

# A Review of Botryosphaeria Stem Blight Disease of Blueberry from the Perspective of Plant Breeding

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**Abstract:** Stem blight of blueberry caused by fungal pathogens in the family *Botryosphaeriaceae* presents a major challenge to global blueberry production. Since its first documented outbreak in North Carolina, USA in the 1950s, *Botryosphaeria* stem blight has been reported in the blueberry production regions of more than nine countries across five continents. The lack of effective management strategies or resistant cultivars makes disease control especially challenging. With the goal of illuminating directions for future *Botryosphaeria* stem blight management, especially through resistant-cultivar development, this review summarizes the latest information on the distribution and causal pathogens of this disease, the pathogenicity of fungal species, disease resistance of blueberry cultivars, and currently recommended management practices. DNA sequencing techniques have revealed multiple fungal species that are associated with this disease. However, a lack of reliable methods to screen cultivars for stem blight resistance remains a major bottleneck for the development of resistant cultivars. Future studies should focus on at least four key areas: (1) the development and adoption of uniform and reliable screening protocols; (2) utilization of diverse and well-characterized *Botryosphaeriaceae* isolates for germplasm screens; (3) field evaluations of cultivar resistance and management practices; and (4) exploration of new tools for disease management and prevention.

**Keywords:** *Botryosphaeriaceae*; stem blight; blueberry; resistance screening

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## 1. Introduction

Rich in antioxidants, fiber, and nutrients and low in calories, blueberry is the perfect fruit for a healthy diet. The health benefits and improved qualities of cultivated blueberries have led to a surge in the global demand for blueberries. Since 1970, global blueberry production has been constantly increasing at an average annual rate of 6.1% [1]. In 2021, the total value of the global blueberry industry was \$938 M [2]. As the No. 1 blueberry producer in the world, the U.S. produced a total value of over \$908 M cultivated blueberries in 2019 [3]. The United States blueberry market is projected to continue to increase at a 2.1% compound annual growth rate between 2020 and 2025 driven by an anticipated increase in consumer demands [4].

*Botryosphaeria* stem blight of blueberry (also referred to as dieback) caused by fungal pathogens in the family *Botryosphaeriaceae*, such as the *Botryosphaeria* and *Neofusicoccum* spp., presents a major challenge to global blueberry production [5,6]. These fungal pathogens can infect blueberry plants through wounds or natural openings (e.g., lenticels, stomata) and cause severe hyperplasia of the vascular system, which can partially or completely block the xylem, leading to drought stress-like symptoms and eventually plant death [7,8] (Figure 1). Internal discoloration is often found on one side of infected stems, and this has been used as a characteristic symptom for disease diagnosis [7]. Spores of the pathogens can spread from infected plants to healthy plants by wind, water splash, and

field equipment [7]. In addition to blueberries, these fungal pathogens can also infect a wide range of economically important crops including apple [9], grape [10], mango [11], peach [12], and pear [13,14].



**Figure 1.** Symptoms of *Botryosphaeria* stem blight, pictures taken from blueberry farms in Alabama, USA in 2022.

*Botryosphaeria* stem blight of blueberry was first reported in North Carolina in 1958 [7] but has since been reported in multiple blueberry-producing regions including Australia [15], China [6,16], Italy [13], Mexico [17], New Zealand [18], Portugal [19], the south-eastern United States [5,8,20], and Korea [21]. Sammonds et al. [22] reported annual losses of up to NZ\$500,000 due to reduced yield and replanting costs in New Zealand blueberry production areas because of the *Botryosphaeria* stem blight. Blueberry growers in Florida, USA voted *Botryosphaeria* stem blight as the most important disease economically [5]. Despite the damage caused by *Botryosphaeria* stem blight, no single management practice or resistant cultivar is currently available that can effectively prevent this disease [7,8]. This review aims to shed light on potential solutions to overcome this disease from the perspective of plant breeding by reviewing current knowledge on (1) the global distribution of pathogens causing *Botryosphaeria* stem blight on blueberry, (2) the virulence of different pathogen species, (3) the natural resistance of blueberry species and cultivars, and (4) the effectiveness of fungicides and cultural practices for *Botryosphaeria* stem blight management.

## 2. Mechanisms of Infection

Wounds caused by abiotic factors such as frost, wind, and artificial damage from pruning, herbicide applications, and harvesting are considered major avenues of stem blight infection [7,23]. In addition, natural openings such as stomata, lenticels, and flower buds also provide the pathogens access to the vascular system [7]. Conidia produced from pycnidia on infected plants and released by rainfall are the main source of inoculum [7]. By contrast, ascospores, which are windborne, are believed to be relatively minor in importance as an inoculum source [7]. Upon infection, plants usually develop symptoms within 4–6 weeks [7]. However, some infected plants may remain asymptomatic until symptoms are triggered by stressors such as drought or nutrient deficiency [24]. Young

plants are most susceptible to this disease as infection of succulent stems can result in rapid plant death, whereas infection in older plants usually results in the loss of individual stems or branches but not the entire plant [7].

### 3. Species of Causal Pathogens

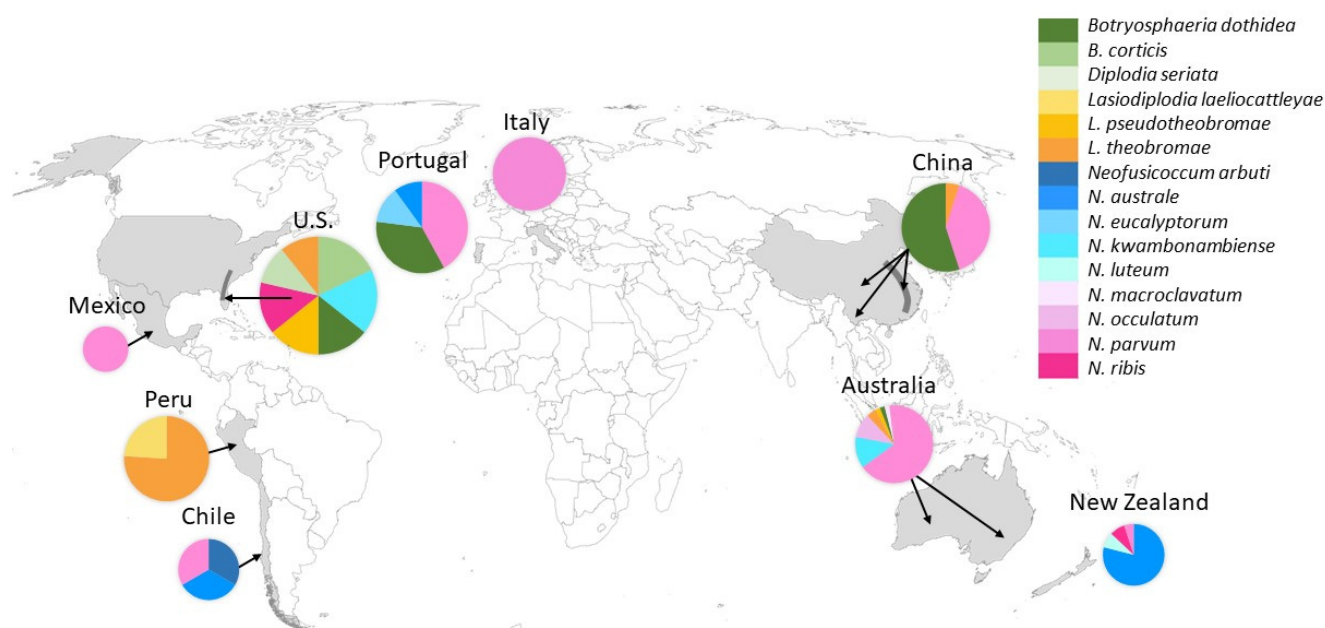
Early studies considered *Botryosphaeria dothidea* as the dominant species infecting blueberries [7,20,25–27], thus the name *Botryosphaeria* stem blight. However, in those studies, *B. dothidea* was identified based only on morphological characteristics [15,20,25–27]. In recent studies, the combined use of DNA sequencing, phylogenetic analysis, and morphological analysis has led to more accurate identification of causal agents, which are primarily within the genera *Botryosphaeria*, *Lasiodiplodia*, and *Neofusicoccum*. The most commonly reported species, based on DNA sequence information, are *N. parvum* (reported in 7 countries), *B. dothidea* (4 countries), *L. theobromae* (4 countries), *N. australe* (4 countries), *L. pseudotheobromae* (2 countries), *N. kwambonambiense* (2 countries), and *N. ribis* (2 countries), while other reported species include *B. corticis*, *D. seriata*, *L. laeliocattleyae*, *N. arbuti*, *N. eucalyptorum*, *N. luteum*, *N. macroclavatum*, and *N. oculatum* (Table 1, Figure 2) [5,6,15,18,19,28–30]. In prior studies, *Botryosphaeriaceae* species were often found to co-exist with other species such as *Alternaria*, *Pestalotiopsis*, *Neopestalotiopsis*, and *Phomopsis* spp. [5,6,19,28,31].

**Table 1.** Common species of *Botryosphaeriaceae* associated with blueberry stem blight worldwide.

Location	Year	Distribution of Causal Species	Sequenced Genomic Regions	Blueberry Type/Cultivar	Reference
Australia >16 orchards in New South Wales and Western Australia	2016	<i>Neofusicoccum parvum</i> (65%) <i>N. kwambonambiense</i> (13%) <i>N. oculatum</i> (10%) <i>L. theobromae</i> (4%) <i>L. pseudotheobromae</i> (2%) <i>Botryosphaeria dothidea</i> (2%) <i>N. australe</i> (2%) <i>N. macroclavatum</i> (2%)	ITS region of rDNA, <i>EF1-α</i> , <i>rpb2</i> , and $\beta$ -tubulin gene	NA	Scarlett et al., 2019 [15]
Chile 8 orchards	2009	<i>N. arbuti</i> (unknown %) <i>N. australe</i> (unknown %) <i>N. parvum</i> (unknown %)	ITS region of rDNA (ITS1, ITS2, and 5.8S)	Aurora (NHB <sup>1</sup> ) Bluegold (NHB <sup>1</sup> ) Brigitta (NHB <sup>1</sup> ) Duke (NHB <sup>1</sup> ) Elliot (NHB <sup>1</sup> ) Liberty (NHB <sup>1</sup> ) Misty (SHB <sup>2</sup> )	Espinoza et al., 2009 [28]
China 20 orchards in 8 provinces	2013–2014	<i>Lasiodiplodia theobromae</i> (5%) <i>N. parvum</i> (40%) <i>B. dothidea</i> (55%)	ITS region of rDNA, partial sequence of <i>EF1-α</i> , and partial sequence of $\beta$ -tubulin (BT2) gene.	Bluecrop (NHB <sup>1</sup> ) Bluegold (NHB <sup>1</sup> ) Darrow (NHB <sup>1</sup> ) Duke (NHB <sup>1</sup> ) Northland (NHB <sup>1</sup> ) Spartan (NHB <sup>1</sup> ) Elliott (NHB <sup>1</sup> ) Anna (SHB <sup>2</sup> ) Legacy (SHB <sup>2</sup> ) Misty (SHB <sup>2</sup> ) O’Neal (SHB <sup>2</sup> ) Brightwell (RE <sup>3</sup> )	Xu et al., 2015 [6]

Italy 4 orchards in Cuneo prov- ince, North- ern Italy	2019	<i>N. parvum</i> (100%)	ITS region of rDNA, <i>EF1-α</i> , and $\beta$ -tubulin ( <i>BT2</i> ) gene.	Last Call (NHB <sup>1</sup> ) Blue Ribbon (NHB <sup>1</sup> ) Top Shelf (NHB <sup>1</sup> )	Guarnaccia et al., 2020 [30]
Mexico High-tunnel and open field in Mi- choacan and Jalisco		<i>N. parvum</i>	ITS region of rDNA	Biloxi (SHB <sup>2</sup> )	Boyzo-Marin et al., 2016 [17]
New Zea- land 7 orchards in multiple re- gions	2013–2014	<i>N. australe</i> (79%) <i>N. luteum</i> (8%) <i>N. ribis</i> (8%) <i>N. parvum</i> (5%)	ITS region of rDNA	NA	Tennakoon et al., 2018 [18]
Peru Olmos Bar- ranca and Huaaura		<i>L. theobromae</i> (76%) <i>L. laeliocattleyae</i> (24%)	ITS region of rDNA, <i>EF1-α</i> , and $\beta$ -tubulin ( <i>BT2</i> ) gene.	Biloxi (SHB <sup>2</sup> ) Emerald (SHB <sup>2</sup> ) Ventura (SHB <sup>2</sup> )	Rodríguez-Gálvez et al., 2020 [31]
Portugal 3 orchards in the center re- gion	2015–2016	<i>N. parvum</i> (42%) <i>B. dothidea</i> (35%) <i>N. eucalyptorum</i> (13%) <i>N. australe</i> (10%) <i>B. corticis</i> (23%)	ITS region of rDNA, <i>EF1-α</i> gene, <i>MAT1-2-1</i> gene	Duke (NHB <sup>1</sup> ) Ozarkblue (SHB <sup>2</sup> )	Hilario et al., 2020 [19]
United States 28 sites of Southeast U.S. (AL, GA, FL, NC, SC)	2009–2010	<i>N. kwambonambiense</i> (23%) <i>B. dothidea</i> (18%) <i>L. pseudotheobromae</i> (18%) <i>N. ribis</i> (18%) <i>D. seriata</i> (14%) <i>L. theobromae</i> (14%)	ITS region of rDNA, <i>EF1-α</i> , $\beta$ -tubulin	SHB <sup>2</sup> and RE <sup>3</sup> culti- vars	Flor et al., 2022 [29]
United States 2 farms in Florida	2007	<i>N. ribis</i> (77%) <i>L. theobromae</i> (23%) <i>B. dothidea</i> (twice out of the survey area)	ITS regions of rDNA, and a partial sequence of <i>EF1-α</i>	SHB <sup>2</sup> (cultivars not specified)	Wright and Har- mon, 2010 [5]

<sup>1</sup>NHB: northern highbush blueberry (*Vaccinium corymbosum* L.). <sup>2</sup>SHB: southern highbush blueberry (*V. corymbosum* L. interspecific hybrids). <sup>3</sup>RE: rabbiteye blueberry (*V. virgatum* Aiton).

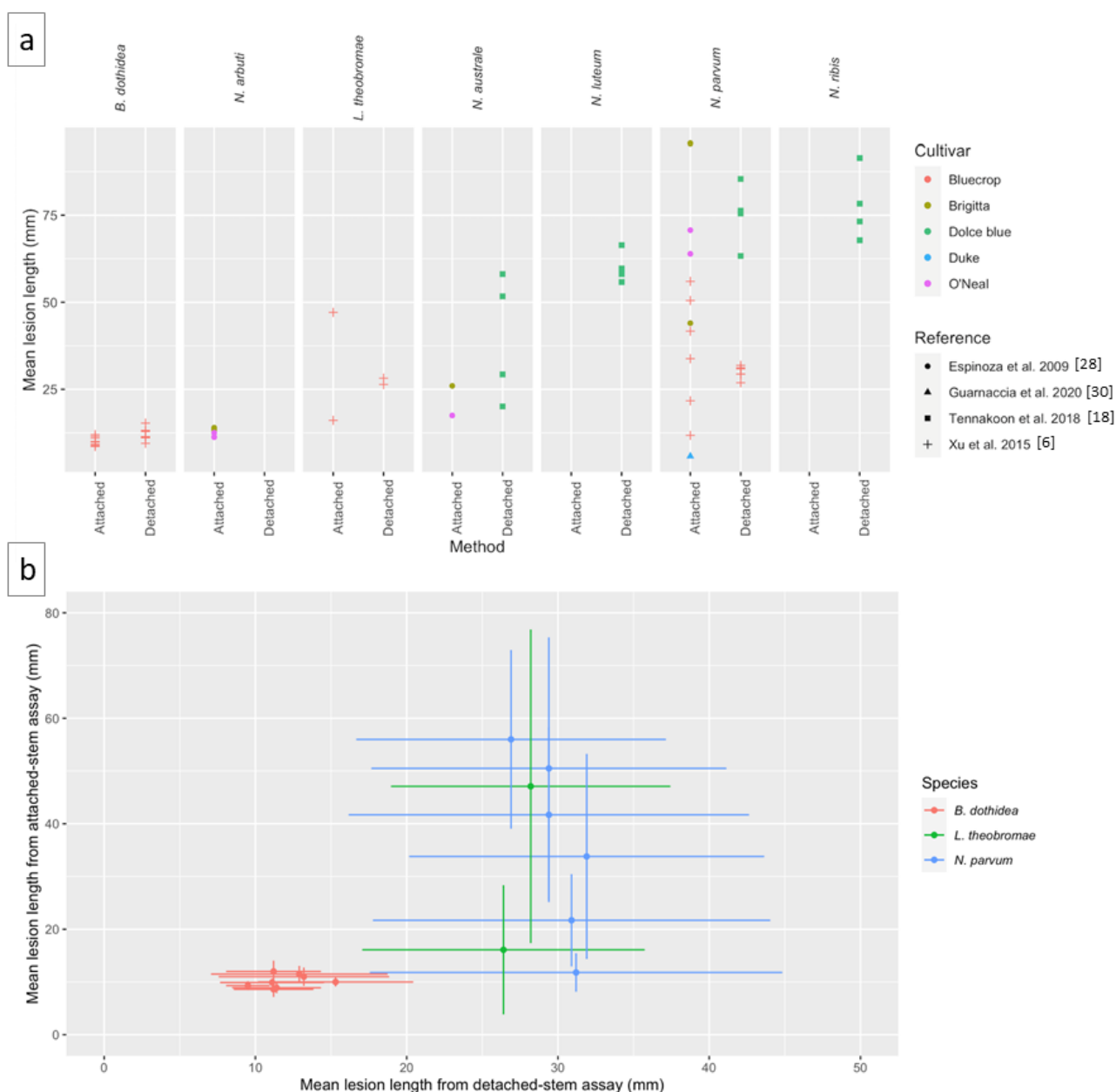


**Figure 2.** Worldwide distribution of causal pathogens of Botryosphaeria stem blight. Size of each pie chart indicates sample size in each study. Only studies which used DNA sequence information to identify causal agents were included in this figure. Detailed data can be found in Table 1 [6,15,17–19,28–31].

#### 4. Pathogenicity and Virulence Tests of *Botryosphaeriaceae* Species and Isolates on Blueberry

Pathogenicity and virulence tests, through either detached- or attached-stem assays, have shown substantial variability in virulence among different species or isolates of the same species [15,18,20,32]. In general, *N. ribis* and *N. parvum* tended to be more aggressive than other species based on several studies [6,18,30] (Figure 3a). However, lesion lengths reported by different studies using the same or different screening assays, isolates and/or species of *Botryosphaeriaceae*, and blueberry cultivars have been inconsistent (Figure 3a). Tennakoon et al. [18] reported that *N. ribis* and *N. parvum* were significantly more virulent than *N. luteum* and *N. australe* in both soft green shoots and hard green shoots. *N. parvum* and *L. theobromae* isolates were found to be significantly more aggressive than *B. dothidea* in both detached and attached stems of cultivar Bluecrop [6]. On the other hand, Scarlett et al. [15] found no significant differences in virulence among eight species, *B. dothidea*, *L. pseudotheobromae*, *L. theobromae*, *N. australe*, *N. kwambonambiense*, *N. macroclavatum*, *N. oculatum*, and *N. parvum*, potentially due to substantial variation among isolates of the same species [15,18]. Significant isolate  $\times$  genotype interactions were also reported in multiple studies [20,32].





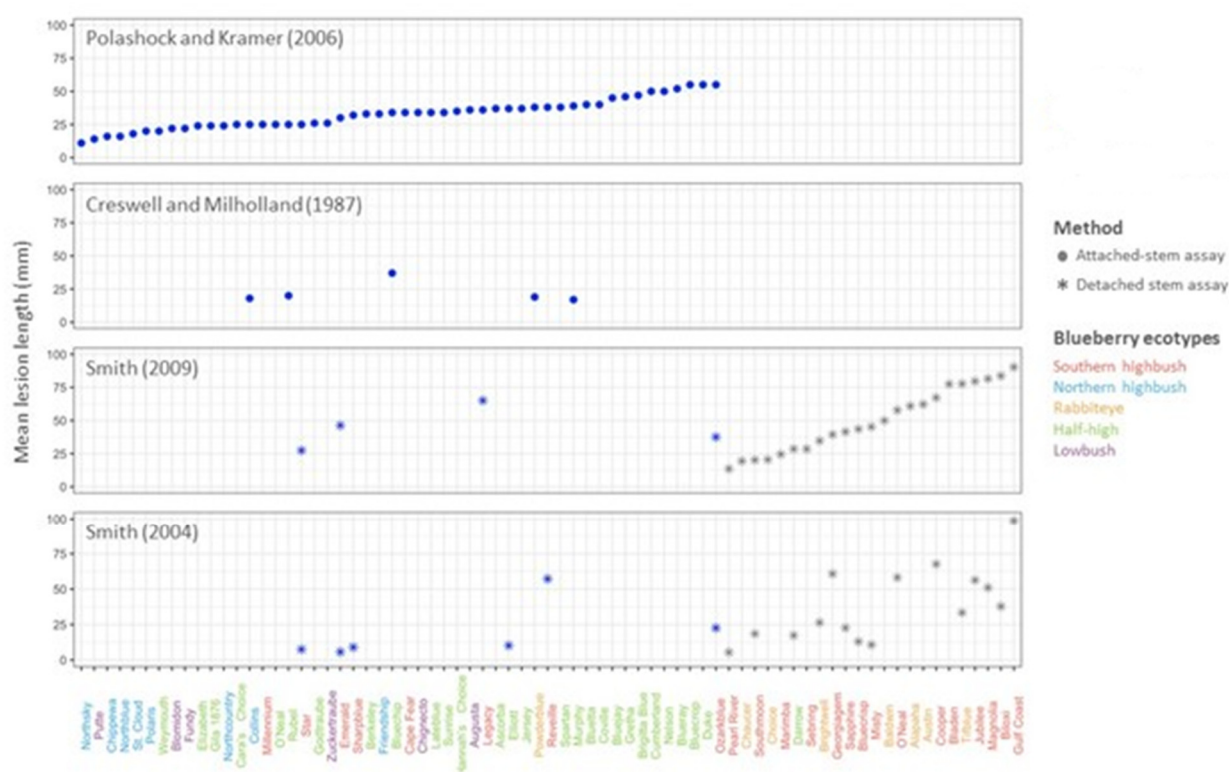
**Figure 3.** Comparisons of mean lesion lengths of pathogenicity and virulence tests. **(a)** Pathogenicity and virulence test results with different methods, isolates, species, and blueberry cultivars from five studies. Attached: attached-stem assay, Detached: detached stem assay. Data for ‘Bluecrop’ was obtained from <sup>1</sup> Xu et al. [6], ‘Brigitta’ and ‘O’Neal’ from <sup>2</sup> Espinoza et al. (2009) [28], ‘Dolce blue’ from <sup>3</sup> Tennakoon et al. [18], ‘Duke’ from <sup>4</sup> Guarnaccia et al. [30]. **(b)** Mean lesion lengths on infected ‘Bluecrop’ using attached- and detached-stem assays. Horizontal error bars represent standard deviation for detached-stem assay, vertical error bars stand for standard deviation for attached-stem assay. Multiple isolates of *B. dothidea*, *L. theobromae*, and *N. parvum* were used to inoculate ‘Bluecrop’. Data were obtained from Xu et al. [6].

Pathogenicity and virulence tests from different reports have revealed large discrepancies between results from detached- and attached-stem assays (Figure 3b) and even between different versions of the same assay (Figure 3a). The major difference between detached- and attached-stem assays is whether the stems are still attached to the plant. In a commonly used detached-stem assay [20,27,33], stems were cut from blueberry plants, wounds were created by scraping away a section of bark, and wounded stems were

inoculated by covering the wound with a mycelial agar plug of fungi secured with a parafilm wrap. The base of each stem was inserted into moistened, sterilized sand and incubated at 25 °C and 100% relative humidity [27]. In an attached-stem assay, such as the one utilized by Polashock and Kramer (2006) [32], shoot tips of container-grown plants were pruned and inoculated with a mycelial agar plug with the mycelium-side down on the fresh-cut surface. These were then covered with parafilm for 3 days to prevent desiccation. Plants were maintained in a greenhouse at ambient temperatures until evaluation. In both detached- [20,27,33] and attached-stem assays [32]), succulent, partially hardened-off stems were inoculated, and the length of the lesion that developed days or weeks post-infection was used as the indicator of resistance or susceptibility. Although inoculation protocols of the same type (e.g., detached-stem assays) were alike, modifications in various aspects (e.g., stem size, stem age, time of evaluation) were common among studies, which, together with variability among isolates/species, isolate × genotype interactions all contributed to inconsistent lesion lengths between studies. Large variations were observed between different inoculation methods and even among isolates inoculated with the same method. For example, ‘Bluecrop’ was screened with both detached- and attached-stem assays by Xu et al. [6] with multiple isolates of *B. dothidea*, *L. theobromae*, and *N. parvum*. A large variation in lesion length was found for each isolate and results from detached- and attached-stem assays showed a weak correlation.

## 5. Resistance in Wild and Cultivated Blueberry Species

A wide range of cultivated and wild blueberry species (*V. corymbosum*, *V. corymbosum* interspecific hybrid, *V. virgatum* Aiton, *V. angustifolium*, *V. corymbosum* × *V. angustifolium*, *V. elliotii*, *V. darrowii*, *V. arboretum*, *V. stamineum*) have been screened for stem blight resistance using detached- [20,27,33] or attached-stem assays [32]. As with the pathogenicity tests described above, the relative lesion lengths reported between studies using the same *Vaccinium* sp. or cultivar were often inconsistent whether the same type of assay or different assays were used (Figure 4). Previous studies reported a higher level of resistance in lowbush and half-high blueberry cultivars compared to cultivated blueberries (highbush and rabbiteye) [32]. Others also reported a higher level of resistance in rabbiteye than in highbush cultivars [7,27]. On the contrary, Babiker et al. [33] screened 39 accessions of 7 blueberry species (*V. corymbosum*, *V. virgatum* Aiton, *V. elliotii*, *V. darrowii*, *V. arboretum*, *V. stamineum*) with a detached-stem assay using two isolates of *Neofusicoccum* spp.; however, no significant differences in lesion length were observed among blueberry species. Discrepancies were also reported between artificial inoculation and field observations, which was likely due to the different environmental conditions in artificial inoculation and field infection experiments. For example, the detached-stem assay may have been conducted in an environment much more conducive than natural field conditions [27,34]. Accordingly, ‘O’Neal’ was considered resistant to stem blight based on field observations [7] but showed susceptibility based on a detached-stem assay [27]. Just as differing methods and parameters can lead to different pathogenicity test results, tests for cultivar resistance to stem blight may also be inconsistent due to pathogenicity or virulence differences between the isolates used, interactions between isolates and blueberry cultivars, and a lack of standardized and reliable screening methods. Since field performance is the ultimate standard for cultivar evaluation, screening protocols that closely align with field observations may more accurately identify cultivars that can minimize field losses to *Botryosphaeria* stem blight.



**Figure 4.** Stem blight resistance of blueberry cultivars of five ecotypes measured in mean lesion length (mm). Southern highbush (*V. corymbosum* L. interspecific hybrids), northern highbush (*V. corymbosum* L.), rabbiteye (*V. virgatum* Aiton), half-high (*V. corymbosum* L.  $\times$  *V. angustifolium* Aiton), and lowbush blueberries (*V. angustifolium* Aiton). Blue dots represents cultivars used in Polashock and Kramer (2006) [32] and grey dots represents additional cultivars in Smith (2009) [20].

## 6. Disease Management

### 6.1. Chemical Management

Fungicide applications immediately after pruning events are strongly recommended to reduce infection of the open pruning wounds with *Botryosphaeria* fungi [8,35]; however, fungicides in general have been very limited in their efficacy for controlling *Botryosphaeria* stem blight in the field. Nonetheless, certain fungicides have shown some degree of protection in in vitro assays or detached stem assays. Using in vitro assays, Smith and Miller-Butler [36] reported significant effects of fungicides (cyprodinil + fludioxonil, pyraclostrobin + boscalid, tebuconazole, pyraclostrobin, propiconazole, fludioxonil, and cyprodinil) on inhibiting the growth of *B. dothidea* and/or *N. parvum* isolates. Using a detached assay, applying fungicide treatment (pyraclostrobin, fosetyl aluminum, or pyraclostrobin + boscalid) after (but not before) wounding and inoculating blueberry stems, significantly reduced lesion length 21 days after the inoculation [36]. Cline & Milholland [34] also reported that dipping roots of blueberry plants into Benomyl-kaolin clay slurry at concentrations between 2000 to 3000  $\mu\text{g/mL}$  can effectively limit stem blight caused by *B. dothidea* for up to 6 months. Additional promising fungicides which have been tested in vitro to control *Botryosphaeria* stem blight are fenhexamid, pyraclostrobin + boscalid, and azoxystrobin + fenbuconazole [20], and azoxystrobin [33]. A recent field evaluation showed significant efficacy of thiophanate-methyl on mitigating *Botryosphaeria* stem blight on mechanically hedged blueberry plants, whereas the other five fungicide tested (azoxystrobin, fludioxonil, penthiopyrad, prothioconazole, and potassium phosphite) did not show consistent efficacy in comparison with an unsprayed control [37].



## 6.2. Cultural Management

In the absence of effective fungicide treatments, the management of *Botryosphaeria* stem blight relies on cultural practices. Among these, ensuring establishment with healthy nursery plants and pruning out symptomatic tissues in a timely manner are considered the most effective approaches [7,8]. Reducing biotic and abiotic stresses via good management practices such as proper irrigation, fertilization, effective disease management, and avoiding wounding of the plants are also recommended for stem blight prevention [7,8]. Excessive nitrogen fertilization or late summer fertilization may increase the occurrence of winter injury [36] which can lead to new *Botryosphaeria* infections. Given the wide host range of *Botryosphaeriaceae* [7], site selection is important when establishing new plantings to avoid areas with high disease pressure. The adoption of disease-resistant cultivars, though desirable, has been difficult in practice as no commercial cultivar has exhibited consistent and sufficient resistance against stem blight in the field [20,33].

## 7. Future Directions

Thanks to modern DNA sequencing efforts, we are now aware that *Botryosphaeria* stem blight can be caused by multiple species of *Botryosphaeriaceae*. However, control of this disease remains a challenge and no major breakthroughs have yet been made regarding disease management or *Botryosphaeria* stem blight-resistant cultivar development. The incidence of stem blight is likely to increase under the changing climate, which is expected to trigger more extreme weather events [38] and result in additional biotic and abiotic stresses. To respond to increasing disease pressures, it is therefore urgent to develop more effective management strategies against stem blight. Limitations of current research are reflected in the inconsistent results from pathogenicity tests and cultivar disease response screenings, a lack of commercial cultivars with a high level of disease resistance, and limited chemical and cultural management options that effectively minimize the impact of this disease. Accordingly, advances in the following directions are of particular importance to address existing challenges:

### 7.1. Development and Adoption of Uniform and Reliable Screening Protocols

Results from pathogenicity tests and germplasm screenings have shown large discrepancies due to the use of different protocols and assay types (including the use of detached- and attached-stem assays) between studies. Significant discrepancies have even been reported when the same type of assay was repeated with identical blueberry cultivars and the same fungal isolates [6]. Inconsistencies between prior studies have made it difficult to translate findings from one experiment to another. Furthermore, discrepancies between artificial inoculation experiments and field observations have also limited the usefulness of current methods for resistant cultivar development. Therefore, it is critical to develop and adopt uniform and reliable protocols for pathogenicity tests and germplasm screenings to enable more successful development of resistant cultivars. Ideally, screening protocols should: (a) provide consistent results on the same genotype when the same isolates are used for inoculation; (b) be designed to reflect the overall resistance or tolerance level of the whole blueberry plant instead of only individual branches; and (c) exhibit desirable correlations with field observations.

### 7.2. Utilization of Diverse and Well-Characterized *Botryosphaeriaceae* Isolates for Cultivar Screens

Given that *Botryosphaeria* stem blight of blueberry is caused by diverse species within *Botryosphaeriaceae*, any concerted breeding effort aimed at producing cultivars resistant to this disease must take into account this diversity when designing germplasm screening protocols. Considering the significant effects of isolates and isolate  $\times$  genotype interactions on lesion length previously observed in multiple pathogenicity and germplasm screening studies [20,32], selection for resistance against a single isolate causing *Botryosphaeria* stem blight of blueberry is not advised. To translate into durable

resistance in the field, cultivar screens should utilize *Botryosphaeriaceae* isolates that represent the diversity of species present in the area where developed cultivars are intended to be deployed. Pathogen diversity studies will be critical to establish which particular species of *Botryosphaeriaceae* are causing Botryosphaeria stem blight of blueberry in a given area or region and enable the collection of appropriately diverse, pathogenic isolates for use in screens to select for resistant cultivars. Genetic identification of isolates is essential, especially in light of past confusion and incorrect assumptions regarding pathogen diversity that resulted from identification of the organisms causing Botryosphaeria stem blight on the basis of morphology alone. Utilization of a diverse collection of genetically well-characterized *Botryosphaeriaceae* isolates for cultivar screens will be instrumental to evaluate the effects of species, isolates, and isolate  $\times$  genotype interactions on stem blight severity. Ideally, isolates used in each study should be made accessible for future researchers to better identify correlations between the virulence of the pathogen isolates and their genetics. This research will likely benefit from whole genome sequencing data, as isolate sequence information from only 2–5 genomic regions, as used in most studies for genetic identification, is limited in its ability to reveal the genetic differences that determine differences in virulence between fungal isolates of the same species on blueberry.

### 7.3. Conducting Field Evaluations of Cultivars, Chemicals, and Cultural Management Practices

Most of the data available on the pathogenicity of *Botryosphaeriaceae*, cultivar resistance, and the effectiveness of fungicides has been based primarily on laboratory or greenhouse experiments. While lab observations provide valuable information on the mechanisms of disease infection, resistance, and management, discrepancies between the results of artificial inoculation experiments and field observations of host susceptibility have been reported in multiple studies [7,27]. Research findings can only benefit growers if they translate into tangible effects on commercial production. Therefore, carrying out experiments such as germplasm screening and fungicide testing under field conditions is essential for developing solutions for this devastating disease.

### 7.4. Other Directions

Planting with clean materials is vital to prevent stem blight outbreaks [7]. However, blueberry growers in the southeastern U.S. and elsewhere still have limited access to tissue culture materials. Therefore, expanding the availability and use of tissue-cultured blueberry nursery materials will be helpful for the prevention of Botryosphaeria stem blight disease. In addition, learning from other crops, biocontrol agents may be useful tools for inhibiting the causal pathogens and providing options for sustainable stem blight management. Biocontrol agents such as *Trichoderma* species or grapevine endophytic fungi have been found to be effective in reducing the incidence of grapevine trunk diseases or were antagonistic to the growth of grapevine trunk disease causal pathogens [39,40]. As the pathogens causing Botryosphaeria stem blight of blueberry are in the same genera as grapevine trunk disease-associated pathogens, biocontrol agents may also have efficacy for controlling stem blight in blueberry.

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## References

1. FAOSTAT. *Production: Crops: Blueberry*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2021; Available online: <https://www.fao.org/faostat/en/#home> (accessed on 22 April 2021).
2. International Blueberry Organization 2021 Report. Available online: <https://www.internationalblueberry.org/2021-report/> (accessed on 8 December 2021).
3. USDA National Agricultural Statistics Service (NASS). Available online: [https://www.nass.usda.gov/Statistics\\_by\\_State/New\\_Jersey/Publications/Blueberry\\_Statistics](https://www.nass.usda.gov/Statistics_by_State/New_Jersey/Publications/Blueberry_Statistics) (accessed on 22 April 2021).
4. United States Blueberry Market—Growth, Trends, and Forecasts (2020–2025). Available online: <https://www.mordorintelligence.com/industry-reports/united-states-blueberry-market> (accessed on 22 April 2021).
5. Wright, A.F.; Harmon, P.F. Identification of species in the *Botryosphaeriaceae* Family causing stem blight on southern highbush blueberry in Florida. *Plant Dis.* **2010**, *94*, 966–971. <https://doi.org/10.1094/PDIS-94-8-0966>.
6. Xu, C.; Zhang, H.; Zhou, Z.; Hu, T.; Wang, S.; Wang, Y.; Cao, K. Identification and distribution of *Botryosphaeriaceae* species associated with blueberry stem blight in China. *Eur. J. Plant Pathol.* **2015**, *143*, 737–752. <https://doi.org/10.1007/s10658-015-0724-6>.
7. Milholland, R.D. Botryosphaeria stem blight. In *Compendium of Blueberry and Cranberry Diseases*; Caruso, F.L., Ramsdell, D.C., Eds.; The American Phytopathological Society Press: St. Paul, MN, USA, 1995; pp. 10–11.
8. Flor, N.C.; Phillips, D.A.; Harmon, P.F. *Botryosphaeria Stem Blight on Southern Highbush Blueberry in Florida*; UF/IFAS Extension. 347; University of Florida: Gainesville, FL, USA, 2019.
9. Tang, W.; Ding, Z.; Zhou, Q.; Wang, Y.Z.; Guo, L.Y. Phylogenetic and pathogenic analyses show that the causal agent of apple ring rot in China is *Botryosphaeria dothidea*. *Plant Dis.* **2012**, *96*, 486–496. <https://doi.org/10.1094/PDIS-08-11-0635>.
10. Urbez-Torres, J.R.; Leavitt, G.M.; Voegel, T.; Gubler, W.D. Identification and distribution of *Botryosphaeria* species associated with grapevine cankers in California. *Plant Dis.* **2006**, *90*, 1490–1503. <https://doi.org/10.1094/PD-90-1490>.
11. Sakalidis, M.L.; Ray, J.D.; Lanoiselet, V.; Hardy, G.E.S.J.; Burgess, T.I. Pathogenic *Botryosphaeriaceae* associated with *Mangifera indica* in the Kimberly region of Western Australia. *Eur. J. Plant Pathol.* **2011**, *130*, 379–391. <https://doi.org/10.1007/s10658-011-9760-z>.
12. Wang, F.; Zhao, L.N.; Li, G.H.; Huang, J.B.; Hsiang, T. Identification and characterization of *Botryosphaeria* species causing gummosis of peach trees in Hubei province, central China. *Plant Dis.* **2011**, *95*, 1378–1384. <https://doi.org/10.1094/PDIS-12-10-0893>.
13. Garibaldi, A.; Bertetti, D.; Poli, A.; Gullino, M.L. First report of fruit rot in pear caused by *Botryosphaeria dothidea* in Italy. *Plant Dis.* **2012**, *96*, 910. <https://doi.org/10.1094/PDIS-02-12-0130-PDN>.
14. Zhai, L.; Zhang, M.; Lv, G.; Chen, X.; Jia, N.; Hong, N.; Wang, G. Biological and molecular characterization of four *Botryosphaeria* species isolated from pear plants showing stem wart and stem canker in China. *Plant Dis.* **2014**, *98*, 716–772. <https://doi.org/10.1094/PDIS-10-13-1060-RE>.
15. Scarlett, K.A.; Shuttleworth, L.A.; Collins, D.; Rothwell, C.T.; Guest, D.I.; Daniel, R. Botryosphaeriales associated with stem blight and dieback of blueberry (*Vaccinium* spp.) in New South Wales and Western Australia. *Australas. Plant Pathol.* **2019**, *48*, 45–57.
16. Zhao, L.; Wang, Y.; He, W.; Zhang, Y. Stem blight of blueberry caused by *Lasiodiplodia vaccinii* sp. nov. in China. *Plant Dis.* **2019**, *103*, 2041–2050. <https://doi.org/10.1094/PDIS-01-19-0079-RE>.
17. Boyzo-Marin, J.; Rebollar-Alvitar, A.; Silva-Rojas, H.V.; Ramirez-Maldonado, G. First report of *Neofusicoccum parvum* causing stem blight and dieback of blueberry in Mexico. *Plant Dis.* **2016**, *100*, 2524. <https://doi.org/10.1094/PDIS-03-16-0381-PDN>.
18. Tennakoon, K.M.S.; Ridgway, H.J.; Jaspers, M.V.; Jones, E.E. *Botryosphaeriaceae* species associated with blueberry dieback and sources of primary inoculum in propagation nurseries in New Zealand. *Eur. J. Plant Pathol.* **2018**, *150*, 363–374. <https://doi.org/10.1007/s10658-017-1283-9>.
19. Hilario, S.; Lopes, A.; Santos, L.; Alves, A. *Botryosphaeriaceae* species associated with blueberry stem blight and dieback in the Center Region of Portugal. *Eur. J. Plant Pathol.* **2019**, *156*, 31–44. <https://doi.org/10.1007/s10658-019-01860-6>.
20. Smith, B.J. Botryosphaeria stem blight of southern blueberries: Cultivar susceptibility and effect of chemical treatments. *Acta Hort.* **2009**, *810*, 385–394.
21. Choi, I.Y. First report of bark dieback on blueberry caused by *Botryosphaeria dothidea* in Korea. *Plant Dis.* **2011**, *95*, 227. <https://doi.org/10.1094/PDIS-05-10-0371>.
22. Sammonds, J.; Billones, R.; Rocchetti, M.; Ridgway, H.J.; Walter, M.; Jaspers, M.V. Survey of blueberry farms for *Botryosphaeria* dieback and crown rot pathogens. *New Zealand Plant Prot.* **2009**, *62*, 238–242. <https://doi.org/10.30843/nzpp.2009.62.4826>.
23. Creswell, T.C.; Milholland, R.D. Responses of blueberry genotypes to infection by *Botryosphaeria dothidea*. *Plant Dis.* **1987**, *71*, 710–713. <https://doi.org/10.1094/PD-71-0710>.
24. Marsberg, A.; Kemler, M.; Jami, F.; Nagel, J.H.; Postma-Smidt, A.; Naidoo, S.; Wingfield, M.J.; Crous, P.W.; Spatafora, J.W.; Hesse, C.N.; et al. *Botryosphaeria dothidea*: A latent pathogen of global importance to woody plant health. *Mol. Plant Pathol.* **2017**, *18*, 477–488. <https://doi.org/10.1111/mpp.12495>.
25. Milholland, R.D. Histopathology and pathogenicity of *Botryosphaeria dothidea* on blueberry stems. *Phytopathology* **1972**, *62*, 654–660.

26. Smith, B.J. Detached stem assay to evaluate the severity of stem blight of rabbiteye blueberry (*Vaccinium ashei*). *Acta Hort.* **1997**, *446*, 457–464.
27. Smith, B.J. Susceptibility of southern highbush blueberry cultivars to Botryosphaeria stem blight. *Small Fruits Rev.* **2004**, *3*, 193–201.
28. Espinoza, J.G.; Briceño, E.X.; Chávez, E.R.; Urbez-Torres, J.R.; Latorre, B.A. *Neofusicoccum* spp. associated with stem canker and dieback of blueberry in Chile. *Plant Dis.* **2009**, *93*, 1187–1194. <https://doi.org/10.1094/PDIS-93-11-1187>.
29. Flor, N.C.; Wright, A.F.; Huguet-Tapia, J.; Harmon, P.F.; Liberti, D. Identification of Fungi in the Botryosphaeriaceae Family Associated with Stem Blight of Vaccinium spp. in the Southeastern United States. *Fungal Biol.* **2022**, *126*, 342–355.
30. Guarnaccia, V.; Martino, I.; Tabone, G.; Brondino, L.; Gullino, M.L. Fungal pathogens associated with stem blight and dieback of blueberry in northern Italy. *Phytopathol. Mediterr.* **2020**, *59*, 229–245. <https://doi.org/10.14601/Phyto-11278>.
31. Rodríguez-Gálvez, E.; Hilário, S.; Lopes, A. Diversity and pathogenicity of *Lasiodiplodia* and *Neopestalotiopsis* species associated with stem blight and dieback of blueberry plants in Peru. *Eur. J. Plant Pathol.* **2020**, *157*, 89–102. <https://doi.org/10.1007/s10658-020-01983-1>.
32. Polashock, J.J.; Kramer, M. Resistance of blueberry cultivars to botryosphaeria stem blight and phomopsis twig blight. *Hortscience* **2006**, *41*, 1457–1461. Available online: <https://journals.ashs.org/hortsci/view/journals/hortsci/41/6/article-p1457.xml> (accessed on 23 June 2021).
33. Babiker, E.M.; Stringer, S.J.; Sakhanokho, H.F.; Smith, B.J. Characterization and pathogenicity of stem blight complex isolates associated with stem blight disease on *Vaccinium* species. *Hortscience* **2019**, *54*, 1199–1203. <https://doi.org/10.21273/HORTSCI14033-19>.
34. Cline, W.O.; Milholland, R.D.; Rooks, S.D.; Ballington, J.R. Techniques in breeding for resistance to blueberry stem blight caused by *Botryosphaeria dothidea*. *Acta Hort.* **1993**, *346*, 107–110. <https://doi.org/10.17660/ActaHortic.1993.346.14>.
35. Sial, A.; Cato, A.; Hale, F.; Pfeiffer, D.; Favre, M.; Stafne, E.; Samtani, J.; Cline, B.; Ferguson, M.H.; Melanson, R.; et al. *Southeast Regional Blueberry Integrated Management Guide*; (Annual Publication 123-2); University of Georgia Cooperative Extension: Athens, GA, USA, 2022; Available online: <https://smallfruits.org/files/2022/02/2022-Blueberry-Spray-Guide.pdf> (accessed on 1 November 2022).
36. Smith, B.J.; Miller-Butler, M.A. Effect of nitrogen fertilization and fungicides on Botryosphaeria stem blight lesion development on detached blueberry stems. *Acta Hort.* **2017**, *1180*, 61–70. <https://doi.org/10.17660/ActaHortic.2017.1180.9>.
37. Holland, R.M. Fruit Microbial Hygiene Associated with Mechanical Harvesting, and Injuries and Stem Blight in Relation to Mechanical Hedging and Crown Bark Inclusions in Blueberry. Ph.D. Thesis, Department of Plant Pathology, University of Georgia, Athens, GA, USA, 2021.
38. Stott, P. How climate change affects extreme weather events. *Science* **2016**, *352*, 1517–1518. <https://doi.org/10.1126/science.aaf7271>.
39. Kotze, C.; Van Niekerk, J.; Mostert, L.; Halleen, F.; Fourie, P. Evaluation of biocontrol agents for grapevine pruning wound protection against trunk pathogen infection. *Phytopathol. Mediterr.* **2011**, *50*, S247–S263.
40. Billar de Almeida, A.; Concas, J.; Campos, M.D.; Materatski, P.; Varanda, C.; Patanita, M.; Murolo, S.; Romanazzi, G.; Félix, M.D.R. Endophytic fungi as potential biological control agents against grapevine trunk diseases in Alentejo region. *Biology* **2020**, *9*, 420. <https://doi.org/10.3390/biology9120420>.

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