

Article

Field Responses of Almond Accessions to *Diaporthe*-Associated Biotic Stress in Southern Italy

Michele Casetta ¹, Lorenzo Manco ¹, Mekides Dugo Bati ² , Crescenza Dongiovanni ^{1,*} , Giuseppe Chiapperino ³, Rita Milvia De Miccolis Angelini ²  and Rita Leogrande ³ 

¹ Centre of Research, Experimentation and Training in Agriculture “Basile Caramia” (CRSFA), Via Cisternino 281, 70010 Locorotondo, Italy; michelecasetta96@gmail.com (M.C.); lorenzomanco@crsfa.it (L.M.)

² Department of Soil, Plant and Food Sciences, University of Bari “Aldo Moro”, 70126 Bari, Italy; yatatu2021@gmail.com (M.D.B.); ritamilvia.demiccolisangelini@uniba.it (R.M.D.M.A.)

³ Council for Agricultural Research and Economics, Research Centre for Agriculture and Environment (CREA-AA), Via C. Ulpiani 5, 70125 Bari, Italy; giuseppe.chiapperino@crea.gov.it (G.C.); rita.leogrande@crea.gov.it (R.L.)

* Correspondence: enza dongiovanni@crsfa.it

Abstract

Phenotypic response to twig canker and shoot blight varies substantially across almond germplasm. We hypothesized that genetically diverse almond accessions grown in a common orchard environment would exhibit genotype-dependent differences in symptom severity under natural disease pressure. Thirty-three almond accessions of different geographic origins were evaluated under field conditions in southern Italy. Disease incidence and severity were assessed, and symptomatic tissues were subjected to mycological and molecular analyses. Significant differences among accessions were detected for both disease incidence (I) and McKinney’s Disease Index (McK). ‘Merced’, ‘Nonpareil’, and ‘Genco’ exhibited high levels of disease incidence and severity, characterized by twig canker, shoot blight, and shoot clustering, with incidence values of 50% or higher, whereas local or less widely cultivated genotypes such as ‘Vesta’ and ‘Rana Gentile’ showed low disease incidence and severity. The *Diaporthe* complex represented the predominant fungal group isolated from symptomatic tissues, with *D. foeniculina* accounting for more than 80% of the identified *Diaporthe* isolates, whereas *D. rudis* was detected at lower frequencies. Other fungal taxa, particularly Botryosphaeriaceae, were recovered at lower frequencies. The observed variability supports the use of almond germplasm collections to identify genotypes with reduced symptom expression that may support breeding programs aimed at improving disease resilience.

Keywords: biotic stress; almond germplasm; agrobiodiversity; fungal pathogens; *Diaporthe foeniculina*



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1. Introduction

Almond (*Prunus dulcis* (Mill.) D.A. Webb; syn. *Prunus amygdalus* Batsch) is one of the most important tree nut crops worldwide, with annual global production exceeding 3.9 million tonnes. In Europe, Italy contributes substantially to almond production, with 74,960 tonnes produced mainly in the south; Sicily and Apulia together account for more than 95% of the national production [1,2]. Almond cultivation has ancient origins in the Mediterranean basin, where traditional seed propagation historically promoted the selection of numerous local ecotypes adapted to diverse pedoclimatic conditions [3]. This

locally adapted germplasm has represented an important reservoir of genetic diversity. However, the progressive replacement of traditional varieties with a limited number of high-yielding commercial cultivars has substantially reduced genetic diversity in cultivated almond populations [4,5].

This trend has significantly eroded the local gene pool, with losses reaching up to 75% in the Mediterranean region [5]. Such genetic erosion poses a concrete risk to conserving the valuable allelic diversity needed for future breeding programs. Therefore, the conservation of genetically diverse germplasm collections is essential for preserving potentially useful traits and supporting the development of improved cultivars adapted to changing environmental and production conditions [6].

Almond diseases represent a major constraint to orchard productivity and sustainability, particularly in regions where this crop plays a key economic role [7–10]. Several fungal diseases affect the aerial parts of almond trees and can compromise vegetative growth and yield [11,12]. In recent decades, increasing attention has been devoted to branch and shoot canker and dieback syndromes, which may cause progressive necrosis of woody tissues, shoot blight, canopy decline, and, in severe cases, reduce orchard longevity and productivity [13–16].

The etiology of branch and shoot cankers in almonds is complex and involves a diverse array of fungal taxa. Among these, species of the genus *Diaporthe* are of particular interest because they include plant pathogens, endophytes, and saprophytes widely distributed across a broad range of hosts [17,18].

In major almond-producing regions such as California, canker diseases have been primarily associated with Botryosphaeriaceae and *Ceratocystis*, with less frequent occurrences of genera such as *Cytospora*, *Collophorina*, *Diaporthe*, *Eutypa*, and *Pallidophorina* [10].

In Europe, particularly within the Mediterranean basin, symptoms such as twig canker, shoot dieback, and decline have increasingly been associated with an emerging disease complex of fungal taxa in which genus *Diaporthe* are frequently reported [14,18,19].

Among the Diaporthales, *Diaporthe amygdali* (first described as *Fusicoccum amygdali*) has been the most extensively studied species and is widely recognized as a major causal agent of twig canker and shoot blight of almond and peach [20]. Recent surveys in Portugal and southern Italy, particularly Sicily, have confirmed the prevalence of *D. amygdali* on almond [18,20]. Moreover, Gusella et al. [18] reported an increased prevalence of this species and suggested a possible expansion of its distribution into areas traditionally considered less affected, potentially linked to changing climatic conditions such as milder winter temperatures and increased humidity. Recent studies indicate that almond canker and shoot blight may involve a more complex assemblage of *Diaporthe* species than previously recognized. In addition to *D. amygdali*, species such as *D. foeniculina* and *D. eres* have increasingly been reported from symptomatic almond tissues [18–22]. Several *Diaporthe* species, such as *D. rudis* and *D. noetheicola*, are also documented as pathogens on other *Prunus* species, including peach, apricot, and cherry, as well as on a broader range of woody hosts [19,20,22,23].

The ongoing erosion of genetic diversity, together with the increasing relevance of emerging and re-emerging pathogens, particularly those associated with branch and shoot dieback, highlights the need to evaluate genotype-dependent responses of almond germplasm to biotic stress. However, information on genotype-dependent field responses of almond germplasm to twig canker and shoot blight under natural disease pressure remains limited, particularly for Mediterranean germplasm collections. Evaluating germplasm collections under common field conditions and natural inoculum pressure allows for robust comparison of genetically diverse materials, using disease incidence and severity as organism-level indicators of biotic stress response. The ex situ almond germplasm collec-

tions maintained at the experimental farms of the Council for Agricultural Research and Analysis of Agricultural Economics—Research Center for Agriculture and Environment (CREA-AA), located in Bitetto and Rutigliano, near Bari (Apulia, southern Italy), represent valuable genetic resources, including local varieties, cultivars from other Italian regions, foreign varieties, and breeding selections from diverse origins. The germplasm conservation program began in the late 1960s with the establishment of the Bitetto collection and expanded further in the early 2000s through the establishment of the Rutigliano collection. Together, these collections preserve a broad genetic base and provide a valuable resource for comparative studies of agronomic traits and disease responses. In the Rutigliano collection, symptoms consistent with infections by species within the order Diaporthales were observed, including shoot, flower, and leaf dieback; necrotic lesions developing into branch cankers; gum exudation; shoot clustering; and branch girdling followed by dieback of distal shoot portions.

Host genotype likely modulates symptom development under uniform orchard conditions and natural *Diaporthe*-associated disease pressure. Specifically, genetically diverse almond accessions were expected to exhibit distinct phenotypic stress responses, measurable as differences in disease incidence and severity. The present study aimed to (i) characterize the fungal taxa associated with shoot clustering, twig blight, and dieback symptoms observed under natural field conditions, and (ii) assess the field response of 33 almond accessions, including local, national, and international cultivars, as well as breeding selections. The goal was to identify germplasm with reduced symptom expression, which may represent promising material for further evaluation in breeding.

2. Results

2.1. Field Survey

The evaluated almond accessions exhibited considerable variability in disease incidence (I%) and McKinney's Disease Index (McK) values. Accessions differed significantly for both parameters (disease incidence: $F = 6.14$; $df = 32, 64$; $p < 0.0001$; $\eta^2 = 0.75$; McKinney's Disease Index: $F = 8.86$; $df = 32, 64$; $p < 0.0001$; $\eta^2 = 0.82$).

Disease incidence and severity were calculated from observations collected on all experimental trees according to the standardized assessment protocol (Table S1). The highest symptom expression was recorded in 'Merced', which showed the maximum severity class (7), with symptoms affecting 100% of examined twigs and an McK value of 100%. High incidence values were also observed in 'Nonpareil' (59.7%), 'Genco' (50.0%), 'SAS 1' (almond sweet seed $\times$ cv. 'Mollese') (50.0%), 'Pizzuta d'Avola' (49.3%), and 'SAS 23' (cv. 'Caputo' $\times$ apricot) (49.0%). In contrast, 'Vesta' and 'Rana Gentile' exhibited only sporadic symptoms, with approximately 2% of twigs infected. Low incidence values (<10%) were recorded in 'SAS 2' (cv 'Filippo Ceo' $\times$ cv. 'Rachele'), 'Fournat de Brezenaud', 'ISA 420' (SAS42 seed – SAS42 = cv. 'Filippo Ceo' $\times$ cv. 'Rachele'), 'Malaguena', and 'Nikitski' (Figure 1). A similar pattern was observed for the McKinney index. Following 'Merced' (100%), the highest values were found in 'Nonpareil' (56.0%), 'SAS 23' (39.7%) and 'Genco' (38.1%). In contrast, very low severity levels (<2%) were recorded in 'Malaguena', 'IXL', and 'Fournat de Brezenaud', with even lower values (<1%) in 'Vesta' and 'Rana Gentile' (Figure 2). Most of the remaining accessions showed intermediate responses for both incidence and McKinney's index, including 'Ferragnès' and 'Tuono', which are among the most widely cultivated almond cultivars worldwide.

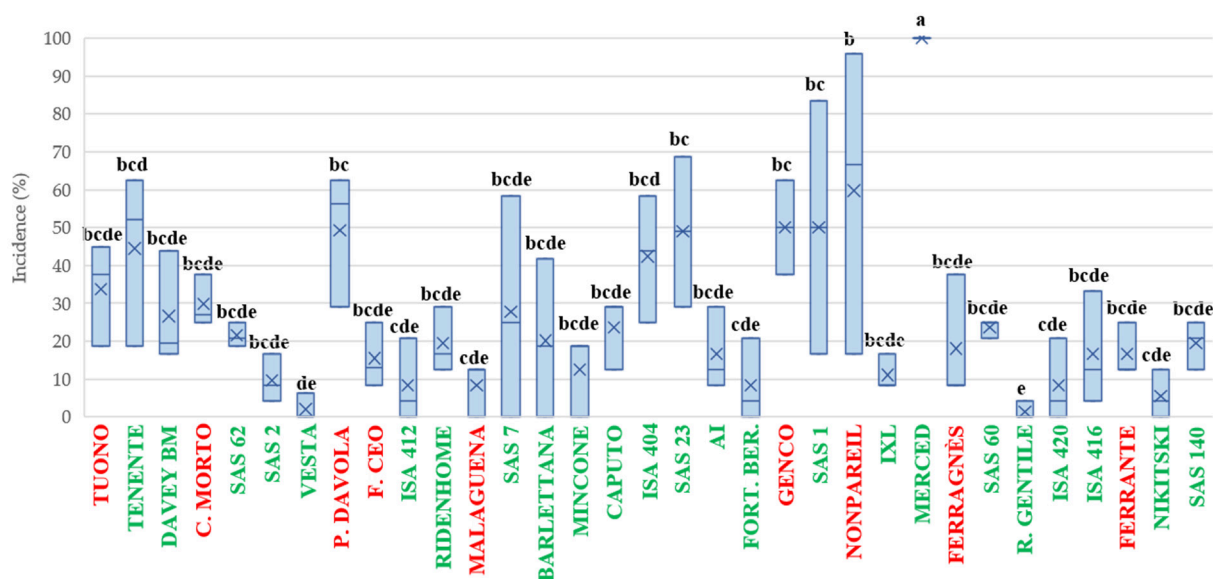


Figure 1. Disease incidence, expressed as the percentage of symptomatic twigs across the analyzed almond accessions. Boxplots show the median, interquartile range, and extreme values; X represents the mean. Different letters indicate statistically significant differences among accessions according to Tukey's post hoc test ($p < 0.05$). Accessions highlighted in red represent commercially widespread cultivars, while those in green represent local varieties, breeding selections, or genotypes with limited commercial cultivation in Southern Italy.

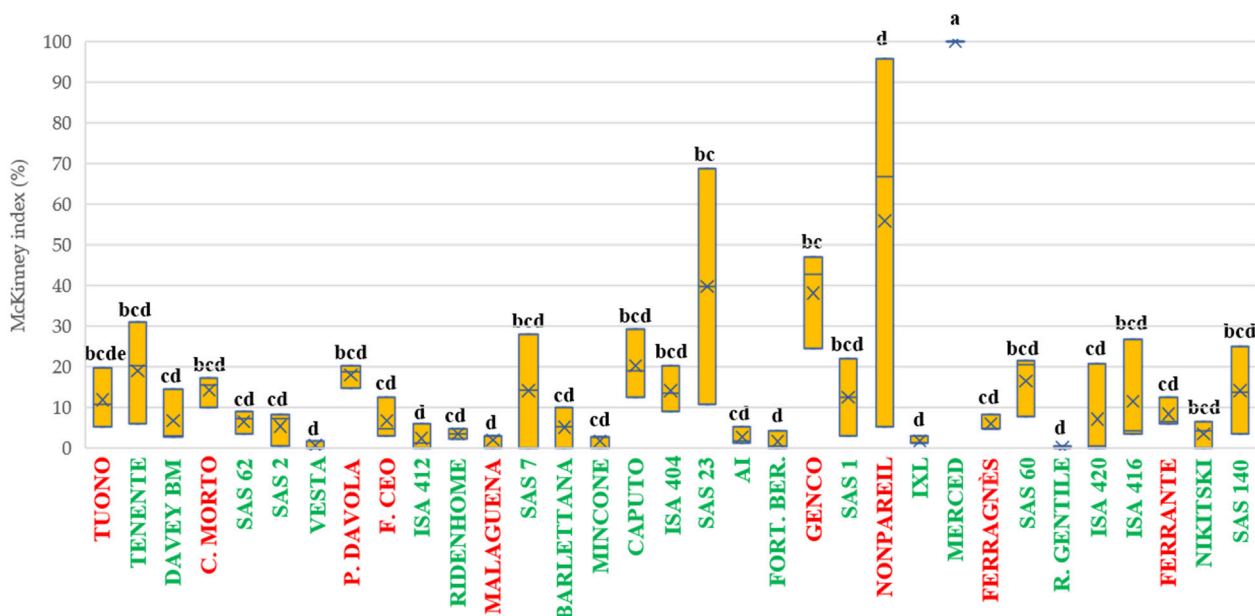


Figure 2. McKinney index values across the analyzed almond accessions. Boxplots show the median, interquartile range, and extreme values; X represents the mean. Different letters indicate statistically significant differences among accessions according to Tukey's post hoc test ($p < 0.05$). Accessions highlighted in red represent commercially widespread cultivars, while those in green represent local varieties, breeding selections, or genotypes with limited commercial cultivation in Southern Italy.

2.2. Field Sampling and Mycological Analysis

Fungal isolation from symptomatic almond tissues yielded 95 isolates assigned to *Diaporthe* spp., *Botryosphaeriaceae*, *Phoma* spp., *Cytospora* spp., and *Stigmina* spp. (Figure 3). *Diaporthe* was the predominant genus, with 56 isolates (58.9% of the total). Among the other fungal groups, *Botryosphaeriaceae* accounted for 22.1% of isolates, followed by

Phoma spp. ($\approx 10\%$), and *Cytospora* spp. ($\approx 8\%$), while *Stigmina* sp. was only sporadically recovered (2.1%).

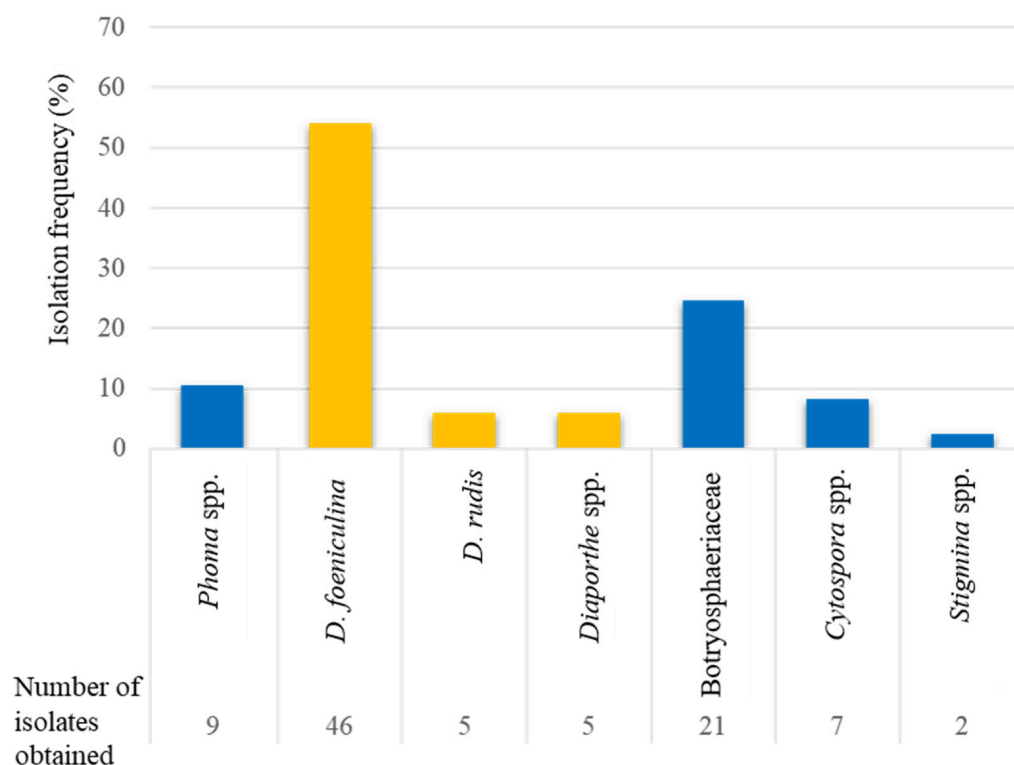


Figure 3. Isolation frequency (%) of fungal taxa recovered from symptomatic almond twigs collected in the germplasm collection field. Percentages were calculated relative to the total number of isolates ($n = 95$); numbers below the bars indicate absolute isolate counts. Yellow bars indicate *Diaporthe* isolates subjected to molecular characterization, whereas blue bars indicate non-*Diaporthe* fungi identified based on morphological characteristics only.

Isolates showing morphological characteristics consistent with the genus *Diaporthe* were subjected to molecular characterization. Detailed morphological observations were available for the predominant species *D. foeniculina*. Representative colonies grown on PDA were initially whitish, then turned light brown, with irregular margins, and produced abundant pycnidia during incubation (Figure 4). Molecular sequence analysis of the internal transcribed spacer (ITS) region and, for representative isolates, partial TEF1- α and β -tubulin sequences supported the identification of *D. foeniculina* and *D. rudis*. For the isolate assigned to *D. foeniculina*, BLAST comparisons showed 100% sequence identity over the aligned regions with *D. foeniculina* reference sequences PV187149 (ITS), MH925319 (TEF1- α), and MT006668 (β -tubulin). Similarly, isolates attributed to *D. rudis* showed 100% identity over the aligned regions with *D. rudis* reference sequences KF955763 (ITS), MZ423146 (TEF1- α), and KX856059 (β -tubulin). Isolates for which molecular analyses did not provide sufficient species-level resolution were conservatively retained as *Diaporthe* spp. Within the genus *Diaporthe*, *D. foeniculina* was the predominant species, accounting for 82.2% of the isolates, whereas *D. rudis* and unresolved *Diaporthe* spp. were detected at substantially lower frequencies (approximately 9%). The remaining fungal taxa were assigned solely on morphological characteristics, as molecular characterization was beyond the scope of this study.

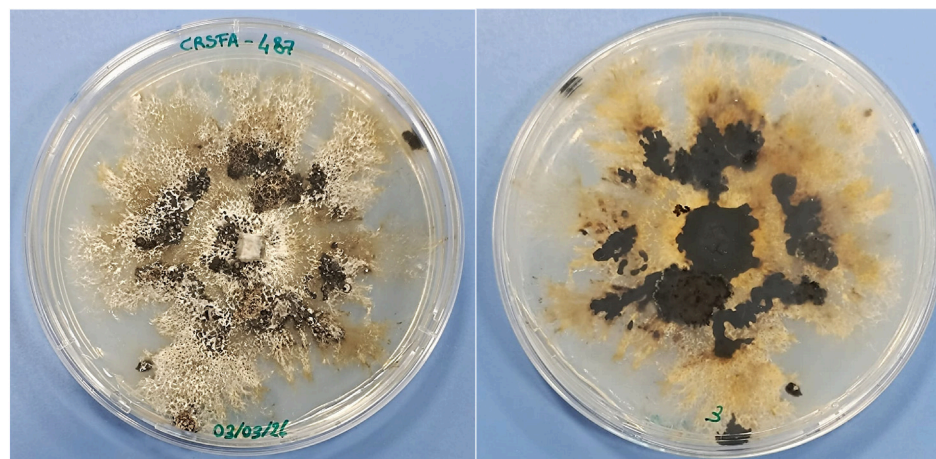


Figure 4. Representative morphological features of *Diaporthe foeniculina* isolate CRSFA. Colony morphology on PDA after 60 days of incubation, obverse (**left**) and reverse (**right**).

3. Discussion

The present study revealed marked genotype-dependent variation in symptom expression among 33 almond accessions exposed to a common orchard environment and natural disease pressure. Significant differences in both disease incidence and McKinney's index support the working hypothesis that host genotype contributes to almond's response to *Diaporthe*-associated biotic stress.

Cultivars such as 'Merced', 'Nonpareil', and 'Genco' exhibited the highest symptom expression, displaying pronounced twig canker and shoot dieback, typical symptoms reported in almond and other *Prunus* species affected by *Diaporthe* spp. infections [18,19]. Additionally, shoot clustering was frequently detected, particularly in 'Merced'. This phenomenon may result from bud and shoot necrosis leading to loss of apical dominance, followed by activation of lateral buds and proliferation of secondary shoots. Comparable witches' broom-like growth has been associated with infections by the *Diaporthe* complex in *Prunus* species [24,25].

Although 'Merced' exhibited the highest disease incidence and the most severe symptom expression among the examined cultivars, its limited worldwide cultivation reduces the immediate commercial relevance of this response. In contrast, the relatively high susceptibility observed in widely cultivated cultivars such as 'Nonpareil' and 'Genco', together with the intermediate response of 'Ferragnès' and 'Tuono', is more relevant to current almond production systems. The widespread reliance on a limited number of genetically uniform cultivars may reduce the range of host responses available within commercial orchards and increase the importance of identifying germplasm with consistently reduced symptom expression.

Another relevant finding concerns the response of local or less widely cultivated varieties. Accessions such as 'Rana Gentile', 'Fournat de Brezenaud', and 'Malaguena', exhibited lower disease incidence and severity under the conditions of the present study. 'Vesta', a hybrid with limited commercial distribution, showed a comparable response. These accessions therefore could represent promising material for future evaluation in breeding studies.

Marked variability in susceptibility to cankers caused by species within the order *Diaporthales*, historically referred to as genus *Fusicoccum* based on their asexual morphs, has been well documented since the 1970s and 1980s [26,27]. More recently, substantial variability among almond cultivars has also been reported under both field conditions and artificial inoculations [28,29]. However, these findings are not always consistent with the present study, which underscores the complexity of host–pathogen interactions. For exam-

ple, Vargas and Miarnau [28], in a field evaluation of a wide panel of cultivars, identified ‘Genco’ as highly tolerant and ‘Ferragnès’ as highly susceptible, whereas the present study recorded high symptom expression in ‘Genco’ and an intermediate response in ‘Ferragnès’. In both studies, ‘Rana Gentile’ showed a consistently low symptom expression. Similarly, Lucchese et al. [29], working under controlled artificial inoculation conditions, reported a wide range of responses in some French cultivars, with high resistance in ‘Ferrastar’ and ‘Ardéchoise’, while ‘Ferragnès’ and ‘Tuono’ were classified as highly susceptible; in the present field study, these cultivars exhibited an intermediate response. Such discrepancies likely reflect the complex nature of host–pathogen interactions, which may be influenced by differences in experimental conditions, pathogen composition, host genetic background, plant age, phenological stage, environmental conditions, and agronomic management [14,17,22,24,30–33]. In particular, several studies have reported that variation in phenological traits, such as flowering and fruit maturation timing, may modify the period of host exposure to favorable infection conditions and thereby contribute to differences in symptom development among cultivars [13,18,19,34].

The frequent recovery of *Diaporthe* spp. from almond canker, bud necrosis, and shoot clustering indicates a strong association between this genus and the observed disease complex, whereas the less frequently isolated fungal taxa represent minor components of the fungal assemblage associated with symptomatic tissues.

Molecular identification was performed only for isolates morphologically assigned to the genus *Diaporthe*, whereas the remaining fungal taxa (Botryosphaeriaceae, *Phoma* spp., *Cytospora* spp., and *Stigmina* sp.) were identified exclusively on the basis of morphological characteristics. Therefore, no species-level or causal inference should be drawn for these additional groups. This interpretation is consistent with recent studies recognizing *Diaporthe* species as established pathogens in Mediterranean almond orchards [18,19]. In contrast, Botryosphaeriaceae, *Cytospora* spp., and *Phoma* spp. were recovered at lower frequencies in the present survey, suggesting that they may play a more limited role in twig canker development, consistent with findings from other pathosystems and regions [10,13,14,35,36].

Diaporthe foeniculina was the predominant taxon among the *Diaporthe* isolates, with 46 of 56 isolates (82.2%) assigned to this species, whereas *D. rudis* was detected at a much lower frequency. A small proportion of isolates could not be reliably resolved at species level and were therefore conservatively retained as *Diaporthe* spp. The frequent occurrence of *D. foeniculina* in this study corroborates recent investigations in Spain that identified this species as part of the *Diaporthe* complex associated with almond canker [19]. *D. rudis*, although reported mainly from woody hosts, including *Quercus* spp. [37], has also recently been identified in symptomatic almond tissues in California as part of a diverse *Diaporthe* complex [38]. In the same study, *D. rudis* produced smaller lesions on almond twigs than *D. amygdali*, indicating comparatively lower aggressiveness on woody tissues. This finding may align with the low frequency of *D. rudis* observed in the present study, although this relationship requires further investigation. *D. amygdali* historically regarded as a major causal agent of almond canker in Mediterranean environments [14,18,19], was not identified among the *Diaporthe* isolates characterized in the present survey. Other *Diaporthe* species, including *D. eres*, *D. phaseolorum* and *D. mediterranea*, have also been reported from symptomatic almond tissues [19,21], highlighting the diversity of *Diaporthe* species associated with almond canker and shoot blight. Variation in the composition of *Diaporthe* communities has also been observed in other *Prunus* pathosystems; studies conducted in China found *D. eres* and related species to predominate in peach twig cankers, with *D. amygdali* absent or only rarely detected [24,39]. Similarly, studies from Japan reported *D. eres*, *D. ambigua*, and *D. ampelina*, with no relevant detection of *D. amygdali* [40]. Overall,

these findings indicate that the species assemblage associated with canker and shoot blight can vary geographically and further support the hypothesis of a complex disease etiology.

Moreover, because molecular characterization focused on *Diaporthe* isolates, future studies should include multilocus identification of the entire fungal community to better describe the diversity and potential role of the additional taxa associated with almond twig canker and shoot blight.

The observed cultivar-specific responses support the use of germplasm collections to identify genotypes with reduced severity symptoms. In this context, ancient and rare almond germplasm serves as a valuable source of agronomically important traits and genes that may help address climate change and facilitate adaptation to evolving environmental conditions. Because field response to woody pathogens may be influenced by genotype-by-environment interactions, future multi-year, multi-location studies are needed to evaluate the stability of the observed differences in symptom expression.

4. Materials and Methods

4.1. Site and Experimental Design

This study considered 33 accessions, including local (autochthonous), Italian, and foreign cultivars, as well as newly developed selections, from the ex situ almond collection of CREA-AA (Table 1). The collection is located at the experimental farm “M.E. Venezian Scarascia” in Rutigliano, near Bari, southern Italy (40°59′35.9″ N, 17°02′06.7″ E, 147 m a.s.l.).

Table 1. Main agronomic and genetic characteristics of the almond accessions evaluated in this study.

Number	Accessions [†]	Origin/Source	Parents	Flowering Period	Flowering Duration	Average Kernel Yield Per Tree
				DOY	d	kg tree ^{−1}
1	Barlettana	Italia	N.A.	37–51	14	>4
2	Caputo	Italia	N.A.	43–57	14	2–3
3	Cristomorto	Italia	N.A.	42–56	14	2–3
4	Ferrante	Italia	N.A.	38–51	13	2–3
5	Filippo Ceo	Italia	N.A.	37–51	14	>4
6	Genco	Italia	N.A.	44–57	13	1–2
7	Mincone	Italia	N.A.	34–47	13	2–3
8	Pidocchioso	Italia	N.A.	18–31	13	3–4
9	Pizzuta d’Avola	Italia	N.A.	21–36	15	3–4
10	Rana Gentile	Italia	N.A.	49–62	13	3–4
11	Tenente	Italia	N.A.	42–57	15	2–3
12	Tuono	Italia	N.A.	44–57	13	3–4
13	Ai	Francia	N.A.	43–57	14	1–2
14	Ferragnès	Francia	Ai × Cristomorto	44–57	13	1–2
15	Fournat de Brezenaud	Francia	N.A.	25–38	13	<1
16	Malaguena	Spagna	N.A.	15–30	15	<1
17	DaveyBM	USA	N.A.	44–57	13	<1
18	IXL	USA	N.A.	42–54	12	1–2
19	Merced	USA	N.A.	44–57	13	1–2
20	Nonpareil	USA	N.A.	40–51	11	2–3
21	Ridenhome	USA	N.A.	39–51	12	1–2
22	Vesta	USA	N.A.	45–57	12	<1
23	Nikitski	Russia	N.A.	45–57	12	2–3

Table 1. *Cont.*

Number	Accessions [†]	Origin/Source	Parents	Flowering Period	Flowering Duration	Average Kernel Yield Per Tree
				DOY	d	kg tree ^{−1}
24	SAS 1	Breeding program	Sweet seed × Mollese	15–31	16	1–2
25	SAS 2	Breeding program	Filippo Ceo × Rachele	38–51	13	2–3
26	SAS 7	Breeding program	Unknown × Catalini	43–57	14	2–3
27	SAS 23	Breeding program	Caputo × Apricot	43–57	14	3–4
28	SAS 60	Breeding program	Mollese × Filippo Ceo	37–51	14	2–3
29	SAS 62	Breeding program	SAS21 ^a × Mollese tonda	41–57	16	1–2
30	SAS 140	Breeding program	Filippo Ceo × Mollese tonda	43–57	14	1–2
31	ISA 404	Breeding program	(Filippo Ceo × Albanese) × Centopezze	36–51	15	<1
32	ISA 416	Breeding program	Zanzanidde open-pollinated	32–45	13	<1
33	ISA 420	Breeding program	(Filippo Ceo × Rachele) open-pollinated	44–57	13	3–4

[†] The rootstock used in the whole field was GF 677; ^a SAS21, agronomic selection with unknown parentage.

The soil at the site is classified as clay under the USDA soil classification, with high total organic carbon (TOC, 20.78 g kg^{−1}) and total nitrogen (1.8 g kg^{−1}) [41]. The site has a shallow arable layer, a relatively homogeneous soil profile, and calcareous parent rock within the upper horizons at an average depth of approximately 0.60 m [42].

The area's climate is classified as accentuated thermo-Mediterranean according to the UNESCO-FAO classification [43]. Winter rainfall predominates, with certain months showing precipitation that exceeds evapotranspiration. In contrast, the summer months are warm and dry, with pronounced moisture deficits that lead to soil drying and seasonal vegetation stress, consistent with a xeric moisture regime [44].

The almond germplasm collection was established in 2008 and covers approximately 2.0 ha. The experimental design followed a randomized complete block structure with three replicates, and each accession was represented by three trees per replicate (three replicates × three trees = nine trees per accession). The experimental unit was the accession within each block, represented by three trees; individual trees were considered subsamples within each experimental unit. Trees were planted at 6.0 m × 5.0 m spacing and managed according to standard local agronomic practices. A schematic representation of the experimental field and block layout is provided in Supplementary Figure S1.

All accessions were exposed to the same natural inoculum pressure and environmental conditions throughout the study period. This experimental layout minimized the influence of environmental heterogeneity and allowed direct comparison of symptom expression among accessions.

4.2. Field Survey

A single-field survey was conducted in spring 2025 (20 May 2025), during the period of maximum symptom expression. This timing allowed all accessions to be evaluated under the same phenological and environmental conditions. At this stage, the observed symptoms were considered suitable for comparing disease incidence and severity among accessions; therefore, no additional field assessments were included in the experimental design. All almond trees included in the germplasm collection field were subjected to visual assessment to determine the presence and severity of symptoms on twigs and buds, consisting of canker, necrotic lesions affecting nodes and buds, as well as shoot clustering. For each tree, eight current-season twigs (approximately 50 cm in length) were randomly selected from the terminal portions of the branches and distributed throughout the canopy, according to the main cardinal directions and including upper and lower portions, to ensure a representative evaluation of the overall phytosanitary status of each plant. Overall, 72 twigs were examined per each accession (9 trees $\times$ 8 twigs), corresponding to 2376 twigs across the 33 accessions. For statistical analysis, data from the three trees within each block (24 twigs) were combined to calculate disease incidence and McKinney Disease Index, yielding three replicate values per accession.

A tree was considered symptomatic when at least one of the eight assessed twigs showed symptoms. Disease incidence (I), however, was calculated at the twig level as the percentage of symptomatic twigs out of the total number of twigs assessed. For statistical analysis, incidence and McKinney Disease Index (McK) were calculated for each accession within each block using the 24 twigs assessed from the three trees.

The scale consisted of eight severity classes:

- Class 0 = no symptoms (healthy twigs);
- Class 1 = 1–5% of twig surface affected;
- Class 2 = 6–10%;
- Class 3 = 11–15%;
- Class 4 = 16–25%;
- Class 5 = 26–50%;
- Class 6 = 51–75%;
- Class 7 = 76–100% of the twig surface affected and/or the presence of extensive shoot clustering along the twig.

The collected data were used to calculate the following parameters:

- Incidence (I), expressed as the percentage of symptomatic twigs out of the total observed, calculated as:

$$I = \left(\frac{n}{N} \right) \cdot 100$$

- Disease severity, expressed as McKinney's Index (McK), calculated as the weighted mean disease severity, according to the following formula:

$$\text{McK} = \left[\frac{\sum(f \cdot v)}{(N \cdot x)} \right] \cdot 100$$

where n is the number of symptomatic twigs, N is the total number of twigs examined, f is the frequency of cases in each severity class, v is the class value, and x is the maximum value of the severity scale. Disease severity was evaluated using McKinney's Index because it integrates both symptom frequency and severity, providing a more comprehensive estimate of disease intensity than Incidence alone.

4.3. Field Sampling and Mycological Analysis

In May 2025, almond twigs exhibiting symptoms of canker, dieback, shoot clustering, and shoot blight (Figure 5a–e) were collected from 70 different symptomatic trees through random sampling, without following a systematic accession-based scheme. The samples were labeled and transported to the phytopathology laboratory of the CRSFA “Basile Caramia” in Locorotondo (BA), Italy.



Figure 5. Representative disease symptoms recorded on almond trees under natural field conditions. (a) General view of an affected tree showing canopy dieback, sparse vegetation, partial defoliation and shoot clustering; (b) detail of clustered shoots with apical twig dieback, partial defoliation and leaf yellowing; (c) wilted and desiccated leaves remaining attached to the twig; (d) shoot blight symptoms on clustered shoots; (e) completely desiccated shoot within a cluster, with visible twig cankers.

All samples were processed within 24 h after collection. Wood fragments (approximately 5×5 mm) were excised from the margins of symptomatic tissues, including the interface between healthy and necrotic areas. Fragments were surface-disinfected by immersion in 5% sodium hypochlorite for 60 s, followed by 70% ethanol for 60 s and rinsed twice in sterile distilled water. After drying on sterile filter paper under a laminar flow hood, fragments were placed onto potato dextrose agar (PDA, infusion of 200 g of peeled and sliced potatoes kept at 60 °C for 1 h, 20 g of D-(+)-glucose, adjusted to pH 6.5, and 20 g agar per liter) in 90 mm Petri dishes. Plates were incubated at 24 ± 1 °C in the dark until fungal colonies developed. Emerging colonies were sub-cultured onto fresh PDA plates to obtain pure cultures. Cultures were periodically examined for the development of reproductive structures, including ascomata and conidiomata. Isolates were preliminarily characterized based on morphological features, such as colony morphology, color, texture, growth pattern, and microscopic features, including conidial characteristics, according to standard mycological keys and taxonomic descriptions [45,46].

Molecular identification was subsequently performed on isolates morphologically attributable to the genus *Diaporthe*. Genomic DNA was extracted as described by De Miccolis Angelini et al. [47]. The internal transcribed spacer (ITS) region was amplified by polymerase chain reaction (PCR) using the primers ITS1 and ITS4 [48]. Representative isolates were selected from the main morphotypes identified among the *Diaporthe* cultures for sequencing of partial fragments of the translation elongation factor 1- α

(TEF1- α) and β -tubulin (TUB) genes, using EF1-728F/EF2 primers [49] and Bt2a/Bt2b primers [50], respectively.

PCR products were sequenced by Sanger sequencing, and the resulting sequences were compared to reference sequences in the GenBank database using the web-based NCBI BLAST service. Species-level assignments were accepted only when morphological characteristics were consistent with the genus *Diaporthe* and sequence comparisons showed high identity with reference sequences of the same species across the available loci. Accordingly, isolates were assigned at the species level only when morphological and molecular evidence provided sufficient taxonomic resolution; otherwise, they were conservatively retained as *Diaporthe* spp. The isolation frequency for each taxon was calculated as the percentage of isolates assigned to each taxonomic group relative to the total number of isolates obtained.

4.4. Data Analysis

Disease incidence and McKinney's Disease Index data were subjected to analysis of variance according to the randomized complete block design (RCBD). The experimental unit was the accession within each block, represented by three trees, and the three block level values were used as replicates in the analysis. Accession was included as the treatment factor and block as the blocking factor. The magnitude of the accession effect was estimated using partial eta squared (η^2).

Before analysis, percentage data were checked for homogeneity of variance and, when required, transformed into angular values according to Bliss [51]. When the ANOVA F-test was significant, mean comparisons among accessions were performed using Tukey's test [52]. All statistical analyses were conducted using CoStat ver. 6.204 software.

5. Conclusions

This study showed that almond germplasm exposed to a shared natural disease environment can display markedly different phenotypic responses to *Diaporthe*-associated biotic stress. Mycological analyses showed that symptomatic tissues were predominantly associated with species of the genus *Diaporthe*, with *D. foeniculina* representing the most frequently identified species among the molecularly characterized isolates. Other fungal taxa, identified based on morphological characteristics, were recovered at lower frequencies, supporting the view that *Diaporthe* spp. were the predominant fungi associated with the observed disease symptoms under the conditions of the present study.

Significant genotype-dependent variation was observed among cultivars. Widely cultivated varieties such as 'Nonpareil' and 'Genco' exhibited relatively high symptom expression, whereas local or less commonly grown genotypes, including 'Rana Gentile', 'Fournat de Brezenaud', 'Malaguena', and 'Vesta', showed reduced symptom expression under the environmental conditions of the present study. These findings highlight the importance of preserving almond biodiversity and genetic resources to safeguard potentially useful alleles that may have been lost from modern commercial cultivars through genetic erosion. Accessions with reduced symptom expression may represent promising material for future evaluation in breeding studies. Furthermore, the results suggest that cultivar choice may represent a useful component of disease management strategies in newly established orchards. Combining field phenotyping with mycological and molecular analyses provides a useful framework for identifying differential host responses under natural biotic pressure. Further research involving pathogenicity tests, multilocus characterization, and long-term field evaluations is needed to clarify the ecological roles and aggressiveness of the different *Diaporthe* species involved.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/stresses6040079/s1>, Figure S1: Aerial image of the experimental site and layout of the randomized block design adopted for the almond germplasm collection. Different colours (black, blue and orange) indicate the three blocks of the design, adopted in the experimental field; Table S1: Plant-level phytopathological assessments carried out on almond cultivars/accessions. For each plant, the table reports the plant identification number, experimental block, number of symptomatic twigs out of 8 assessed, and mean disease severity score, calculated on a 0–7 scale. Mean severity values include asymptomatic twigs/plants, which are scored as 0; therefore, low mean values may reflect both reduced symptom occurrence and lower symptom severity. ND indicates not determined.

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