Fungal Research

SCIEPublish

Communication

Morphological and Genome Characterization of *Alternaria alternata* Causing Blueberry (*Vaccinium corymbosum* L.) Leaf Spot in Peru

Ricardo Velasquez [1,†], Valentina Osorio [2,†], Ana Maria Leiva [2], Juan Manuel Pardo [2], Alejandra Gil-Ordóñez [2], Ida Bartolini [3] and Wilmer J. Cuellar [2,*]

[1] Laboratorio de Entomología y Manejo Integrado de Plagas, Instituto Nacional de Innovación Agraria (INIA), Av. Chancay—Huaral KM 5.500, Lima 15026, Peru; rvelasquez@inia.gob.pe (R.V.)

[2] Crop Protection Research Area, Cassava Program, Crops for Nutrition and Health, International Center for Tropical Agriculture (CIAT), the Americas Hub, Km 17, Recta Cali-Palmira, Palmira 763537, Colombia; v.osorio@cgiar.org (V.O.); a.m.leiva@cgiar.org (A.M.L.); j.m.pardo@cgiar.org (J.M.P.); a.gil@cgiar.org (A.G.-O.)

[3] Laboratorio de Biología Molecular del Servicio Nacional de Sanidad Agraria (SENASA), La Molina Nº 1915, La Molina Lima 12, Lima 15026, Peru; ibartolini@senasa.gob.pe (I.B.)

* Corresponding author. E-mail: w.cuellar@cgiar.org (W.J.C.)

† These authors contributed equally to this work.

Received: 26 January 2026; Revised: 10 April 2026; Accepted: 9 May 2026; Available online: 20 May 2026

ABSTRACT: Blueberries (*Vaccinium corymbosum* L.), valued for their nutritional benefits and economic significance, have become Peru's leading agro-export crop. However, intensive cultivation can lead to phytosanitary problems if not addressed promptly, posing a serious threat to blueberry production. This study aimed to isolate and identify the causal agent of leaf spot symptoms initially observed in blueberries cultivated in Peru, marking the first formal documentation of its presence in the country. In 2022, leaf spot symptoms were recorded on *V. corymbosum* cv. Biloxi, in the north of Lima, Peru. Field observations revealed necrotic, sunken spots on leaves and fruits, with 4.84% of leaves diseased and 1.28% of fruits affected. Pathogen isolation and microscopic studies identified *Alternaria alternata* as the primary causal agent, which was confirmed by genome sequencing using Oxford Nanopore Technology. Pathogenicity tests demonstrated the fungus' ability to reproduce symptoms identical to those observed in the field, fulfilling Koch's postulates. Under experimental conditions, disease severity increased over time, with the affected leaf area ranging from 9.35% to 25.61% between 7 and 14 days post-inoculation. This study establishes *A. alternata* as a pathogen of blueberries in Peru and provides essential insights for future research and strategies to mitigate its impact on the industry.

Keywords: Fungal identification; Conidiophores; Conidia; Nanopore; Pathogenicity; Crop protection

1. Introduction

Blueberries (*Vaccinium corymbosum* L.), a perennial crop native to North America and part of the Ericaceae family, are highly valued for their nutritional properties and global economic importance. The increasing global demand for blueberries has driven expansion in Peru's production. Although commercial

production began in 2007, the industry experienced remarkable growth following a national campaign launched in 2012, expanding from just 50 hectares to over 18,000 hectares in 2022. That year, blueberries had become Peru's top agro-export crop, generating $1.435 billion in revenue and accounting for approximately 14% of the nation's total agro-export earnings [1]. Today, Peru ranks as the world's third-largest blueberry producer, following the United States (U.S.) and Canada, and is the leading producer in Latin America. The country exports to major markets, including the U.S., Europe, and China [2,3].

Nevertheless, this crop is highly susceptible to fungal pathogens that cause severe economic losses. Among them, *Alternaria* spp. are a broad-host-range pathogen responsible for yield reductions of 20% to 80% widely [4], and postharvest losses of up to 46% in China. Economic losses in the blueberry industry due to *Alternaria* spp. infections are estimated to be between $230 million and $1 billion globally [5]. These substantial financial impacts underscore the critical importance of early detection and timely implementation of management to contain the spread of the disease and lower the associated costs of disease management at such scales.

Globally, the most frequently reported *Alternaria* species affecting blueberries are *A. alternata* and *A. tenuissima*, which have been associated with leaf spot in major producing countries such as the U.S., China, South Korea, and Australia [6–9]. Additionally, these species have been linked to fruit rot in blueberries in the U.S. and Argentina [10,11]. These are polycyclic pathogens that persist in soil and plant debris through various survival structures. Under favorable environmental conditions, conidia initiate infections through natural openings and wounds [12,13]. However, despite this extensive global documentation of *Alternaria* species affecting blueberries, no formal reports have documented these pathogens in blueberry crops in Peru. Although *Alternaria* spp. have been reported affecting other crops in Peru, such as citrus and tomato [14,15], this knowledge gap represents a phytosanitary concern that limits effective disease management.

Therefore, the objective of this study was to isolate, identify, and characterize the causal agent of leaf spot symptoms observed in blueberry plants in Peru, integrating morphological, molecular, and biological approaches (including fulfillment of Koch's postulates). By scientifically documenting its identity, this work provides a robust foundation for future epidemiological studies and targeted disease management strategies.

2. Materials and Methods

2.1. Sample Collection and Pathogen Isolation

In 2022, leaf spots were first observed on *V. corymbosum* cv. Biloxi in a field in northern Lima (–11.51478, –77.23869). Plants were evaluated for symptoms by examining lesion characteristics, including color, shape, pattern distribution, and presence of fungal signs. Disease incidence, calculated as the percentage of affected leaves per plant, was assessed using a stratified random sampling of 30 plants across three homogeneous field strata (10 plants per stratum) [16], covering a total area of 1800 m^2. Fruit rot incidence was evaluated by collecting 9250 fruits from the same 30 plants across three harvest periods and expressing it as the percentage of symptomatic fruits. For pathogen isolation, five plants showing moderate symptom severity were selected to avoid advanced lesions potentially colonized by secondary microorganisms. From these plants, ten symptomatic leaves were collected from the middle canopy, as this location represents the active disease front, ensuring that tissues retain cellular integrity and nutrients necessary for pathogen metabolism, unlike lower leaves already compromised by advanced senescence or upper leaves that may not yet have been colonized [17]. Small tissue fragments (5 mm diameter) were excised from the margins of necrotic lesions using a sterile scalpel.

Samples were surface disinfected by sequential immersion in 1% NaClO for 1 min, followed immediately by 70% ethanol for 1 min, and then rinsed three times with sterile distilled water. All procedures were conducted under aseptic conditions without interruption. Disinfected tissues were placed

on potato dextrose agar (PDA) and incubated at 25 °C under continuous darkness. Fungal growth was observed after three days, and single-spore purification was subsequently performed to obtain pure cultures.

2.2. Morphological Characterization

A total of five fungal isolates were obtained from symptomatic tissues, all showing consistent macroscopic and microscopic characteristics typical of *Alternaria alternata*. Due to this uniformity, one representative isolate was selected for detailed characterization. Macroscopic characteristics were evaluated on PDA, including colony color (surface and reverse), texture, margin type, and mycelial appearance. For microscopic characterization, cultures were grown on potato carrot agar (PCA), a medium known to promote sporulation in *Alternaria* spp., and incubated at 25 °C under an 8 h light/16 h dark photoperiod for 7 days. Conidia were examined using a light microscope (BA210, Motic, Xiamen, China) at 40× magnification, and measurements were obtained from 50 randomly selected conidia following the criteria described by Simmons (2007) [18].

2.3. Genome Characterization

A single representative isolate obtained by single-spore purification was selected for genomic analysis and designated as *Alternaria alternata* isolate Per1. For DNA extraction, isolate Per1 was grown on PDA medium at 25 °C under continuous darkness for 2–4 weeks. Approximately 0.03 g of fresh mycelium was scraped from agar plates and processed according to the protocol described by Gil-Ordóñez et al. (2024) [19]. DNA integrity was confirmed by gel electrophoresis, and concentration was determined using Qubit™ dsDNA HS Assay Kit (Invitrogen, Life Technologies, Carlsbad, CA, USA). Whole-genome sequencing was performed using Oxford Nanopore Technology (FLO-MIN114 R10 chemistry, LSK114 kit). Genome assembly was conducted using Flye v2.9 [20] with default parameters, specifying only the input read (--nano-raw) and computational settings. Assembly and sequencing coverage metrics were calculated using BBMap v38.18 [21] and Qualimap v2.2.2 [22], respectively. Genome completeness and quality were assessed with BUSCO v5.2.2 using the eukaryota_odb10 (8 January 2024) and pleosporales_odb10 (8 January 2024) datasets asreferences [23]. Subsequently, in silico prediction of coding regions was performed using AUGUSTUS, previously trained with the genome of *A. alternata* (GenBank: GCA_036971565). Orthologous gene clusters were inferred using OrthoVenn3 (https://orthovenn3.bioinfotoolkits.net/home, accessed on 20 January 2025) and compared with the reference genomes of *A. alternata* (GenBank: GCA_036971565), *A. arborescens* (GenBank: GCA_004154835), and *A. tenuissima* (GenBank: GCA_041430775) using the OrthoMCL algorithm (e-value = 1×10^{-10}), with all other parameters set to default. Phylogenetic relationships were inferred based on conserved single-copy orthologs using a maximum likelihood approach implemented within the OrthoVenn3 pipeline [24].

2.4. Pathogenicity Test

Pathogenicity tests were conducted on whole blueberry plants grown in pots under screen-house conditions to fulfill Koch's postulates [8,9,25]. The experiment followed a completely randomized design using five healthy six-month-old blueberry plants (biological replicates), with six leaves per plant (technical replicates), totaling 30 leaves per treatment. The experiment was conducted twice. Inoculations were done using a conidial suspension (3.0×10^5 conidia mL^{-1}) of *A. alternata* obtained from a 10-day-old single-spore culture grown on PDA, suspended in sterile distilled water containing 0.025% Tween 20. Before inoculation, four wounds per leaf were made using a sterile needle to ensure consistent infection. The conidial suspension was applied using sterile brushes to cover the entire leaf surface, while control leaves received sterile water containing 0.025% Tween 20. All leaves remained attached to living plants

throughout the experiment. Plants were maintained at >70% relative humidity and 26 ± 2 °C for 14 days. Disease severity was evaluated at 0, 7, and 14 days post-inoculation (DPI) using paired measurements from five leaves per plant, with one additional leaf per plant reserved for re-isolation and documentation. Necrotic leaf area was quantified through manual analysis using the R package *pliman* [26]. Data were analyzed descriptively using means and standard deviations.

2.5. Re-Isolation and Validation

Following pathogenicity testing, re-isolation was attempted from both inoculated symptomatic tissues and mock-inoculated leaves using the same isolation procedure described in Section 2.1. Re-isolated organisms were subjected to morphological characterization following the same methodology described in Section 2.2, to confirm identity with the original isolate. Rapid molecular validation was conducted by amplification of the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA using the primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [27]. PCR reactions were performed in 25 µL volumes containing GoTaq® Green Master Mix (12.5 µL, 1× final concentration), upstream and downstream primers (0.5 µL each, 10 µM), DNA template (3 µL, 20 ng), and nuclease-free water to a final volume. Thermal cycling conditions included initial denaturation at 94 °C for 4 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, with final extension at 72 °C for 10 min. PCR products were sequenced using the Sanger method at Macrogen Co., Ltd. (Daejeon, Korea). Additionally, we carried out whole-genome sequencing and assembly of the re-isolated pathogen as previously described for multilocus validation (including regions *gapdh*, *rpb2*, *tef1-α*, and *Alt-a1*). Sequences were compared with those from the original isolate using BLASTn analysis (e-value < 1×10^{-25}).

3. Results and Discussion

3.1. Field Symptoms and Disease Incidence

Leaf spot symptoms were characterized by brown, circular or irregular necrotic lesions with sunken, dark-brown centers and concentric rings, often surrounded by yellow halos and small black dots (Figure 1a). Lesions initially appeared as small spots (approximately 3–7 mm in diameter), predominantly on the abaxial leaf surface, and became evident on both surfaces as the disease progressed. Fruit rot symptoms were also observed in the field, characterized by flattening of mature fruits with a characteristic dry appearance, later covered by a mass of mycelium. Damage assessment at the field level revealed that 4.80% of fruits were affected during the first production cycle and 1.28% in the last production cycle of the year. Additionally, leaf spot incidence was recorded at 4.8%, a level comparable to previous reports of leaf spot incidence in South Korea [8].

3.2. Morphological Characterization

Five isolates were obtained that showed consistent morphology, with simple conidiophore architecture and short conidia chains (up to seven conidia) characteristic of *A. alternata*. Fungal colonies exhibited a dark olive color on the surface and a dark gray color on the reverse. The colonies had white filamentous margins, a circular form, and a velvety texture. They produced loose, aerial hyphae on the culture surfaces, with a cottony mycelial appearance (Figure 1b), consistent with the characteristics reported in other studies [28,29]. Single suberect or erect pale brown conidiophores were observed, ranging in size from 23.7 to 66.6 µm (mean: 36.5 ± 1.7 µm) and producing up to seven chained conidia (Figure 1c). Each conidium measured from 20.2 to 43.7 µm in length, with a mean of 28.6 µm ± 4.7 µm (n = 50), and 8.2 to 16.9 µm in width, with a mean of 11.7 µm ± 1.9 µm (n = 50). They contained three to six transverse septa and up to two longitudinal septa. Conidia were olivaceous or dull green-brown in color, ellipsoid to ovoid, often featuring

a short conical or cylindrical beak (Figure 1d). The first conidium produced on a conidiophore was also narrowly elliptical, with three transverse septa.

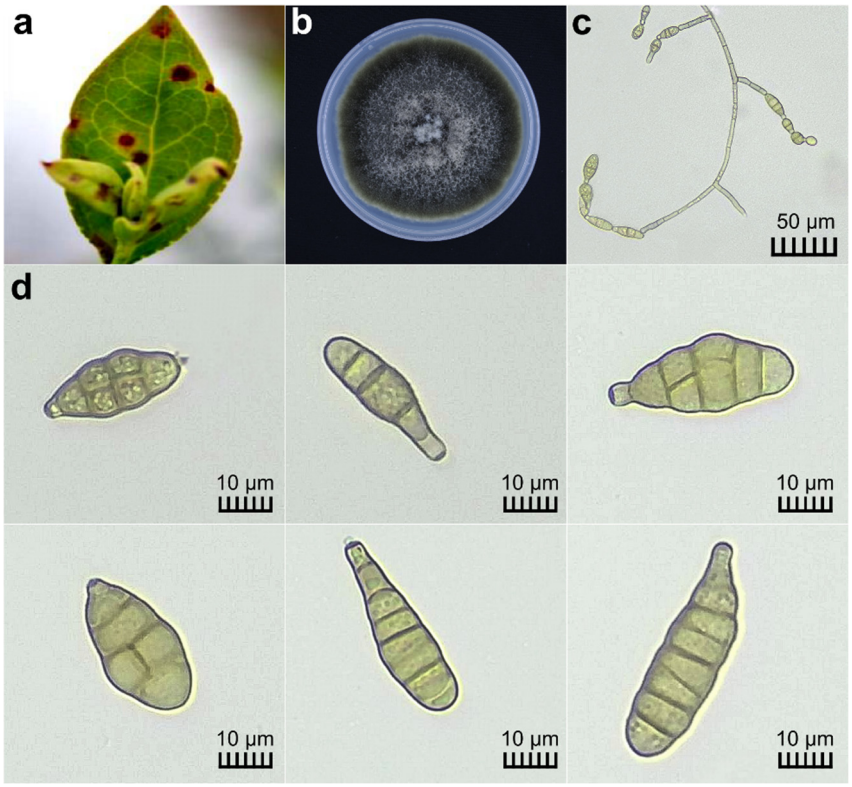

Figure 1. Disease symptoms on *V. corymbosum* caused by *A. alternata* and its cultural and morphological characteristics. (**a**) Necrotic lesions on blueberry leaves taken from the field; (**b**) Colony morphology after 10 days of growth on PDA medium; (**c**) Conidiophores and conidia after 7 days of growth on PCA medium; (**d**) Microscopic view showing the diverse shapes of macroconidia (40×).

Based on these key features, the isolate was identified as *A. alternata*, distinct from closely related species in the *Alternaria* complex, according to Simmons' taxonomic key [18]. Key distinguishing features included conidial dimensions consistent with the ellipsoid form, simple conidiophore architecture with short chains (up to 7 conidia) typical of Group J species rather than the longer unbranched chains (10–25+ conidia) characteristic of *A. tenuissima* (Group H), and typical beak morphology and septation patterns. The observed morphological traits are consistent with previous reports describing conidia with 3 to 6 transverse septa and dimensions ranging from 16 to 42 × 7 to 16 µm [7]. Likewise, other authors have described conidia as obclavate to elliptical [30]. However, differences were observed when comparing conidial dimensions with isolates from California, which showed average measurements of 22.04 µm in length and 8.7 µm in width [11]. Such variation may be influenced by geographic factors or local adaptation, emphasizing the importance of regional studies for understanding pathogen variability and its possible implications in disease management.

A. alternata has been confirmed as the causal agent of leaf spots on blueberries in South Korea and southern Poland [8,31]. Similarly, in California, 61.5% of *Alternaria* isolates from decayed blueberries were identified as *A. alternata* [11]. However, other studies have reported *A. tenuissima* as responsible for leaf spots in Western Australia and Argentina [9,32]. These results underscore the complexity of *Alternaria* spp. in different regions and highlight the need for accurate identification to develop precise, pathogen-targeted disease management strategies.

In other crops, *Alternaria alternata* has been reported causing brown spots on citrus crops across several Peruvian provinces [14]. Given that citrus crops are well-established in Peru, particularly in regions such as

Lima [33], they could serve as a potential reservoir for *A. alternata* populations affecting blueberries. The widespread occurrence of *A. alternata* across Latin America, including citrus in Argentina, alfalfa in Brazil, quinoa in Colombia, hazelnut in Chile, broccoli in Ecuador, and weeds in Uruguay, underscores its adaptability to different environmental conditions and hosts [34–39]. The presence of many of these crops in Peru may have facilitated the expansion and adaptation of *A. alternata* to the more recently introduced blueberries.

3.3. Genome Characterization

DNA had high integrity and yielded 90.4 ng/µL before sequencing. Whole-genome sequencing yielded approximately 6 Gb of estimated bases (506,720 reads, average read length: 11.8 kb, N50 raw reads: 20.04 kb, mean quality score: Q10), and resulted in an assembled genome of 34.2 Mb with a coverage of ~166×, comprising 17 contigs (N50: 435,246 bp, L50: 18, largest contig: 6,834,098 bp) and a GC content of 49.93%. BUSCO analysis revealed 98.8% completeness (98.8% complete, 0.8% fragmented) and 95.3% completeness (94.8% complete, 0.5% fragmented) based on eukaryota_odb10 and pleosporales_odb10 databases, respectively. The genome size of *A. alternata* Per1 (34.2 Mb) and GC content fall within the expected range for this species, which has been reported to vary between 30–35 Mb and ~50%, respectively, across isolates from different host plants using various sequencing technologies, including Nanopore [6,40]. Protein-based gene cluster analysis was performed using the entire predicted proteome (all protein-coding genes) derived from the assembled genome of isolate Per1 (12,169 protein-coding genes). Orthologous gene cluster analysis identified 11,720 clusters and showed strong clustering with reference genomes of *A. alternata* (Figure 2).

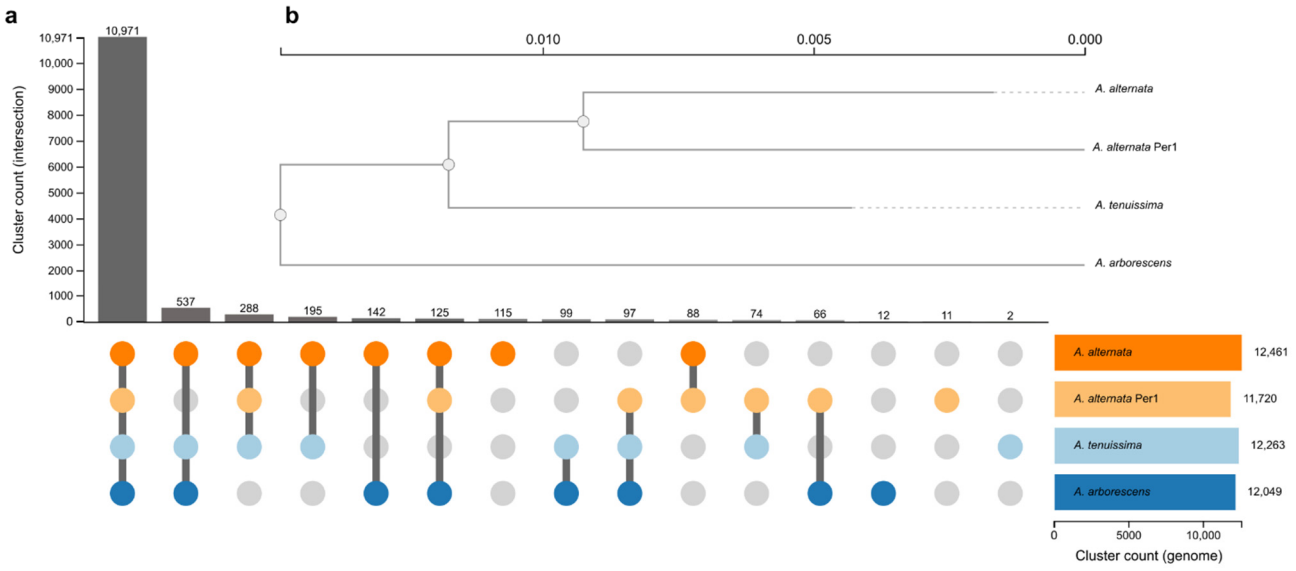

Figure 2. Phylogenetic relationships and orthologous cluster analysis of an *Alternaria* isolate from Peruvian blueberries. (**a**) Diagram showing distinct and shared orthologous clusters among *A. alternata* Per1 (orange dots) and the reference genomes of *A. alternata*, *A. tenuissima*, and *A. arborescens* available in the OrthoVenn3 web service. The bar graph on the left displays the count of orthologous clusters. (**b**) Maximum likelihood phylogenetic tree illustrating evolutionary relationships and genetic distances based on single-copy conserved genes. The tree was constructed using the JTT+CAT evolutionary model. This analysis provides insights into the genetic relationships between the Peruvian Alternaria isolate and other reference genomes.

3.4. Pathogenicity Test

Symptoms began to appear on inoculated leaves seven days post-inoculation, and by day 14, all inoculated leaves had developed necrotic spots identical to those observed in the field. In contrast, control plants remained asymptomatic (Figure 3). These results are consistent with previous studies reporting

symptom onset between 7 and 8 days after inoculation [27,41]. Similarly, leaf spot symptoms observed after 15 days were comparable to those occurring under natural conditions [10]. Disease severity increased over time, with the affected leaf area ranging from 9.35 ± 0.71% at 7 days to 25.61 ± 6.2% at 14 days post-inoculation (Figure 3).

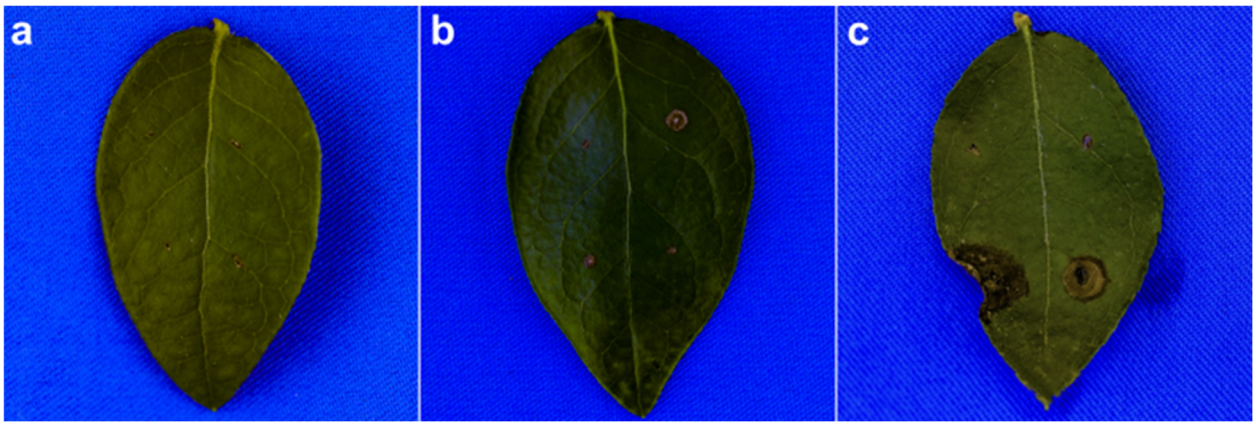

Figure 3. Results of the pathogenicity test showing *A. alternata* causing leaf spots 14 days post-inoculation. (**a**) No symptoms on a control leaf at 14 days post-inoculation; (**b**) Necrotic symptoms on a leaf 7 days post-inoculation; (**c**) Necrotic symptoms on a leaf 14 days post-inoculation. All leaves shown were attached to living, whole plants at the time of inoculation and symptom development.

The pathogen was successfully re-isolated from 86.7% of symptomatic tissues, confirming pathogenicity and fulfilling Koch's postulates. *Fusarium* spp. were occasionally recovered as contaminants; however, contamination was controlled through colony purification and morphological differentiation, as *Fusarium* spp. colonies exhibited white coloration distinct from the characteristic olive-green appearance of *A. alternata* isolates.

Morphological and cultural comparisons confirmed the re-isolated organism as *A. alternata*, consistent with the inoculated pathogen (Figure 4). Comprehensive molecular validation included multilocus sequence analysis (extracted from whole-genome data) of ITS, *gapdh*, *rpb2*, *tef1-α*, *Alt-a 1* locus, which confirmed the identity of the re-isolated fungus with that of the inoculated isolate (sequences for all loci shared 100% identity; GenBank nros: PV069777, PZ316321-PZ316324). These results fully satisfy Koch's postulates through both morphological and robust molecular confirmation.

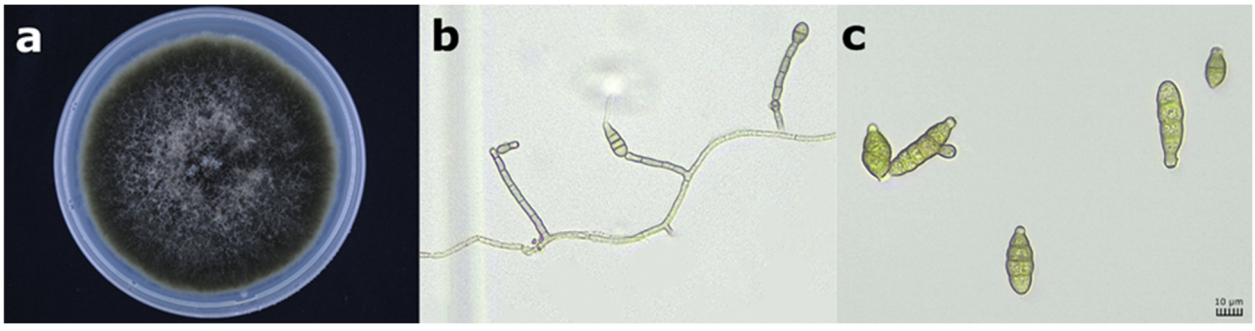

Figure 4. Morphological confirmation of re-isolated *A. alternata*. (**a**) Colony morphology after 10 days of growth on PDA medium; (**b**) Conidiophores and conidia after 7 days of growth on PCA medium; (**c**) Microscopic view of conidia (40×).

4. Conclusions

Alternaria alternata has been identified as the causal agent of leaf spot symptoms in blueberries in Peru. The combined use of morphological, pathogenicity, and whole-genome analyses enabled precise identification of the pathogen and confirmed its pathogenicity. These results establish a foundation for targeted disease management and inform the selection of specific control strategies.

Statement of the Use of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this manuscript, the authors used Grammarly in order to improve the English language and writing clarity. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Acknowledgments

We extend our gratitude to David Pavel Casanova Núñez Melgar of INIA–Peru; Jesús Iván Chumpitaz Fernández at the Peruvian Institute of Agroexporters; and Jacobo Trujillo, Technical Support at INIA EEA Donoso–Huaral and the National Agrarian Health Service (SENASA), for their valuable assistance in molecular laboratory work, with special thanks to Arturo Olortegui Livia. We also acknowledge the technical support provided by Viviana Domínguez and Ronal Montes from CIAT.

Author Contributions

Conceptualization, R.V., J.M.P. and W.J.C.; Biological test, R.V., V.O. and J.M.P.; Formal Analysis, R.V., V.O., A.M.L. and A.G.-O.; Validation, R.V. and V.O.; Data Curation, V.O., A.M.L. and A.G.-O.; Writing—Original Draft Preparation, All authors; Writing—Review & Editing, W.J.C.; Supervision, J.M.P. and W.J.C.; Project Administration, R.V., I.B. and W.J.C.

Ethics Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

The genome assembly and associated data of isolate Per1 and re-isolate have been deposited in NCBI under accession JBMHEI000000000, BioProject PRJNA1189477, and BioSample SAMN44971619 and SAMN57370065 and SAMN57370065.

Funding

This research was funded by the Ministry of Agriculture of Peru (MINAGRI) through the Secretaría Técnica de Cooperación del CGIAR (STC-CGIAR).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Bell MG, Zimmerer KS, Tinoco OSR. Following the water uphill? The spread of blueberry cultivation to the mountains of Áncash, Peru. *J. Lat. Am. Geogr.* **2023**, *22*, 156–166. DOI:10.1353/lag.2023.a899558
2. FAO. FAOSTAT: Crops and Livestock Products. Available online: https://www.fao.org/faostat/en/#data/QCL/visualize (accessed on 10 January 2026).
3. Montes Ninaquispe JC, Arbulú Ballesteros MA, Cruz Salinas LE, García Juárez HD, Farfán Chilicaus GC, Martel Acosta R, et al. A strategy for the sustainability of Peru's blueberry exports: Diversification and competitiveness. *Sustainability* **2024**, *16*, 6606. DOI:10.3390/su16156606

4. Deka B, Upamanya GK, Baruah RMB. Eco-friendly management of Alternaria blight disease: A review. *Asian J. Adv. Agric. Res.* **2024**, *24*, 34–43. DOI:10.9734/ajaar/2024/v24i10554
5. Cong K, Cheng J, Li T, Wu C, Fan G, Li X, et al. Alternaria in berry: Effects on its quality, pathogenic mechanisms, and potential control strategies. *Plant Pathol.* **2024**, *73*, 1317–1333. DOI:10.1111/ppa.13916
6. Gai Y, Ma H, Chen Y, Li L, Cao Y, Wang M, et al. Chromosome-scale genome sequence of *Alternaria alternata* causing citrus brown spot. *Mol. Plant-Microbe Interact.* **2021**, *34*, 726–732. DOI:10.1094/MPMI-10-20-0278-SC
7. Luan YS, Feng L, Xia XY, An LJ. First report of *Alternaria tenuissima* on blueberry in China. *Plant Dis.* **2007**, *91*, 464. DOI:10.1094/PDIS-91-4-0464A
8. Kwon JH, Kang DW, Cheon MG, Kim J. First report of *Alternaria* leaf spot on highbush blueberry in South Korea. *Plant Dis.* **2014**, *98*, 1434. DOI:10.1094/PDIS-04-14-0344-PDN
9. You MP, Lanoiselet V, Wang CP, Barbetti MJ. First report of *Alternaria tenuissima* on blueberry in Western Australia. *Plant Dis.* **2014**, *98*, 423. DOI:10.1094/PDIS-07-13-0737-PDN
10. Greco M, Patriarca A, Terminiello L, Pose G. Toxigenic *Alternaria* species from Argentinean blueberries. *Int. J. Food Microbiol.* **2012**, *154*, 187–191. DOI:10.1016/j.ijfoodmicro.2012.01.004
11. Zhu XQ, Xiao CL. Characterization of *Alternaria* species associated with fruit rot of blueberry in California. *Phytopathology* **2015**, *105*, 1555–1567. DOI:10.1094/PHYTO-05-15-0122-R
12. Neugebauer KA, Mattupalli C, Hu M, Oliver JE, VanderWeide J, Lu Y, et al. Managing fruit rot diseases of blueberry. *Front. Plant Sci.* **2024**, *15*, 1428769. DOI:10.3389/fpls.2024.1428769
13. Salotti I, Giorni P, Battilani P. Biology, ecology, and epidemiology of *Alternaria* species affecting tomato. *Front. Plant Sci.* **2024**, *15*, 1430965. DOI:10.3389/fpls.2024.1430965
14. Marín JE, Fernández HS, Peres NAR, Andrew M, Peever TL, Timmer LW. First report of citrus brown spot in Peru. *Plant Dis.* **2007**, *90*, 686. DOI:10.1094/PD-90-0686C
15. Schmey T, Auxier B, Krebs S, Patneedi SK, Ahmad F, Habig M, et al. Evolutionary divergence in sympatric populations of the fungal pathogen *Alternaria alternata* across wild tomato hosts. *bioRxiv* **2025**, 2025-10. DOI:10.1101/2025.10.01.678058
16. Delp BR, Stowell LJ, Marois JJ. Evaluation of field sampling techniques for estimation of disease incidence. *Phytopathology* **1986**, *76*, 1299–1305. DOI:10.1094/Phyto-76-1299
17. Dai YF, Wu XM, Wang HC, Li WH, Cai LT, Li JX, et al. Spatio-temporal variation in the phyllospheric microbial biodiversity of alternaria alternata-infected tobacco foliage. *Front. Microbiol.* **2022**, *13*, 920109. DOI:10.3389/fmicb.2022.920109
18. Simmons EG. *Alternaria: An Identification Manual*; CBS Fungal Biodiversity Centre, Ed.; ASM Press: Washington, DC, USA, 2007.
19. Gil-Ordóñez A, Pardo JM, Sheat S, Xaiyavong K, Leiva AM, Arinaitwe W, et al. Isolation, genome analysis, and tissue localization of *Ceratobasidium theobromae*. *Sci. Rep.* **2024**, *14*, 18139. DOI:10.1038/s41598-024-69061-8
20. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. Assembly of long, error-prone reads using repeat graphs. *Nat. Biotechnol.* **2019**, *37*, 540–546. DOI:10.1038/s41587-019-0072-8
21. Bushnell B. BBMap: A Fast, Accurate, Splice-Aware Aligner. 2014. Available online: https://escholarship.org/uc/item/1h3515gn (accessed on 4 February 2025).
22. García-Alcalde F, Okonechnikov K, Carbonell J, Cruz LM, Götz S, Tarazona S, et al. Qualimap: Evaluating next-generation sequencing alignment data. *Bioinformatics* **2012**, *28*, 2678–2679. DOI:10.1093/bioinformatics/bts503
23. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. BUSCO: Assessing genome assembly completeness. *Bioinformatics* **2015**, *31*, 3210–3212. DOI:10.1093/bioinformatics/btv351
24. Sun J, Lu F, Luo Y, Bie L, Xu L, Wang Y. OrthoVenn3: Orthologous data visualization platform. *Nucleic Acids Res.* **2023**, *51*, W397–W403. DOI:10.1093/nar/gkad313
25. Haq IU, Ijaz S, Khan IA. A journey from Koch's postulates to molecular systems biology. In *Phytomycology and Molecular Biology of Plant–Pathogen Interactions*; CRC Press: Boca Raton, FL, USA, 2022; pp. 1–25.
26. Olivoto T. Lights, camera, pliman! An R package for plant image analysis. *Methods Ecol. Evol.* **2022**, *13*, 789–798. DOI:10.1111/2041-210X.13803
27. Glass NL, Donaldson GC. Development of primer sets for conserved genes in filamentous ascomycetes. *Appl. Environ. Microbiol.* **1995**, *61*, 1323–1330. DOI:10.1128/aem.61.4.1323-1330.1995
28. Hu LJ, Li Y, Xuan JP, Gu YH, Li DL, Mo ZH, et al. A new leaf spot disease caused by *Alternaria alternata* on *Atractylodes lancea*. *Plant Dis.* **2024**, *108*, 3658. DOI:10.1094/PDIS-07-24-1349-PDN
29. Liu Z, Zhang R, Sun D, Tuerdi M, Zhu T, Chen X. *Alternaria alternata* causing brown spot on walnut in China. *Plant Dis.* **2023**, *107*, 2224. DOI:10.1094/PDIS-07-22-1536-PDN

30. Olmez S, Mutlu N, Kaba A. First report of *Alternaria alternata* on cotton in Türkiye. *Plant Dis.* **2023**, *107*, 3296. DOI:10.1094/PDIS-04-23-0724-PDN
31. Nadziakiewicz M, Kurzawińska H, Mazur S, Tekielska D. *Alternaria alternata*—The main causal agent of disease symptoms in juniper, rose, yew and highbush blueberry in nurseries in southern Poland. *Folia Hortic.* **2018**, *30*, 15–25. DOI:10.2478/fhort-2018-0002
32. Wright ER, Rivera MC, Esperón J, Cheheid A, Rodríguez Codazzi A. Alternaria diseases of blueberry in Argentina. *Plant Dis.* **2004**, *88*, 1383. DOI:10.1094/PDIS.2004.88.12.1383B
33. Ruiz Camacho W, Julca Otiniano A. Comportamiento del cultivo de naranja (Citrus sinemis) variedad Valencia en dos zonas agroecológicas de la provincia de Chanchamayo, Junín, Perú. *Idesia* **2022**, *40*, 89–94. DOI:10.4067/S0718-34292022000300089
34. Durán P, Barra PJ, Mora MLL, Morina F, Viscardi S, Meriño-Gergichevich C. First report of fungal complex causing grey necrosis of hazelnut in Chile. *New Dis. Rep.* **2020**, *42*, 7. DOI:10.5197/j.2044-0588.2020.042.007
35. Fonseca-Guerra IR, Beltrán Pineda ME, Benavides Rozo ME. Characterization of *Alternaria alternata* and *Alternaria scrophulariae* brown spot in Colombian quinoa. *J. Fungi* **2023**, *9*, 947. DOI:10.3390/jof9090947
36. Kaspary TE, García MA, Stewart S, Casaroto G, Ramos RF, Bellé C. First report of *Alternaria alternata* causing leaf spot on *Rumex crispus* in Uruguay. *Plant Dis.* **2019**, *103*, 2139. DOI:10.1094/PDIS-03-19-0590-PDN
37. Miguel DA, Mariana RA, José AM, Gerarda BPS, Raquel SC, José ARS, et al. First report in Southern Brazil of *Alternaria alternata* causing Alternaria leaf spot in alfalfa (*Medicago sativa*). *Afr. J. Agric. Res.* **2015**, *10*, 491–493. DOI:10.5897/AJAR2014.9205
38. Peres NAR, Agostini JP, Timmer LW. Outbreaks of citrus brown spot in Brazil and Argentina. *Plant Dis.* **2003**, *87*, 750. DOI:10.1094/PDIS.2003.87.6.750C
39. Ramirez-Villacis DX, Barriga-Medina N, Llerena-Llerena S, Pazmino-Guevara C, Leon-Reyes A. First report of *Alternaria alternata* on broccoli in Ecuador. *Plant Dis.* **2023**, *107*, 2866. DOI:10.1094/PDIS-03-23-0598-PDN
40. Dettman JR, Eggertson Q. Phylogenomic analyses of *Alternaria* section *Alternaria*. *Mycologia* **2021**, *113*, 1218–1232. DOI:10.1080/00275514.2021.1950456
41. Shahid AA, Iftikhar S, Nawaz K, Anwar W, Ali M. First report of *Alternaria alternata* causing brown leaf spot of potato in Pakistan. *J. Plant Pathol.* **2017**, *99*, 300. Available online: https://www.cabidigitallibrary.org/doi/full/10.5555/20183088575 (accessed on 4 February 2025).