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Cross-infection and asymptomatic colonization by *Botryosphaeriaceae* fungi on lignified stems of apple and olive, and dormant cuttings of grapevine

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Summary. *Botryosphaeriaceae* pathogens have broad host ranges and can move between hosts, particularly those with overlapping geographic distributions. Cross-infection potential and virulence were assessed for 40 isolates of *Botryosphaeria*, *Diplodia*, and *Neofusicoccum* (11 species), originally isolated from apple, olive, or grapevine crops. Progression of asymptomatic colonization beyond visible necrotic lesions was also assessed, to determine minimum pruning distances required for effective pathogen removal. The assays were conducted using detached lignified stems of apple and olive, and dormant cuttings of grapevine. All the isolates cross-infected and colonized stems or cuttings of the three potential hosts, confirming host-independence of these pathogens. Most of the *Neofusicoccum* isolates consistently caused the largest lesions across the three inoculated hosts. Asymptomatic colonization was not detected at distances of 20 or 30 cm beyond visible lesions. However, at 10 cm, one isolate of *N. parvum* colonized the three hosts, and one isolate of *D. seriata* colonized olive host. These results highlight the challenges for managing these pathogens in fruit crops growing in close proximity, and emphasize the urgency of revising the minimum pruning distances required for successful pathogen removal.

Keywords. *Botryosphaeria*, *Diplodia*, *Neofusicoccum,* host jumping, sanitation practices.

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INTRODUCTION

Botryosphaeriaceae includes fungi widely recognized for abilities to infect a broad range of woody hosts, both native and introduced (Slippers and Wingfield 2007; Phillips *et al.,* 2013; Batista *et al.,* 2021; Guarnaccia *et al.,* 2022). These fungi pathogens frequently act as latent pathogens, becoming virulent when their hosts are in stress conditions (Slippers and Wing-

field 2007; Slippers *et al.,* 2013; Hrycan *et al*., 2020; Batista *et al.,* 2021). The most common symptoms caused by *Botryosphaeriaceae* include fruit rot, leaf spot, dieback, and canker (Slippers and Wingfield 2007; Phillips *et al.,* 2013; Yang *et al.,* 2017), with cankers being the most destructive, as they can lead to the death of stems, branches or whole trees (Slippers and Wingfield 2007; Delgado *et al*., 2016; Úrbez-Torres *et al*., 2016; Hernández *et al*., 2022; Valdez-Tenezaca *et al*., 2025).

Botryosphaeriaceae are known for their broad host ranges and ability for host jumping, particularly among hosts with overlapping geographic distributions (Amponsah *et al*., 2011; Cloete *et al*., 2011; Úrbez-Torres *et al*., 2013; Sessa *et al*., 2016; Zlatković *et al*., 2018; Silva-Valderrama *et al*., 2024). Recent studies have confirmed the capacity of various host species to be inoculum sources for other susceptible hosts (Mojeremane *et al*., 2020; Díaz *et al*., 2022; Hernández *et al*., 2025).

In Uruguay, *Botryosphaeriaceae* have been isolated from healthy and symptomatic tissues of commercial and native *Myrtaceae* species (Pérez *et al*., 2009; 2010), and from cankers and fruit rots of several fruit crops, including grapevine (Abreo *et al*., 2013), apple (Delgado *et al*., 2016; Sessa *et al*., 2016), pear, peach (Sessa *et al*., 2016), blueberry (Sessa *et al*., 2018), and olive (Hernández *et al*., 2022). Most of these crops are cultivated in close proximity, highlighting the risks of cross-infection occurrence (Silva-Valderrama *et al*., 2024). Furthermore, the majority of identified *Botryosphaeriaceae* in *Botryosphaeria*, *Diplodia*, and *Neofusicoccum*, and have been found infecting most of these hosts (Abreo *et al*., 2013; Delgado *et al*., 2016; Pérez *et al*., 2009; 2010; Sessa *et al*., 2016; 2018; Hernández *et al*., 2022).

Multiple studies of pathogen virulence have shown that species of *Neofusicoccum* are the most virulent, regardless of their origins. For example, most isolates of *N. luteum* and *N. parvum* originating from grapevine (Úrbez-Torres *et al*., 2011; Belleé *et al*., 2017), apple (Delgado *et al*., 2016), almond (Olmo *et al*., 2016), or walnut (Antony *et al*., 2024), caused longer necrotic wood lesions compared to other related species when inoculated into the respective hosts. A similar pattern was observed in cross-inoculation studies, where most *Neofusicoccum* species exhibited the greatest levels of virulence (Sessa *et al*., 2016; Diaz *et al*., 2022; Hernández *et al*., 2025).

Once wood is infected and cankers become visible, removing diseased stems, branches or trunks is the most appropriate recommendation to extend the productive life of the trees (Úrbez-Torres, 2011; Alaniz *et al*., 2012; Delgado *et al*., 2016). Pruning should extend several centimeters beyond the visible margins of necrotic lesions, as it has been documented that *Botryosphaeriaceae* hyphae can colonize asymptomatic tissues, mainly xylem, beyond visible lesions (Brown and Hendrix, 1981; Muniz *et al*., 2011; Obrador-Sánchez and Hernández-Martínez, 2020; Antony *et al*., 2024). For example, in nut crops, Moral *et al*., (2019) recommended pruning approx. 5 to 6 cm below external canker margins, whereas Úrbez-Torres (2011) suggested removing all infected grapevine wood at least 10 cm below visible vascular symptoms. However, no studies have established the precise amount of tissue that must be removed to ensure complete pathogen elimination. Incomplete removal will lead to canker recurrence, as residual pathogens can continue to colonize host tissues.

The aims of the present study were: i) to investigate the occurrence of cross-infections by *Botryosphaeria*, *Diplodia* and *Neofusicoccum* species originating from apple, olive or grapevine, and to evaluate their virulence in these hosts; ii) to estimate the asymptomatic colonization distances of *Botryosphaeria*, *Diplodia*, and *Neofusicoccum* species from visible lesion margins in apple, olive, and grapevine fruit crops. Experiments were conducted using detached lignified stems of apple and olive, and dormant cuttings of grapevine, which were inoculated with local isolates of *Botryosphaeriaceae*.

MATERIAL AND METHODS

Fungal isolates

Forty *Botryosphaeriaceae* isolates, representing eleven species of *Botryosphaeria*, *Diplodia* or *Neofusicoccum* (maximum of three isolates per species, according to availability) were used for direct and cross inoculation assessments. Twelve isolates of the seven most relevant species from these genera, with a maximum of three isolates per species, were used for asymptomatic colonization progression assessments (Table 1). The isolates were obtained from apple stem cankers, dieback, or fruit rot (Delgado *et al*., 2016), olive stem canker (Hernández *et al*., 2022), or from grapevine wood canker samples collected from commercial orchards and vineyards in the south of Uruguay (Supplementary material 1). *Botryosphaeriaceae* isolates from grapevine were initially identified by their fast-growing mycelium, which was white and cottony in early growth in culture, on the first days and turning to grey or grey-green a few days later. Isolates were subcultured on water agar with sterilized pine needles on the agar surface, and incubated at 25°C under near UV-light with a 12-h photoperiod. When mature pycnidia were formed, conidium shapes and colour were assessed. For species

Table 1. Designations, hosts, localities, and relevant references for Uruguayan isolates of *Botryosphaeria*, *Diplodia* and *Neofusicoccum* used in this study.

Species	Isolate	Host	Locality	Reference
B. dothidea	B14 a	Apple	Melilla/Montevideo	Delgado *et al.,* 2016
	B49	Apple	Villa Nueva, Canelones	Delgado *et al.,* 2016
	B108	Apple	Canelón Chico, Canelones	Delgado *et al.,* 2016
	O28	Olive	19 de Abril, Rocha	Hernández *et al.,* 2022
	V2	Grapevine	n/d	Present study
	V13	Grapevine	Las Brujas, Canelones	Present study
	V22 a	Grapevine	Progreso, Canelones	Present study
B. wangensis	O7 a	Olive	Garzón, Maldonado	Hernández *et al.,* 2022
	O22	Olive	Melilla, Montevideo	Hernández *et al.,* 2022
D. intermedia	B5	Apple	Melilla, Montevideo	Delgado *et al.,* 2016
	B118	Apple	Melilla, Montevideo	Delgado *et al.,* 2016
	B144	Apple	Progreso, Canelones	Delgado *et al.,* 2016
D. mutila	O36	Olive	Melilla, Montevideo	Hernández *et al.,* 2022
D. pseudoseriata	V 14	Grapevine	Las Brujas, Canelones	Present study
D. seriata	B27	Apple	El Colorado, Canelones	Delgado *et al.,* 2016
	B69	Apple	Kiyu, San José	Delgado *et al.,* 2016
	B157 a	Apple	Juanicó, Canelones	Delgado *et al.,* 2016
	O14 a	Olive	San Jacinto, Canelones	Hernández *et al.,* 2022
	O19	Olive	Montevideo, Melilla	Hernández *et al.,* 2022
	V1 a	Grapevine	n/d	Present study
	V5	Grapevine	n/d	Present study
	V23	Grapevine	Progreso, Canelones	Present study
N. australe	B112	Apple	Melilla, Montevideo	Delgado *et al.,* 2016
N. cryptoaustrale	O6	Olive	Garzón, Maldonado	Hernández *et al.,* 2022
	O21 a	Olive	Melilla, Montevideo	Hernández *et al.,* 2022
	O24	Olive	Villa Nueva, Canelones	Hernández *et al.,* 2022
N. luteum	B55	Apple	Melilla, Montevideo	Delgado *et al.,* 2016
	B107	Apple	Canelón Chico, Canelones	Delgado *et al.,* 2016
	B129 a	Apple	Juanicó, Canelones	Delgado *et al.,* 2016
	O10 a	Olive	Garzón, Maldonado	Hernández *et al.,* 2022
	O20	Olive	Melilla, Montevideo	Hernández *et al.,* 2022
	O27	Olive	Villa Nueva, Canelones	Hernández *et al.,* 2022
N. occulatum	O12 a	Olive	Garzón, Maldonado	Hernández *et al.,* 2022
	O29	Olive	19 de Abril, Rocha	Hernández *et al.,* 2022
N. parvum	B60	Apple	Melilla, Montevideo	Delgado *et al.,* 2016
	B146	Apple	Progreso, Canelones	Delgado *et al.,* 2016
	B168 a	Apple	Melilla, Montevideo	Delgado *et al.,* 2016
	V4	Grapevine	C. de Sierra, Canelones	Present study
	V28	Grapevine	Las Brujas, Canelones	Present study
	V35 a	Grapevine	Las Brujas, Canelones	Present study

[a] Isolates used to study progression of infections in asymptomatic host tissues.
n/d no data.

identification, the isolate genomic regions translation elongation factor 1-α (TEF), beta-tubulin (TUB2), and internal transcribed spacer regions (ITS) were analyzed (Supplementary material 2).

Plant material and inoculation method

Apparently healthy 1-year-old detached lignified stems or dormant cuttings of lengths approx. 50 or 80 cm and 1 cm diam. of apple 'Red Delicious', olive 'Arbe-

quina', or grapevine 'Marseland', were used for pathogenicity assessments. These cultivars are among the most planted of the respective fruit crops in Uruguay. The stems and cuttings were collected during winter, from mature commercial orchards with no known previous *Botryosphaeriaceae* infections, that were located in Canelones and Montevideo Department, of southern Uruguay. The climate of the region where these orchards are established is characterized by persistent high humidity, frequent rainfall (approx. 1,100 mm per year) and moderate temperatures. The collected stems or cuttings were immediately placed in 200 mL capacity glass jars each containing 50 mL of sterile moist sand.

For direct and cross-inoculation assays, a central internode of each stem or cutting was surface-disinfected with cotton soaked in 70% ethanol. For asymptomatic colonization progression assays, the upper internode of each stem or cutting was disinfected under the same conditions. A wound (5 mm diam.) was immediately made by removing the bark with a sterile scalpel to expose the cambium. Mycelium plugs (5 mm diam.) were cut from the margins of *Botryosphaeriaceae* colonies on PDA growing at 25°C in darkeners for 1 week, and were each placed onto a wound with the mycelium surface facing the stem cambium. Cotton soaked in sterile water was attached to the inoculated wound and wrapped with parafilm, to prevent desiccation.

Direct and cross inoculations on detached host stems and cuttings

For each fruit crop host, eight detached 50-cm-long stems or cuttings were inoculated with each *Botryosphaeriaceae* isolate, while eight stems or cuttings were inoculated with sterile agar plugs as inoculation controls. Glass jars (see above) containing the inoculated stems or cuttings were randomly arranged in a temperature-controlled room (24 ± 2°C), and the sand in each jar was periodically moistened. After five weeks, the plant stem or cutting bark was removed, and lengths of necrotic lesions as discolored wood extending from the inoculation sites, were measured using a digital caliper (Kamasa, EEUU). The experiment was repeated once.

Lesion length data were analyzed using generalized linear models, assuming a gamma distribution for the variable, with natural Log as link function. The GLIMMIX procedure in SAS program (Statistical Analysis System, version 9.4, SAS Institute Inc.) was used. The model included the following factors: host origins of the isolates, *Botryosphaeriaceae* species within host origin, and isolates within *Botryosphaeriaceae* species and host origin. Means were compared using the Tukey-Kramer test and a significance level of $P \leq 0.05$.

Asymptomatic colonization progression on detached host stems and cuttings

For each fruit host, five detached 80-cm-long stems or cuttings were inoculated with each *Botryosphaeriaceae* isolate, while five stems or cuttings inoculated with sterile agar plugs were used as inoculation controls. Glass jars (see above) containing the inoculated stems or cuttings were randomly arranged in a temperature-controlled room (24 ± 2°C), and the sand in each jar was periodically moistened. After 4 weeks, the bark of each stem or cutting was removed, and isolations were made by removing tissue pieces from the lower edges of visible necrotic lesions, as well as from 10, 20 and 30 cm towards the lower parts of stems or cuttings (Figure 1).

For re-isolations, five 1–2 mm cross-sectional discs were cut at each distance, and were placed on potato dextrose agar (PDA) and incubated at 25°C in darkness. After 3 to 6 d, the presence of *Botryosphaeriaceae* colonies was recorded, and the re-isolated cultures were compared with the original isolates based on colony morphology and conidium characteristics. The proportion of stems or cuttings with positive re-isolations was estimated for each fruit crop and isolate combination at

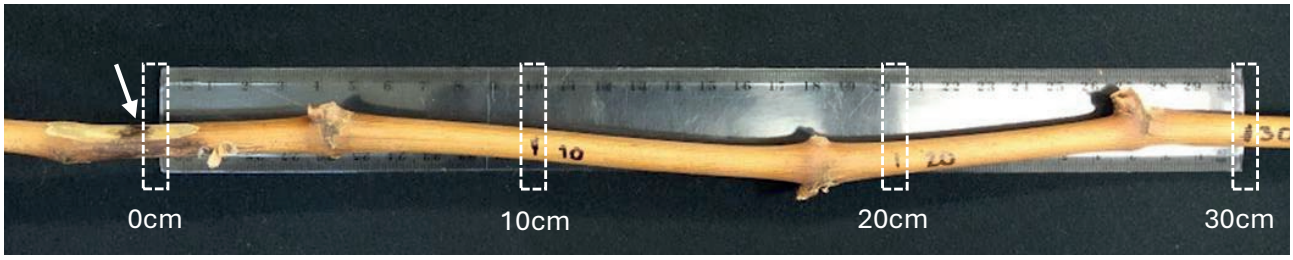

Figure 1. Illustration showing the four distances on detached lignified stems of apple and olive or detached dormant cuttings of grapevine, used for re-isolations of *Botryosphaeriaceae* isolates inoculated onto host tissues. The distances were from the necrotic lesion margin (indicated by the white arrow).

the four specified re-isolation distances (0, 10, 20 and 30 cm) from inoculation points. At least one of the five stem discs plated in PDA yielding a *Botryosphaeriaceae* colony was considered as a positive infection. The experiment was repeated once.

RESULTS

Direct and cross inoculations on detached host stems and cuttings

The forty inoculated *Botryosphaeriaceae* isolates caused necrotic lesions on detached stems of apple and olive, and detached cuttings of grapevine, extending upwards and downwards from the points of inoculation. Cicatrized wounds developed on detached stems and cuttings used as inoculation controls. Necrotic lesions consisted of dark discolouration and internal wood streaking (Figure 2). For all the inoculated isolates, the mean necrotic lesions lengths ranged from 1.3 to 29.4 cm on apple stems, from 2.1 to 19.6 cm on olive stems, and from 2.4 to 20.3 cm in grapevine cuttings.

For each fruit crop, statistical analyses indicated statistically significant effects of host origin of isolates ($P < 0.0001$), *Botryosphaeriaceae* species within host origin ($P < 0.0001$), and isolates within species and host origin ($P < 0.0001$). However, regardless of isolate host origin or inoculated host, most *Neofusicoccum* spp. isolates caused the longest lesions on all three hosts, with statistically significant differences in most cases compared with the mean lesion lengths caused by isolates of *Diplodia* spp. or *Botryosphaeria* spp. The mean necrotic lesion lengths caused by *Neofusicoccum* spp. ranged from 3.3 to 28.3 cm on apple stems, from 8.3 to 15.9 cm on olive stems, and from 7.5 to 13.8 cm on grapevine cuttings. *Diplodia* spp. isolates produced necrotic lesions averaging from 1.3 to 12.8 cm on apple stems, from 1.9 to 4.7 cm on olive stems, and from 4.5 to 9.9 cm on grapevine cuttings. *Botryosphaeria* spp. isolates caused necrotic lesions averaging from 3.9 to 6.8 cm on apple stems, 3.4 to 8.1 cm on olive stems, and 3.8 to 8.1 cm on grapevine cuttings (Figure 3).

Asymptomatic colonization progression on detached hosts stems or cuttings

All the detached apple and olive stems and detached grapevine cuttings inoculated with the 12 *Botryosphaeriaceae* isolates developed necrotic lesions on the host wood. Inoculated fungi were consistently re-isolated from the advancing margins of lesions on all the host stems or cuttings, exhibiting the morphological characteristics of the inoculated isolates.

Asymptomatic colonization was detected in re-isolations from 10 cm beyond the visible lesion margins. The isolate V35 of *N. parvum* was re-isolated from 60% of inoculated olive stems and from 20% of inoculated apple stems and grapevine cuttings. Isolate M157 of *D. seriata* was re-isolated from 20% of inoculated olive stems. No isolates were recovered at 20 or 30 cm from the lesion margins, in any of the three plant hosts. Additionally, no *Botryosphaeriaceae* growth was observed from inoculation control stems or cuttings, either at the callused wound margins (0 cm) or at any of the three distances (10, 20 and 30 cm) from inoculations (Table 2).

DISCUSSION

In this study, *Botryosphaeriaceae* fungi capacities to cross-infect wood plant tissues beyond their original host species were assessed. Several isolates of *B. dothidea*, *B. wangensis*, *D. intermedia*, *D. mutila*, *D. pseudoseriata*, *D. seriata*, *N. australe*, *N. cryptoaustrale*, *N. luteum*, *N. occulatum*, and *N. parvum*, were evaluated for ability to infect and colonize lignified detached stems of apple and olive, and detached cuttings of grapevine, that were both the original and alternative fruit crop hosts for each isolate. The forty inoculated isolates caused necrotic lesions on detached stems or cuttings of the three hosts, providing evidence that host origin does not affect the ability of these pathogens to infect multiple hosts, and showing their capacities for cross-infection.

This knowledge poses significant challenges for fruit crop production in Uruguay, as *Botryosphaeriaceae* hosts are typically cultivated in close proximity, frequently on the same farm, facilitating the spread of these pathogens. While this study was focused on apple, olive, and grapevine, which are among the most economically important fruit crops in Uruguay (MGAP 2023), other co-cultivated fruit crops such as pear, peach, or blueberry are also known hosts of *Botryosphaeriaceae* (Sessa *et al*., 2016; Sessa *et al*., 2018), further highlighting the relevance of these results.

As in the present study, ability of *Botryosphaeriaceae* to infect multiple fruit hosts was previously demonstrated by Cloete *et al*., (2011) in South Africa and Amponsah *et al*., (2011) in New Zealand, who found that isolates of *Botryosphaericeae* from pear, apple, olive, broom, and pine trees could infect grapevine. Similarly, in Uruguay Sessa *et al*., (2016) provided evidence that isolates of *Diplodia*, *Lasiodiplodia* and *Neofusicoccum* isolated from apple, peach, or pear cross-infected these hosts.

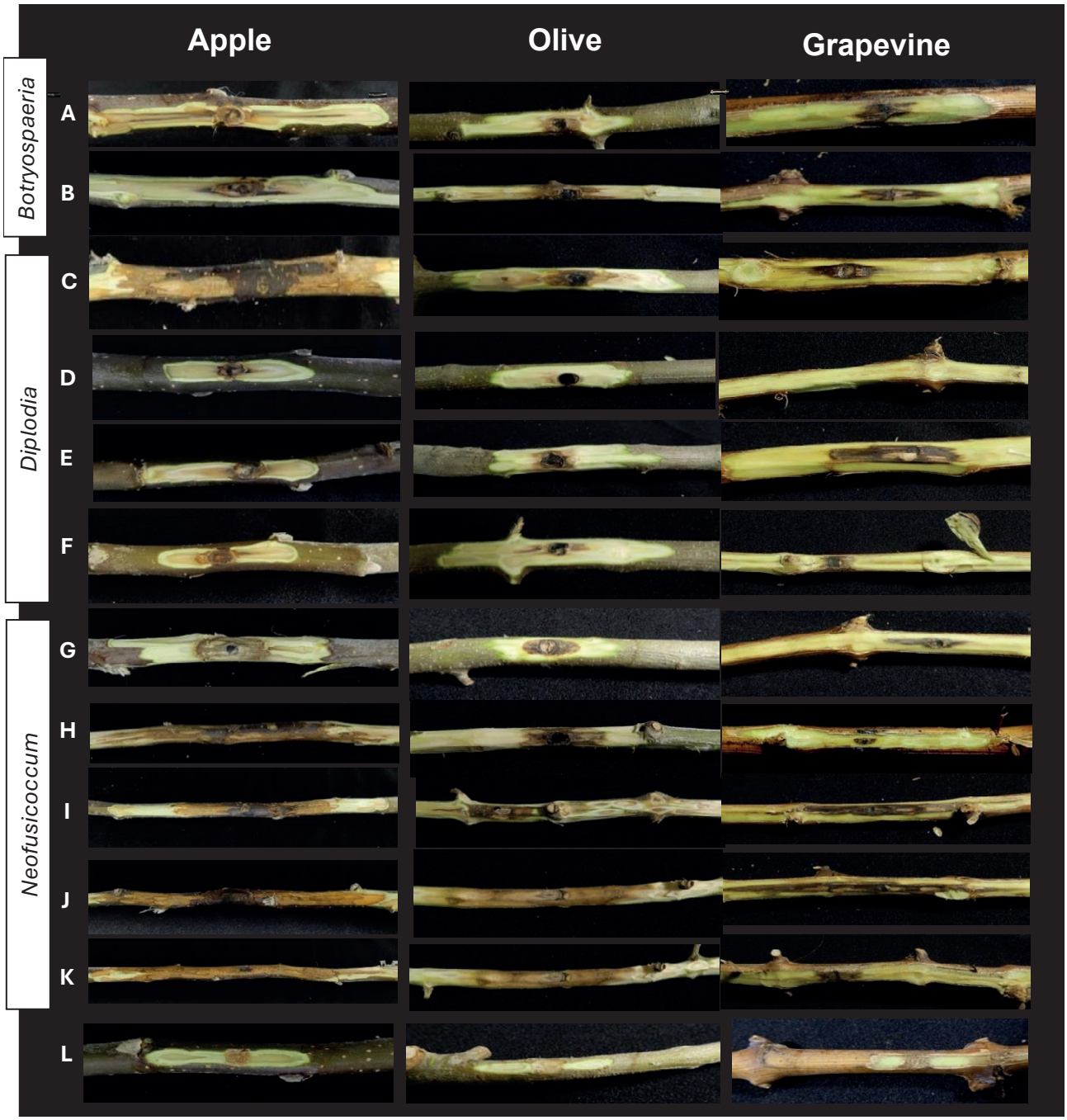

Figure 2. Necrotic lesions developed on detached lignified stems of apple and olive, or detached cuttings of grapevine, 5 weeks after inoculations with *Botryosphaeriaceae* isolates obtained from these three fruit crops. A) *Botryosphaeria dothidea* (B14); B) *B. wangensis* (O22); C) *Diplodia intermedia* (B5); D) *D. mutila* (O36); E*) D. pseudoseriata* (V14); F) *D. seriata* (O19); G) *Neofusicoccum australe* (B112); H) *N. cryptoaustrale* (O6); I) *N. luteum* (B107); J) *N. parvum* (V18); and K) *N. occulatum* (O12). L) Inoculation controls.

Mojeremane *et al*., (2020) in South Africa showed that isolates of *N. australe* and *N. stellenboschiana* obtained from grapevine, plum, apple, olive, Peruvian pepper, and fig were pathogenic on these hosts. In Chile, Díaz *et al*., (2022) and Hernández *et al*., (2025) confirmed cross-infection potential of isolates belonging to *Diplodia*, *Dothiorella*, *Lasiodiplodia*, and *Neofusicoccum*, after induction of necrotic lesions in pear, walnut, and grape-

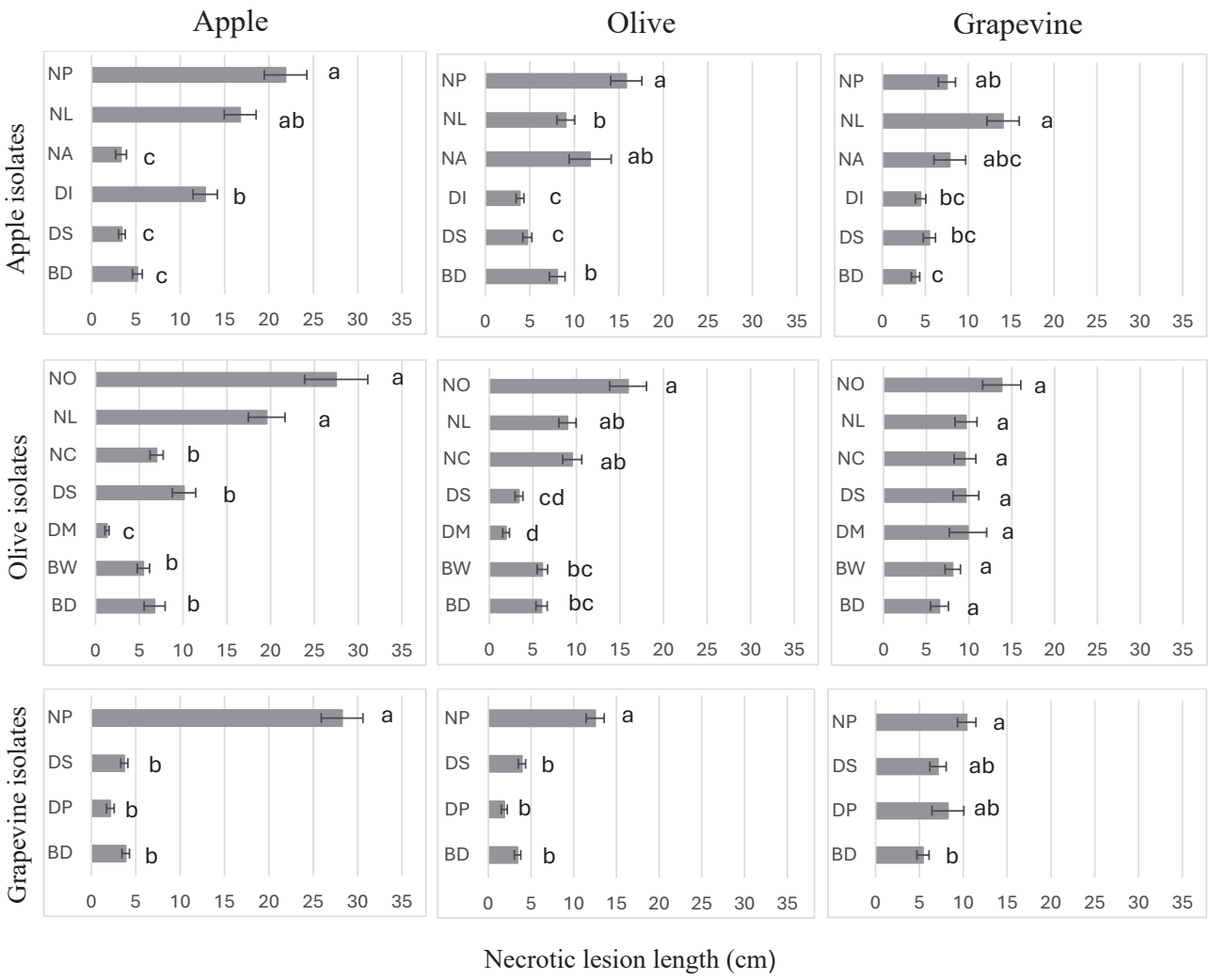

Figure 3. Mean necrotic lesion lengths measured in detached lignified stems of apple and olive, or detached dormant cuttings of grapevine, 5 weeks after direct and cross inoculations with 40 isolates of 11 species of *Botryosphaeriaceae* isolated from apple, olive, or grapevine crops. NP: *Neofusicoccum parvum*, NL: *N. luteum*, NA: *N. australe*, NO: *N. occulatum*, NC: *N. cryptoaustrale*, DS: *Diplodia seriata*, DI: *D. intermedia*, DM: *D. mutila*, DP: *D. pseudoseriata*, BD: *Botryosphaeria dothidea*, and BW: *B. wangensis*. Vertical bars indicate standard errors of the means. Values followed by different letters differ significantly ($P \geq 0.05$), according to the Tukey test.

vine using grapevine-derived isolates, and caused cankers in grapevine using isolates obtained from, respectively, apple, blueberry, or walnut. Some of these studies also examined hosts beyond fruit crops, including forestry, horticultural, and ornamental species, demonstrating that the host ranges and potential for cross-infection extend across a wide and diverse variety of woody plants. Collectively, these findings underscore the epidemiological complexity of this group of *Botryosphaeriaceae* pathogens (Silva-Valderrama *et al*., 2024).

In the present study, virulence of *Botryosphaeriaceae* species varied both among and within species. However, most *Neofusicoccum* isolates consistently caused the longest lesions in the three inoculated fruit hosts, confirming that their high virulence is independent of *Neofusicoccum* origin. These results are consistent with previous studies that have identified *Neofusicoccum* species as among the most virulent *Botryosphaeriaceae*, whether inoculated on their original hosts (Pérez *et al*., 2010; Úrbez-Torres *et al*., 2011; Delgado *et al*., 2016; Olmo *et al*., 2016, Antony *et al*., 2024) or on alternative hosts (Cloete *et al*, 2011; Amponsah *et al*., 2011; Sessa *et al*., 2016; Díaz *et al*., 2022; Hernández *et al*., 2025).

The virulence of *Neofusicoccum* species may be attributed to the expansion of gene families linked to virulence, specifically those encoding carbohydrate-

Table 2. Percentages of positive re-isolations from four distances from necrotic lesion margins for seven species of *Botryosphaeria*, *Diplodia* or *Neofusicoccum* inoculated into detached lignified stems of apple and olive, or detached dormant cuttings of grapevine. Each datum is the percentage for five host stems or cuttings.

Specie	Isolate	Percentage of re-isolation from different distances from lesion margins											
		Apple				Olive				Grapevine			
		0 cm	10cm	20cm	30cm	0cm	10cm	20cm	30cm	0cm	10cm	20cm	30cm
B. dothidea	M14	100	0	0	0	100	0	0	0	100	0	0	0
	V22	100	0	0	0	100	0	0	0	100	0	0	0
B. wangensis	O7	100	0	0	0	100	0	0	0	100	0	0	0
D. seriata	M157	100	0	0	0	100	20	0	0	100	0	0	0
	O14	100	0	0	0	100	0	0	0	100	0	0	0
	V1	100	0	0	0	100	0	0	0	100	0	0	0
N. cryptoaustale	O21	100	0	0	0	100	0	0	0	100	0	0	0
N. luteum	M129	100	0	0	0	100	0	0	0	100	0	0	0
	O10	100	0	0	0	100	0	0	0	100	0	0	0
N. occulatum	O12	100	0	0	0	100	0	0	0	100	0	0	0
N. parvum	M168	100	0	0	0	100	0	0	0	100	0	0	0
	V35	100	20	0	0	100	60	0	0	100	20	0	0
Control (non-inoculated)		0	0	0	0	0	0	0	0	0	0	0	0

active enzymes (CAZymes), peptidases, and components of secondary metabolism. These genetic features give fungi enhanced capacities to degrade lignified tissues and circumvent plant defense mechanisms (Morales-Cruz *et al*., 2015, Belleé *et al*., 2017). Supporting this, Belair *et al*. (2023) conducted a comparative genomic analysis across six *Botryosphaeriaceae* genera, and found that *Neofusicoccum,* particularly a specific isolate of *N. parvum,* exhibited enrichment in genes encoding CAZymes and peptidases, highlighting its superior pathogenic potential.

The present study also assessed the ability of *Botryosphaeriaceae* isolates to colonize asymptomatic woody tissues beyond the margins of visible lesions. One month after inoculation, inoculated isolates were absent at distances of 20 and 30 cm beyond visible necroses in all inoculated stems and cuttings of the three fruit hosts assessed. However, although only occasionally, the inoculated pathogens were re-isolated from up to 10 cm beyond the visible lesion edges in stems of apple and olive, and cuttings of grapevine. In apple, this latent colonization may explain, at least in part, the reactivation of disease that can be observed in commercial apple crops after pruning sanitization (Alaniz *et al*., 2012). This finding highlights the need to revise current pruning recommendations of removing up to 10 cm below visible lesion edges (Alaniz *et al*., 2012) to establish the minimum pruning lengths necessary for effective pathogen eradication. Additionally, the susceptibility of pruning wounds at different ages, and the effect of pruning timing on infection, should be included in studies to establish successful sanitation pruning recommendations (Valdez-Tenezaca *et al*., 2025).

One isolate of *N. parvum* was the only pathogen recovered from asymptomatic tissues across all three host species. This supports that particular isolates of *N. parvum* may have ability to colonize woody tissues beyond the visible lesions more rapidly than other members of this family. This may be through vascular pathways such as xylem vessels (Muniz *et al*., 2011; Han *et al*., 2016; Obrador-Sánchez and Hernández-Martínez 2020; Antony *et al*., 2024).

In summary, the present study has highlighted the challenges of managing diseases caused by *Botryosphaeriaceae* where multiple susceptible hosts are grown in close proximities, as is common in fruit production systems in Uruguay. In these situations, effective disease management should account for the potential for cross-infection among co-cultivated species, recognizing that each host can serve as an inoculum source for others. For example, the status of *Botryosphaeriaceae* cankers in neighbouring fruit orchards should be evaluated before establishing new plantings. Additionally, pruning distance required to ensure the complete elimination of these pathogens needs to be revised. Susceptibility of pruning wounds, effects of pruning time, and protection of pruning wounds should be evaluated under local conditions to prevent infections or re-infections by these

pathogens. Environmentally friendly treatment alternatives, such as fungicides approved for use in integrated management programs, biological control agents, or natural fungicidal compounds, should be considered in these investigations.

AUTHOR CONTRIBUTIONS

Laura Hernández was responsible for experimental assays, data analyses, and preparation of the draft manuscript. María Julia Carbone and Victoria Moreira assisted in experimental assays and contributed to the data analyses and manuscript review. Oscar Bentancur was responsible for experimental designs and statistical analyses of data. Pedro Mondino provided project conceptualization, and draft manuscript review. Sandra Alaniz provided project conceptualization, data analyses, preparation of the draft manuscript, and its review and editing. All authors approved the final version of the manuscript.

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