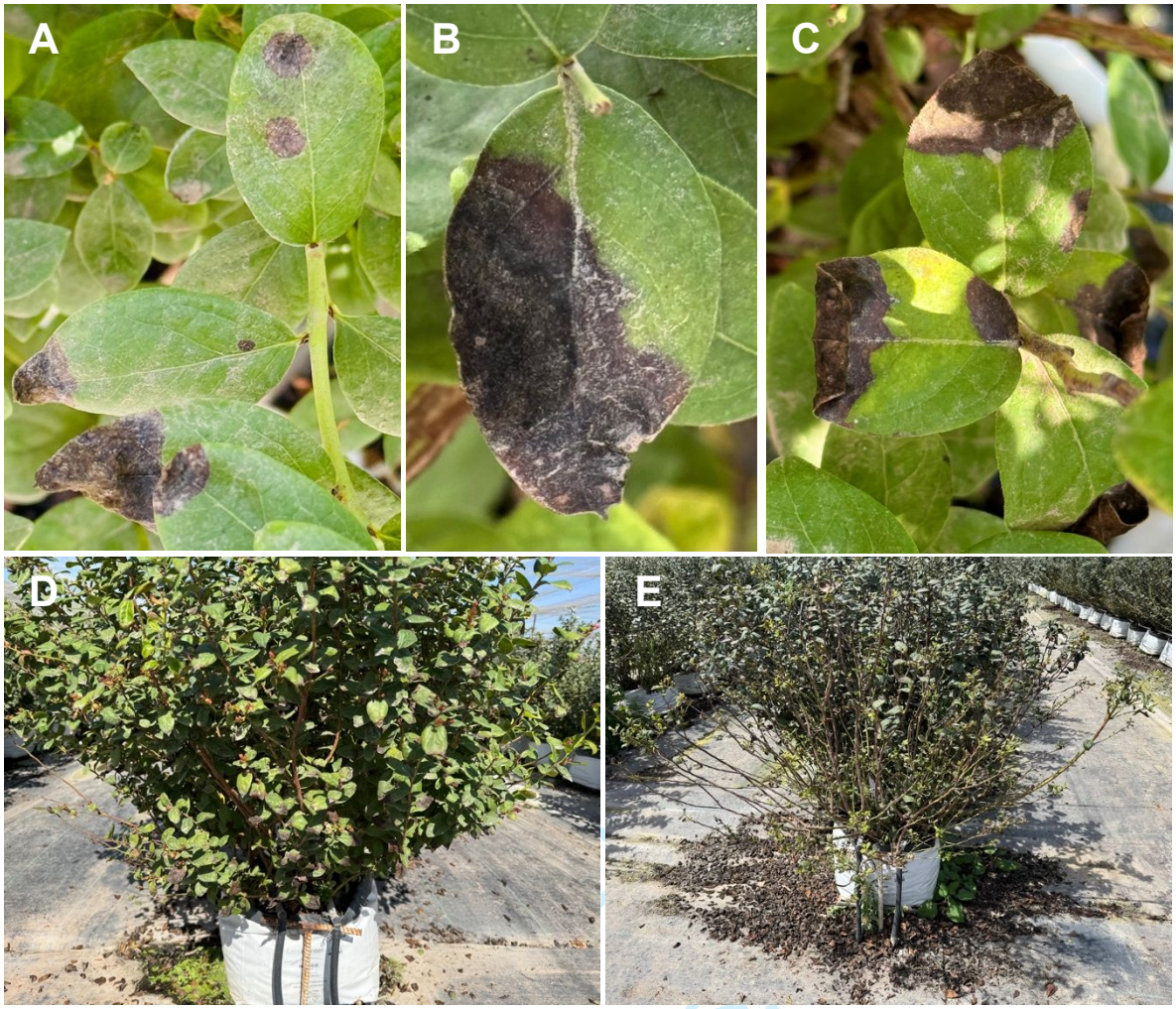

FIGURE 1

Blueberry leaf blight symptoms caused by *Alternaria alternata*. **A,** Initial black lesions. **B-C,** Leaf blight with dark sporulation. **D,** Plant with low severity symptoms. **E,** Plant severely affected, showing defoliation.

FIGURE 2

Colony and spore characteristics of *Alternaria alternata*. **A-B,** Colonies grown on PDA medium after 8 days. **C-E,** Conidia. Scale bars= 20 µm.

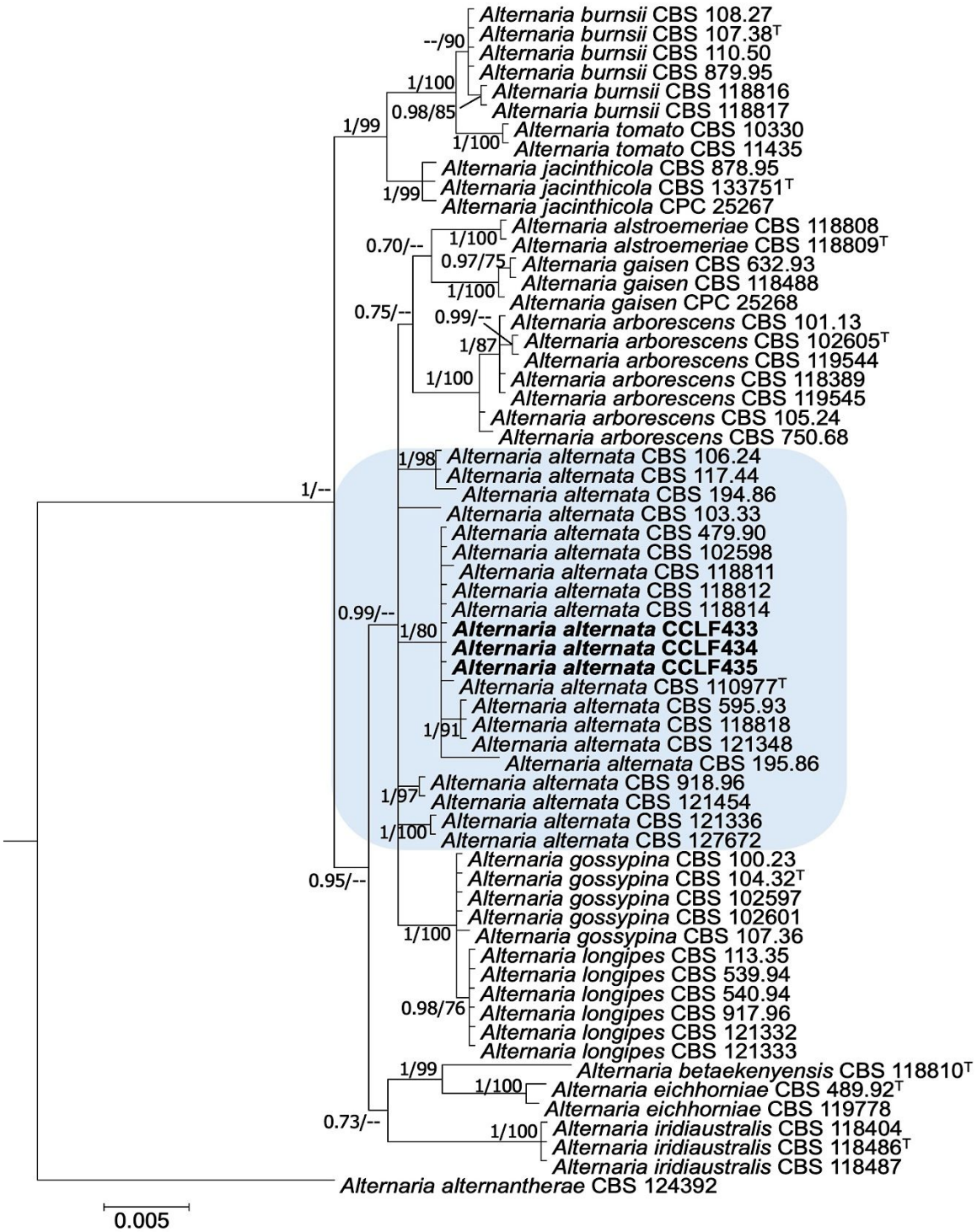

FIGURE 3

Phylogenetic tree constructed based on maximum likelihood of *gapdh*, *rpb2*, and *tef1-α* sequence data of taxa from the *Alternaria* spp. Posterior probabilities (>0.7) and Bootstrap support (>70%) values for Bayesian Inference and Maximum likelihood are shown in the nodes. Ex-type isolates are marked with a superscript T. The tree was rooted with *Alternaria alternantherae* CBS124392. Sequences generated in this study are shown in bold.