

Review



Secondary Metabolites Produced by *Neofusicoccum* Species Associated with Plants: A Review

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Abstract: The genus *Neofusicoccum* is comprised of approximately 50 species with a worldwide distribution and is typically associated with plants. *Neofusicoccum* is well-known for the diseases it causes on economically and ecologically relevant host plants. In particular, members of this genus are responsible for grapevine diseases, such as leaf spots, fruit rots, shoot dieback, bud necrosis, vascular discoloration of the wood, and perennial cankers. Many secondary metabolites, including (–)-botryoisocoumarin A, botryosphaerones, cyclobotryoxide and isosclerone, were identified from species of *Neofusicoccum* and their structural variability and bioactivities might be associated with the role of these compounds in the fungal pathogenicity and virulence. In this review, we summarize the secondary metabolites from *Neofusicoccum* species focusing on the role of these compounds in the interaction between the fungus and host plant.



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). **Keywords:** *Botryosphaeria; Botryosphaeriaceae;* phytopathogens; plant–fungus interaction; Botryosphaeria dieback

1. Introduction

The genus *Neofusicoccum* (Dothideomycetes, Botryosphaeriales, *Botryosphaeriaceae*) was introduced in 2006 to reassign species that were previously included in the genus *Fusicoccum* and its sexual morph *Botryosphaeria* [1]. Initially, the genus included 13 species but it has grown considerably, due mainly to the description of a large number of cryptic species, and currently contains about 50 distinct species listed in MycoBank [2] and Index Fungorum [3].

Species in the genus *Neofusicoccum* are typically associated with plants, being common in a wide range of woody hosts, from forest trees to crops and ornamentals [4,5]. The majority of these species have a worldwide distribution, a plurivorous nature, and exhibit an endophytic or latent pathogen lifestyle. Some *Neofusicoccum* species are plant pathogens of increasing relevance, causing diseases, most commonly dieback and canker, on economically and ecologically relevant host plants, such as grapevine, olive, eucalypts, among many others [4,5]. Grapevine is a particularly relevant crop worldwide, and several species of *Neofusicoccum* have been reported to be pathogens of this host. In fact, *N. parvum* is regarded as one of the most aggressive causal agents of Botryosphaeria dieback of grapevine [6].

In the last decade, literature concerning metabolites produced by *Neofusicoccum* species has been significantly enriched by many reports dealing with the biosynthetic potential of this fungal genus.

This review is intended as a reference guide to secondary metabolites produced by *Neofusicoccum* species, emphasizing their role in the fungal association with plants. The main part of this paper comprises over 90 compounds identified as products of *Neofusicoccum* species and collected in 10 classes on the basis of their chemical structures. Furthermore, some aspects relating to the biosynthesis of these metabolites and related compounds, as well as their bioactivities, are also discussed in dedicated subparagraphs. A final section includes information on the relevance of phytotoxic secondary metabolites produced by *Neofusicoccum* species associated to grapevine trunk diseases.

2. Secondary Metabolites

Fungi from the genus *Neofusicoccum* are a promising source of secondary metabolites which can be classified according to their chemical structures. As can be seen from Table 1, compounds produced by *Neofusicoccum* species belong to different classes of natural products, including 5,6-dihydro-2-pyrones, melleins, myrtucommulones and naphthalenones, which have peculiar biological and chemical features.

Code	Compound	Formula	Nominal Mass (U)			
Cyclohexenones						
1	Cyclobotryoxide	C ₈ H ₁₀ O ₄	170			
2	(–)-Terremutin	C ₇ H ₈ O ₄	156			
3	(+)-Terremutin hydrate	$C_7 H_{10} O_5$	174			
4	(–)-4-Chloro-terremutin hydrate	C ₇ H ₉ ClO ₄	192			
5	(+)-4-Hydroxysuccinate-terremutin hydrate	$C_{11}H_{14}O_8$	274			
6	(+)-epi-Sphaeropsidone	$C_7H_8O_4$	156			
	5,6-Dihydro-2-pyro	nes				
7	Asperlin	C ₁₀ H ₁₂ O ₅	212			
8	(6R,7S)-Dia-asperlin	$C_{10}H_{12}O_5$	212			
9	Luteopyroxin	$C_9H_{12}O_4$	184			
	Fatty acids					
10	Elaidic acid	C ₁₈ H ₃₄ O ₂	282			
11	Ethyl elaidate	$C_{20}H_{38}O_2$	310			
12	Linoleic acid	$C_{18}H_{32}O_2$	280			
13	Ethyl linoleate	$C_{20}H_{36}O_2$	308			
14	Methyl linoleate	$C_{19}H_{34}O_2$	294			
15	9,12,15-Octadecatrienoate ethyl ester	$C_{20}H_{34}O_2$	306			
16	Ethyl oleate	$C_{20}H_{38}O_2$	310			
17	Methyl oleate	$C_{19}H_{36}O_2$	296			
18	Palmitic acid	$C_{16}H_{32}O_2$	256			
19	Methyl palmitate	$C_{17}H_{34}O_2$	270			
20	Palmitoleic acid	$C_{16}H_{30}O_2$	254			
21	Stearic acid	$C_{18}H_{36}O_2$	284			
22	Ethyl stearate	$C_{20}H_{40}O_2$	312			
23	Undecan-2-one	C ₁₁ H ₂₂ O	170			
Melleins						
24	(R)- $(-)$ -Mellein	$C_{10}H_{10}O_3$	178			
25	(R)-(–)-3-Hydroxymellein	$C_{10}H_{10}O_4$	194			
26	(3 <i>R</i> ,4 <i>R</i>)-(–)-4-Hydroxymellein	$C_{10}H_{10}O_4$	194			
27	(3 <i>R,</i> 4 <i>S</i>)-(–)-4-Hydroxymellein	$C_{10}H_{10}O_4$	194			
28	(3R)-5-Hydroxymellein	$C_{10}H_{10}O_4$	194			
29	7-Hydroxymellein	$C_{10}H_{10}O_4$	194			
30	(\pm)-Botryoisocoumarin A	$C_{11}H_{12}O_4$	208			
31	(+)-Neoisocoumarin	$C_{10}H_{10}O_5$	210			

Table 1. List of secondary metabolites produced by *Neofusicoccum* species.

 Table 1. Cont.

Code	Compound	Formula	Nominal Mass (U)				
	Myrtucommulones						
32	Myrtucommulone A	C ₃₈ H ₅₂ O ₁₀	668				
33	Myrtocommulone B	$C_{24}H_{30}O_6$	414				
34	Myrtucommulone D	C38H50O9	650				
	Naphthalenone	s					
35	Botryosphaerone A	C13H16O6	268				
36	Botryosphaerone B	$C_{13}H_{16}O_{6}$	268				
37	Botryosphaerone C	C ₁₄ H ₁₈ O ₆	282				
38	Botryosphaerone D	$C_{13}H_{16}O_5$	252				
39	Isosclerone	$C_{11}H_{12}O_2$	176				
40	O-Methylasparvenone	C13H16O4	236				
41	O-Methylaspmenone	$C_{10}H_{10}O_3$	178				
42	(3R,4R)-3-Methoxyl-botryosphaerone D	$C_{14}H_{18}O_5$	266				
43	3,4,5-Trihydroxy-1-tetralone	$C_{10}H_{10}O_4$	194				
44	(35,45)-3,4,8-Trihydroxy-6-methoxy-3,4- dihydro-1(2H)-naphthalenone	$C_{11}H_{12}O_5$	224				
	Naphthoquinon	es					
45	Botryosphaenin	C ₁₅ H ₁₆ O ₆	292				
46	Neofusnaphthoquinone A	C ₂₇ H ₂₄ O ₁₀	508				
47	6-Ethyl-2,7-dimethoxyyjuglone	$C_{14}H_{14}O_5$	262				
48	6-(1-Hydroxyethyl)-2,7-Dimethoxyjuglone	$C_{14}H_{14}O_{6}$	278				
49	6-(1-Methoxylethyl)-2,7-dimethoxyjuglone	$C_{15}H_{16}O_{6}$	292				
50	6-(1-Hydroxyethyl)-2,7-dimethoxyjuglone	C ₁₆ H ₁₆ O ₇	320				
	5 Hudrovy 27 dimethovynaphthalono 14						
51	dione	$C_{12}H_{10}O_5$	234				
	Phenols and alcoh	ols					
52	2-Ethyldecan-1-ol	$C_{12}H_{26}O$	186				
53	2-Hydroxypropyl salicylic acid	$C_{10}H_{12}O_4$	196				
54	2-Methylbutan-1-ol	$C_5H_{12}O$	88				
55	3-Methylcatechol	$C_7H_8O_2$	124				
56	6-Methyl-salicylic acid	$C_8H_8O_3$	152				
57	Isobutanol	$C_4H_{10}O$	74				
58	Isopentyl alcohol	$C_5H_{12}O$	88				
59	<i>p</i> -Cresol	C_7H_8O	108				
60	Phenylethyl alcohol	$C_{8}H_{10}O$	122				
61	Tyrosof	$C_8 H_{10} O_2$	138				
Sesquiterpenes							
62	Aristolene	$C_{15}H_{24}$	204				
63	Aristolochene	$C_{15}H_{24}$	204				
64	δ-Amorphene	C ₁₅ H ₂₄	204				
65	Botryosterpene	$C_{16}H_{22}O_3$	262				
66	Calarene	$C_{15}H_{24}$	204				
67 62	α -Cadinol	$C_{15}H_{26}O$	222				
68	α -Cearene epoxide	$C_{15}H_{24}O$	220				
69 70	p-Cearen-9-one	$C_{15}H_{24}$	204				
/U 71	a-Copaene 5 Noo codronal	$C_{15}\Pi_{24}$	∠0 4 209				
/ I 70	o-meo-ceuranoi		200 204				
72	γ-Caulmente δ Cadinana	$C_{15}\Pi_{24}$	20 4 204				
73 74	R-Flomono	C_{15} Γ_{24}	204 204				
/4	р-шешене	C151124	204				

Code	Compound	Formula	Nominal Mass (U)			
75	Ermophylene	C ₁₅ H ₂₄	204			
76	Germacrene D	$C_{15}H_{24}$	204			
77	Globulol	$C_{15}H_{26}O$	222			
78	Guaiol acetate	C ₁₇ H ₂₈ O ₂	264			
79	Juniper camphor	$C_{15}H_{26}O$	222			
80	α-Selinene	$C_{15}H_{24}$	204			
81	Trans-cadina-1(2)-4-diene	$C_{15}H_{24}$	204			
82	Valecene	$C_{15}H_{24}$	204			
83	Zonarene	$C_{15}H_{24}$	204			
Miscellaneous						
84	Azelaic acid	$C_9H_{16}O_4$	188			
	5-(Carboxymethyl)-7-hydroxy-1,4a-					
85	dimethyl-6-methylene decahydron	$C_{16}H_{24}O_5$	296			
	aphthalene-1-carboxylic acid					
86	(–)-Terpestacin	C ₂₅ H ₃₈ O ₄	402			
87	Luteoxepinone	$C_9H_{12}O_4$	184			
88	Luteoethanone A	C ₁₁ H ₁₅ NO ₃	209			
89	Luteoethanone B	C ₁₀ H ₁₃ NO ₃	195			
90	Neoanthraquinone	$C_{18}H_{16}O_{4}$	296			
91	(\pm) -Nigrosporione	$C_8H_{10}O_4$	170			

Table 1. Cont.

Members of the genus *Neofusicoccum* are often symbiotically associated with different plant species. Symbiotic association between a fungus and a plant is a widespread phenomenon in nature and several symbiotic lifestyles have been defined considering the benefits to or impacts on host and symbiont [7]. Recent studies have indicated that botryosphaeriaceous fungi may express different symbiotic lifestyles in response to host internal signals or environmental factors which also influence the secondary metabolites production [8–12]. In fact, secondary metabolites may have a crucial role in the fungal lifestyles because these compounds have a broad number of biological functions, including mediating communication, nutrient acquisition and acting as virulence factors [13,14].

Neofusicoccum species found as endophytes or pathogens of plants, produce structurally different metabolites. The variety of secondary metabolites produced by diverse species of *Neofusicoccum* is reported in Table 2, and many compounds showed interesting biological activities (e.g., antibacterial, cytotoxic, phytotoxic).

Diverse species, such as *Neofusicoccum australe*, *Neofusicoccum luteum*, *Neofusicoccum parvum*, and *Neofusicoccum vitifusiforme*, have been isolated from grapevine (*Vitis vinifera*) and shown to be pathogenic to this host [15–23]. In fact, a long list of secondary metabolites has been identified from these strains and the structural variability might be associated with the role of these compounds in the grapevine diseases. In particular, the capability of *N. parvum* to colonize woody tissue, combined with the secretion of phytotoxic compounds, is thought to underlie its pathogenicity and virulence [24]. For this reason, a section of this review is dedicated to the phytotoxic activities of secondary metabolites produced by pathogenic strains from grapevine (see Section 3).

Table 2. Occurrence of secondary metabolites in Neofusicoccum species.

Species	Strain	Host (Lifestyle)	Identified Compounds	Bioactivity	Ref.
N. australe (=Botryosphaeria australis)	AMCL7 BL24 SYSU-SKS024 E54ML	<i>Avicennia marina</i> (endophyte) <i>Juniper phoenicea</i> (pathogen) <i>Kandelia candel</i> (endophyte) <i>Myrtus communis</i> (endophyte)	40 *, 41, 45, 47, 51, 65, 85 38, 44, 61 36, 38, 40, 42, 46–50 32, 34	Antibacterial, cytotoxic Phytotoxic IDO inhibitory Antiproliferative	[25] [17] [26] [27]

Species	Strain	Host (Lifestyle)	Identified Compounds	Bioactivity	Ref.
	A1304B	M. communis (endophyte)	33	Phytotoxic	[28]
N. australe	ZJ12-1A	Sonneratia apetala (epiphyte)	35-38, 40, 41, 47, 50		[29]
(=Botryosphaeria	BOT48	Vitis vinifera (pathogen)	1, 55, 61		[17]
australis)	VP13	V. vinifera (pathogen)	24, 61		[16]
	DAR79506	V. vinifera (pathogen)	24, 59, 90, 61	Phytotoxic	[30]
N. batangarum	CBS143023	<i>Opuntia ficus-indica</i> (pathogen)	24, 26, 27, 30, 31, 86	Phytotoxic	[15]
N. cordaticola	434	(endophyte)	23, 52, 54, 57, 58, 60, 62–64, 66–70, 73–76, 78, 80, 82, 83		[31]
N lutaum	B175	V. vinifera (pathogen)	24, 26, 27, 61		[16]
1 v. <i>iutcum</i>	DAR81016	V. vinifera (pathogen)	9, 24, 26, 27, 61, 87–91	Phytotoxic	[18,30]
	-	<i>Eugenia jambolana</i> (endophyte)	24, 28, 29		[32]
	600	(endophyte)	23, 52, 54, 57, 58, 60, 63, 66–69, 72, 74, 76, 77–80		[31]
N	JS-0968	Vitis rotundifolia (endophyte)	28	Anti-atherosclerotic	[33]
N. paroum (=B.	2S-16	V. vinifera (pathogen)	2-8, 24-27, 53, 56,	Phytotoxic	[21]
paroa)	CBS 121486	V. vinifera (pathogen)	26, 27, 39, 61	Phytotoxic	[20]
	B19	V. vinifera (pathogen)	12, 13, 21, 35, 38, 39, 43,	Phytotoxic	[19,23]
	Bt67	V. vinifera (pathogen)	24	Phytotoxic	[22]
	S-116	V. vinifera (pathogen)	24	Phytotoxic	[22]
	DAR80004	V. vinifera (pathogen)	24, 26, 27, 61		[30]
	UCD646So	-	11, 13–19, 22		[34]
N. ribis	683	(endophyte)	23, 54, 57, 58, 60, 62, 64, 66–69, 71, 73–83		[31]
N. vitifusiforme	B8	V. vinifera (pathogen)	10, 12, 18, 20, 21, 84		[23]

Table 2. Cont.

* In the original paper the authors report the structure of botryosphaerone D instead of O-methylasparvenone.

2.1. Cyclohexenones

Several compounds belonging to the group of cyclohexenones (Figure 1) were produced by a strain of *N. parvum* from grapevine. Among them, the most representative is (–)-terremutin (2), a cyclohexanone oxide, identified together with its analogue (+)-*epi*-sphaeropsidone (6) and the new compound 4-chloro-terremutin hydrate (4) [21]. Cyclobotryoxide (1) is a new metabolite structurally related to 2 produced by a strain of *N. australe* associated with grapevine dieback [17].



Figure 1. Structures of cyclohexenones: cyclobotryoxide, (–)-terremutin, (+)-terremutin hydrate, 4-chloro-terremutin hydrate, (+)-4-hydroxysuccinate-terremutin hydrate, (+)-*epi*-sphaeropsidone (**1–6**).

The biosynthesis of (-)-terramutin is particularly relevant because this compound is a precursor of terreic acid, a potential anticancer drug capable of inhibiting Bruton's tyrosine kinase [35]. This polyketide is biosynthesized from the condensation of three units of malonyl CoA and a unit of acetyl CoA. 6-Methylsalicylic acid is a cyclic intermediate produced, whose subsequent decarboxylation produces 6-methylcatechol and then terremutin. Interestingly, two precursor phenolic compounds were isolated from cultures of strain producers of **1** and **2** (Table 2).

2.2. 5,6-Dihydro-2-Pyrones

The 6-substituted derivatives of 5,6-dihydropyran-2-ones (or 5,6-dihydro- α -pyrones) are polyketides produced by microorganisms and plants [36,37]. These compounds are characterized by the presence of an α , β unsaturated- δ -lactone, with an alkyl, alkenyl or aryl substituent at C-6 and occasionally a varied substitution pattern around the ring. Many of these products are biologically active, exhibiting phytotoxicity, cytotoxicity against tumor cells and antifungal or antimicrobial activity [36,37].

Asperlin (7) and its diastereomer dia-asperlin (8) (Figure 2) have been isolated from a strain of *N. parvum* associated with grapevine plants cv. Chardonnay from nursery, showing decline symptoms. (+)-Asperlin was previously reported from cultures of *Aspergillus nidulans*, while (+)-(6*R*, 7*S*)-dia-asperlin was originally reported from *Aspergillus caespitosus* [21].



Figure 2. Structures of 5,6-dihydro-2-pyrones: asperlin, dia-asperlin, luteopyroxin (7-9).

Luteopyroxin (9) was obtained from grapevines showing symptoms of Botryosphaeria dieback (see Section 3) in Australian vineyards. This compound was characterized as a (3*S*,3a*R*,7a*S*)-4-methoxy-3-methyl-2,3,3a,7atetrahydro- 6H-furo [2,3b]pyran-6-one. In particular, its relative and absolute configurations have been determined by nuclear Overhauser effect spectroscopy and experimental and calculated electronic circular dichroism data [30]. This metabolite is structurally related to astepyrone isolated from *Aspergillus terreus* [38].

2.3. Fatty Acids

Fatty acids are essential storage molecules and the starting material for the synthesis of many secondary metabolites [39]. Some long chain fatty acids and their esters (Figure 3) have been detected in culture extracts of strains of *N. vitifusiforme* and *N. parvum* [23,34]. Particularly relevant is the finding of linoleic acid (**12**) as a product of species of *Neofusicoccum* isolated from grapevine [23]. In fact, this octadecanoid acid may take part in the biosynthesis of jasmonic acid participating in the signaling pathway during pathogen attack and plant colonization [40].



Figure 3. Structures of fatty acids: elaidic acid, ethyl elaidate, linoleic acid, ethyl linoleate, methyl linoleate, 9,12,15-octadecatrienoate ethyl ester, ethyl oleate, methyl oleate, palmitic acid, methyl palmitate, palmitoleic acid, stearic acid, ethyl stearate, undecan-2-one (**10–23**).

2.4. Melleins

Melleins, also known as 3,4-dihydroisocoumarins, are lactonic natural compounds commonly produced by fungi, bacteria and plants representing a subgroup of the isocoumarins. A large majority of isocoumarins belong to this subgroup characterized by having one carbon substituent at C-3. (-(-)-(R)-Mellein is the founding product of this subgroup first isolated from the fungus *Aspergillus melleus* (1933) [41]. Several melleins with hydroxyl, methoxyl, alkyl or acetate groups in diverse positions have been subsequently isolated from natural sources [42].

Some compounds belonging to melleins have been reported as phytotoxins produced by pathogens from the family Botryosphaeriaceae [9,11]. Melleins have also been frequently found in cultures of *Neofusicoccum* species, such as *N. australe*, *N. parvum*, *N. batangarum*, *N. luteum* (Figure 4) [15,16,32,33]. In particular, (-)-(R)-mellein (24), (3R,4R)-hydroxymellein (26), (3R,4S)-hydroxymellein (27), botryoisocoumarin A (30), and (+)-neoisocoumarin (31) have been isolated from *N. batangarum* associated with scabby canker of Cactus pear (*Opuntia ficus-indica*). These compounds showed phytotoxicity on the host and non-host (tomato) plants when tested using the leaf puncture assay and the cladode puncture method. Compounds 30 and 31 are the most active at concentrations in a range from 10^{-3} to 10^{-4} M, inducing a necrosis area around the inoculation points [15]. (-)-(R)-Mellein, (3R,4S)- and (3R,4R)- hydroxymelleins were isolated from the first time from a phytopathogenic strain of *N. luteum*, representing the first detection of phytotoxins from this species [16].



Figure 4. Structures of melleins: (*R*)-(-)-mellein, (*R*)-(-)-3-hydroxymellein, (3*R*,4*R*)-(-)-4-hydroxymellein, (3*R*)-5-hydroxymellein, 7-hydroxymellein, (\pm)-botryoisocoumarin A, (+)-neoisocoumarin (**24–31**).

Interestingly, (3*R*)-5-hydroxymellein (**28**), isolated from *N. parvum*, has an effect in the suppression of the oxidation of LDL and HDL through the inhibition of lipid peroxidation, the decrease in negative charges, the reduction in hyperchromicity and carbonyl contents, and the prevention of apolipoprotein A-I (ApoA-I) aggregation and apoB-100 fragmentation. Furthermore, **28** significantly reduced foam cell formation induced by oxidized LDL (oxLDL). These findings show that it could be a potential preventive agent of atherosclerosis via obvious anti-LDL and HDL oxidation and the inhibition of foam cell formation [33].

2.5. Myrtucommulones

Myrtucommulone A (**32**) is the founding product of the group of structurally related compounds named myrtucommulones whose structures are characterized by a phloroglucinol nucleus coupled with syncarpic acid residues (Figure 5) [28]. Myrtucommulones and related acylphloroglucinols are frequently reported as products of plant species belonging to the family Myrtaceae (e.g., *Eucalyptus globulus* [43], *Kunzea ericoides* [44], *Myrtus communis* [45–47], *Melaleuca citrina* [48], *Rhodomyrtus tomentosa* [49–51]) spread in the Australasian region and these compounds are well-known for their antimicrobial, antioxidant and anti-inflammatory activities [28]. Some endophytic fungi from the genus *Neofusicoccum* are able to produce myrtucommulones. This finding represents a new frontier in the study of myrtucommulones because the availability of strains to be cultured in vitro may provide access to increased amounts of these products for further investigations. To date, myrtucommulones A, B and D (**32–34**) were identified in cultures of endophytic strains of *N. australe* of myrtle (*Myrtus communis*) [27,28]. Antiproliferative activity effects on the human prostatic cancer cell lines DU145 and PC3 were observed for a mixture of **32** and **34** [27].



Figure 5. Structures of myrtocommulones: myrtucommulone A, B and D (32-34).

2.6. Naphthalenones

Naphthalenones (Figure 6) are a group of compounds strictly related to napthoquinones. In fact, depending upon prevalent redox conditions, para-quinones can be fully reduced (hydroquinone; QH2), fully oxidized (*p*-quinone; Q), or in an intermediate (*p*-semiquinone radical; QH_) oxidation state [52]. It was hypothesized that several naphthoquinones (e.g., flaviolin, juglone, 3-hydroxyjuglone) could be linked to the biosynthesis of naphthalenones (e.g., isosclerone, 4-hydroxyscytalone) through the reduction in the carbonylic group at C-4. Moreover, several naphthalenones epimers at C-4 were isolated as natural products, such as (–)-regiolone and (+)-isosclerone, respectively, isolated from walnut tree (*Juglans regia*) and the fungus *Sclerotinia sclerotium* [53], (–)- and (+)leptothalenones A from the marine-derived fungus *Leptosphaerulina chartarum* [54]. Finally, as can be seen from Table 2, compounds from both naphthalenones and naphthoquinones groups were isolated as products of the secondary metabolism of diverse species of *Neofusicoccum*. In particular, 6-ethyl-2,7-dimethoxyyjuglone (47) and its 4-dihydroderivate, (3*R*,4*R*)-3-methoxyl-botryosphaerone D (42), were isolated from *N. australe* [26].

Among the fungal naphthalenones producers, a strain of *Botryosphaeria australis* (=*N. australe*) originally obtained from the epidermis of the mangrove plant *Sonneratia apetala*, is particularly relevant for the capacity to synthesize some new tri-substituted naphthalenones named botryosphaerones A–D (**35–38**) [29] which showed cytotoxicity against HeLa, HepG-2, and A-549 cells at a concentration of 10 μ g mL⁻¹, and antimicrobial activities against pathogenic bacteria or yeasts at a concentration of 50 μ g mL⁻¹. Subsequently, naphthalenones were isolated from cultures of *Neofusicoccum* species involved in grapevine diseases. This is the case of a strain of *N. australe* involved in branch dieback of *Juniperus phoenicea* in Sardinia (Italy) which produced botryosphaerone D (**37**) and (3*S*,*4S*)-3,*4*,8-trihydroxy-6-methoxy-3,4-dihydro-1(2H)-naphthalenone (**44**) [17], a phytopathogenic strain of *N. parvum* isolated from declining vines in Sicily (Italy) which produced botryosphaerones A (**35**) and D (**38**), isosclerone (**39**), and 3,4,5-trihydroxy-1-tetralone (**42**) [19] and a strain of *N. parvum* obtained from a cankered branch of grapevine growing in Catalogna (Spain) which produced **39** representing the first detection of this compound as a product of botryosphaeriaceous fungi [20].



Figure 6. Structures of naphthalenones: botryosphaerones A–D, isosclerone, *O*-methylasparvenone, *O*-methylaspmenone, (3*R*,4*R*)-3-methoxyl-botryosphaerone D, 3,4,5-trihydroxy-1-tetralone, (3*S*,4*S*)-3,4,8-trihydroxy-6-methoxy-3,4-dihydro-1(2H)-naphthalenone (**35**–**44**).

2.7. Naphthoquinones

As can be seen from Table 2, different species of *Neofusicoccum* show the production of 1,4-naphtoquinones. Natural naphthoquinone derivatives have been widely identified as functional metabolites from various plants, microbes, and marine organisms [55]. For their notable activities, this class of compounds is the prime target of a vigorous research activity focused on the development of new therapeutic agents [56]. Furthermore, naphtoquinones are also relevant because of their bioactivities. In fact, naphthoquinones have a very interesting spectrum of biological activities, including antibiotic, antiviral, anti-inflammatory, antipyretic, antiproliferative and cytotoxic effects, which are related to several mechanisms of action, such as redox cycles, arylation of the thiol groups of proteins, intercalation, induction of breaks in the DNA chain, generation of free radicals and other reactive oxygen species (ROS) and bioreductive alkylation via the formation of quinone methide [57].

Seven naphthoquinones have been isolated from *Neofusicoccum* species (Figure 7). The most significant structural differences are the presence of hydroxyl, methoxyl, alkyl or acetate groups in different positions on the 1,4-naphthalenoid ring, but these compounds share the hydroxy group at C-5 and the methoxy groups at C-2 and C-7. These groups are all present in the 5-hydroxy-2,7-dimethoxynaphthalene-1,4-dione (**50**) [25], which was identified as a product of an endophytic strain of *B. australis* (=*N. australe*) isolated from the mangrove plant *Avicennia marina*, together with botryosphaenin (**45**), a new naphtoquinone. This latter compound and its 6-ethyl analogue (6-ethyl-5-hydroxy-2,7-dimethoxynaphthalene-1,4-dione **47**) showed antibiotic and antiproliferative activities with a minimum inhibitory concentration (MIC) ranging from 2 to 32 μ g mL⁻¹ when tested on different cell lines [25].



Figure 7. Structures of naphthoquinones: botryosphaenin, neofusnaphthoquinone A, 6-ethyl-2,7-dimethoxyyjuglone, 6-(1-hydroxyethyl)-2,7-dimethoxyjuglone, 6-(1-methoxylethyl)-2,7-dimethoxyjuglone monoacetate, 5-hydroxy-2,7-dimethoxynaphthalene-1,4-dione (**45–51**).

New ethylnaphthoquinones have been isolated from a mangrove endophytic strain of *N. australe*. In particular, an unsymmetrical naphthoquinone dimer, 6-(1-methoxylethyl)-2,7-dimethoxyjuglone (**49**), and a naphthalenone named (3*R*,4*R*)-3-methoxyl-botryosphaerone (**42**), together with some known analogues. Indoleamine 2,3-dioxygenase (IDO) inhibitory activity tests showed that, among the isolated compounds, the most active was 6-(1-methoxylethyl)-2,7-dimethoxyjuglone (**49**) with an IC₅₀ value of 0.11 μ M [26].

2.8. Phenols and Alcohols

Phenol derivative compounds (Figure 8) are among the most common metabolites of microorganisms and plants. In particular, phenyl ethanol (60) and tyrosol (61) are produced by fungi via the Shikimate biosynthetic pathway, while 2-hydroxypropylsalicylic acid (53) and 6-methylsalicylic acid (56) are produced through the Polyketide biosynthetic pathway. Tyrosol is one of the most frequently detected metabolites in cultures of botryosphaeraceous fungi [58]. In fact, it was identified in culture extracts of several phytopathogenic strains of *N. australe* [16,17,30], *N. parvum* [30,32] and *N. luteum* [18,30] (Table 2).

As reported above, 6-methylsalicylic acid, together with (-)-terremutin (2), takes part in the biosynthesis of the terric acid [35] and, interestingly, these compounds were both identified as products of a strain of *N. parvum* isolated from grapevine [21].



Figure 8. Structures of phenols and alcohols: 2-ethyldecan-1-ol, 2-hydroxypropyl salicylic acid, 2-methylbutan-1-ol, 3-methylcatechol, 6-methyl-salicylic acid, isobutanol, isopentyl alcohol, p-cresol, phenylethyl alcohol, tyrosol (**52–61**).

2.9. Sesquiterpenes

Fungal sesquiterpenes are essentially hydrocarbons possessing a multitude of different carbons skeletons. Starting from farnesol-pyrophosphate, the sesquiterpene skeleton is cyclized by different sesquiterpene cyclases producing the cations germacryl, humulyn, bisabolyn [59,60]. As often reported for secondary metabolites [8–11], sesquiterpenes biosynthesis is highly dependent on growth conditions (e.g., temperature, pH, humidity, growth substrate), but little is known about the fungal biosynthesis. On the contrary, there is ample literature on the biosynthesis of sesquiterpenes by plants [61–63].

The volatile fraction of sesquiterpenes consists mainly of lipophilic compounds and this suggests that their principal targets are cell membranes causing toxic effects due to the loss of osmotic control [64]. Another possibility is that volatile sesquiterpenes facilitate the passage of other toxins through membranes by acting as solvents and synergizing their effects [65].

The headspace-solid phase micro-extraction (HS-SPME) approach was used by Oliveira et al. [31] to efficiently extract the volatile fraction of metabolites in vitro produced by different Botryosphaeriaceae species. Among them, endophytic strains of Neofusicoccum cordaticola, Neofusicoccum ribis and N. parvum were investigated. The fungal extracts were analyzed via gas chromatography-mass spectrometry (GC-MS) and identified by comparing their mass spectrum with those present in mass spectral libraries. These three *Neofusic*occum strains showed the production of different subgroup of sesquiterpenes, including eudesmane, guaiane, cadinane, cedrane, aristolane, valencane, copaene and eremophylane skeletons, and two monocyclic sesquiterpenes (i.e., β -elemene and germacrene D) (Figure 9). The non-oxygenated sesquiterpenes belong mostly to the cadinane group while the oxygenated ones possess the guaiane and cedrane skeleton. Interestingly, in this study, many sesquiterpenes were reported for the first time as fungal metabolites, such as aristolene (62), aristolochene (63), α -cadinol (67), α -cedrene epoxide (68), β -cedren2-one (69), 5-neo-cedranol (71), γ -cadinene (72), δ -cadinene (73), eremophylene (75), guaiol acetate (78), juniper camphor (79), trans-cadina-1(2)4-diene (81). As said above, sesquiterpenes are mainly reported as plant metabolites and their production by endophytes can be explained considering that these fungi are able to produce many plant-derived compounds through the so-called horizontal gene transfer (HGT) [66,67]. In fact, it has been hypothesized that plant-microbe interactions may lead to HGT or genetic recombination, from the host plant to its endophytic counterpart or vice versa, that subsequently, lead to endophytes capable of accumulating certain metabolites specific to the host plants themselves [68].



Figure 9. Structures sesquiterpenes: aristolene, aristolochene, δ-amorphene, botryosterpene, calarene, α -cadinol, α -cedrene epoxide, β -cedren-9-one, α -copaene, 5-neo-cedranol, γ -cadinene, δ -cadinene, β -elemene, ermophylene, germacrene D, globulol, guaiol acetate, juniper camphor, α -selinene, *trans*-cadina-1(2)-4-diene, valecene, zonarene (**62–83**).

Sesquiterpenes seem interesting in terms of chemotaxonomy [31]. In fact, *N. parvum* and *N. ribis* are very closely related species not easy to distinguish even through the use of molecular techniques. Hence, the different production of the sesquiterpenes α -cedrene epoxide (68) and guaiol acetate (78) by these species can help the species identification.

2.10. Miscellaneous

A number of products of *Neofusicoccum* species are placed in a miscellaneous category (Figure 10) because they have no structural affinity with previous groups. This is the case



Figure 10. Structures of compounds from the group "miscellaneous": azelaic acid, 5-(carboxymethyl)-7-hydroxy-1,4adimethyl-6-methylene decahydronaphthalene-1-carboxylic acid, (-)-terpestacin, luteoxepinone, luteoethanones A-B, luteoethanone B, neoanthraquinone, (\pm)-nigrosporione (**84–91**).

Furthermore, a strain of *N. luteum* isolated from grapevines showing symptoms of Botryosphaeria dieback produced a new trisubstituted oxepi-2(7H)-one named luteoxepinone (87) and several known compounds [30], including the cyclopentenone (\pm) -nigrosporione (91) [69].

Another interesting compound is (–)-terpestacin isolated from *Neofusicoccum batangarum*, pathogenic to cactus pear [15]. (–)-Terpestacin has also been isolated from several fungal cultures, in particular from *Rutstroemia capillus-albis*, the causal agent of bleach blonde syndrome on the *Bromus tectorum* [70]. In fact, phytotoxic tests showed that this compound is toxic for both host and non-host (tomato) plants confirming its potential role in fungal pathogenesis [15].

3. Phytotoxicity of Neofusicoccum Metabolites on Grapevine

A large number of species of the family *Botryosphaeriaceae* have been associated with canker and dieback of grapevines. Grapevine trunk diseases (GTDs) are among the prime causes worldwide of serious damages on vineyards and significant economic losses [71,72]. One of the major concerns in GTDs control is the slow progression of the host xylem colonization by pathogenic fungi or the absence of symptoms for long periods [71,73,74]. In the broad spectrum of GTDs, species of *Botryosphaeriaceae* are the disease causative agent of the Botryosphaeria dieback [12,58,75] and several of these species belong to genus *Neofusicoccum*, causing diverse symptoms in infected grapevines, such as leaf chlorosis, bud and wood necrosis, weak spring growth, and vascular cankers primarily in the shape of wedges [72,76,77]. Many symptoms, particularly the foliar ones, can be attributed to the production of toxic secondary metabolites by the fungus [11,78].

As reported in Table 3, strains of *N. australe*, *N. luteum*, *N. parvum*, *N. vitifusiforme* associated with Botryosphaeria dieback produce phytotoxic metabolites. In fact, the phytotoxic activity of some of them has been investigated on leaves, leaf discs or detached leaves of grapevines showing concentration dependent necrosis. As reported by Abou-

Mansour et al. [21], (*R*)-(-)-3-hydroxymellein (**25**) is the most active mellein tested with 62% leaf disc necrosis. Among cyclohexenones, the compounds with an epoxydic ring are the most active (i.e., cyclobotryoxide (**1**), (-)-terremutin (**2**), (+)-*epi*-sphaeropsidone (**6**)), while (+)-(6*R*,7*S*)-dia-asperlin (**8**) is responsible for 33% of leaf disc necrosis. Among naphthalenones, the principal phytotoxic compound is botryosphaerone D (**38**) whose activity is photosensitive [19].

Table 3. Phytotoxicity of metabolites produced by *Neofusicoccum* species on grapevine.

Code	Compound	Assay	Concentration	Activity	Ref.
1	Cyclobotryoxide	Leaf puncture	5.9 mM	24.3 ± 1.1 (Area lesions mm ²)	[17]
2	(–)-Terremutin	Leaf disk	1.3 mM	55% (% of necrotic area)	[21]
6	(+)-epi-Sphaeropsidone	Leaf disk	1.3 mM	47% (% of necrotic area)	[21]
8	(+)-(6R,7S)-Dia-asperlin	Leaf disk	0.9 mM	33% (% of necrotic area)	[21]
9	Luteopyroxin	Detached leaf	2.5 mM	Withering and necrotic spots	[30]
24	(R)-(-)-Mellein	Leaf disk Calli	1.0 mM 2.5 mM	50% (% of necrotic area) slight partial necrosis	[21] [22]
25	(R)-(–)-3- Hydroxymellein	Leaf disk	1.0 mM	62% (% of necrotic area)	[21]
26	(3 <i>R,</i> 4 <i>R</i>)-(–)-4- hydroxymellein	Leaf disk	1.0 mM	34% (% of necrotic area)	[21]
27	(3 <i>R,</i> 4 <i>S</i>)-(—)-4- hydroxymellein	Leaf disk	1.0 mM	33% (% of necrotic area)	[21]
35	Botryosphaerone A	Leaf puncture *	3.7 mM	4.74 (Area lesions mm ²)	[19]
38	Botryosphaerone D	Leaf puncture Leaf puncture *	3.9 mM, 3.9 mM	11.9 (Area lesions mm ²) 9.11 (Area lesions mm ²)	[17] [19]
39	Isosclerone	Leaf puncture *	5.6 mM	2.73 (Area lesions mm ²)	[19]
43	3,4,5-Trihydroxy-1- tetralone	Leaf puncture *	5.2 mM	2.13 (Area lesions mm ²)	[19]
55	3-Methylcatechol	Leaf puncture	8.1 mM	4.9 (Area lesions mm ²)	[17]
59	p-Cresol	Detached leaf	2.5 mM	Low phytotoxicity	[30]
61	Tyrosol	Leaf puncture	7.2 mM	8.3 ± 1.3 (Area lesions mm ²)	[17]
87	Luteoxepinone	Detached leaf	2.5 mM	Withering and necrotic spots	[30]
88-89	Luteoethanones A and B	Detached leaf	2.5 mM	Large necrotic spots, severe shriveling, and distortion of the leaf lamina	[18]
90	Neoanthraquinone	Detached leaf	2.5 mM	Large necrotic spots, severe shriveling and distortion of the leaf lamina	[30]
91	(\pm) -Nigrosporione	Detached leaf	2.5 mM	Withering and necrotic spots	[30]

* Assays conducted under dark conditions.

Furthermore, luteoethanones A and B (88–89), luteopyroxin (9), neoanthraquinone (90) and (\pm) -nigrosporione (91) are responsible for large necrotic spots, severe shriveling, and distortion of the leaf when tested at a concentration of 2.5 mM [18,30].

Concerning the potential involvement of phytotoxins in the symptoms expression by grapevines, a simplified model using grapevine cells (calli) from *V. vinifera* cv. Chardonnay was employed. In this respect (–)-terremutin and mellein are responsible for the expression of some genes involved in the defense mechanisms of grapevine (e.g., cellular detoxification, jasmonic acid pathway, synthesis of secondary metabolites of the phenylpropanoid

pathway, flavonoid synthesis), after 1–6 days of exposure on calli [21,22]. Moreover, these findings confirm the presence and the phytotoxic effects of (–)-terramutin and mellein in grapevine wood from plants showing Botryosphaeria dieback symptoms [21,22]. It seems possible that there is a synergistic action between different fungal metabolites, but the different hypotheses still need to be examined.

4. Conclusions

In this review, the available literature on secondary metabolites produced by *Neofusic*occum species has been analyzed. A total of 91 chemically defined compounds from over 20 isolates belonging to 7 species of *Neofusicoccum* have been reported. These compounds were classified based on their structure in nine groups (i.e., cyclohexenones, 5,6-dihydro-2pyranones, fatty acids, melleins, myrtocommulones, naphthalenones, naphthoquinones, phenols and alcohols, sesquiterpenes). Furthermore, an additional group of miscellaneous compounds was added in order to gather metabolites that have no structural affinity with compounds present in the reported groups.

An aptitude of members of the *Neofusicoccum* genus to colonize different plant species, essentially as a latent pathogen, arises from an accurate examination of the existing literature. The pathogenicity and virulence of these fungi seem to be related, at least in part, to the capacity to produce secondary metabolites. Several strains from different species caused similar effects on plants, perhaps due to the production of specific metabolites. In fact, many disease symptoms (e.g., bud necrosis, vascular discoloration of the wood, and perennial cankers) might be caused by metabolites which were detected in cultures of phytopathogenic species of *Neofusicoccum*. For instance, mellein and its derivatives have been detected in most strains isolated from grapevines showing symptoms of grapevine trunk diseases (GTDs). This evidence is supported by the phytotoxic activity of these compounds on host and non-host plants.

Moreover, metabolites produced by *Neofusicoccum* species exhibit additional biological activities, including antibacterial, cytotoxic, antiproliferative and indoleamine 2,3dioxygenase (IDO) inhibitory effects. In this respect, the genus *Neofusicoccum* can be regarded as a source of bioactive products to be used in diverse biotechnological fields.

The knowledge present in this review can be applied where recommendations for *Neofusicoccum* disease management strategies are required. In fact, enriching the existing literature with data on secondary metabolites produced by *Neofusicoccum* isolates from different hosts might be useful for several purposes, such as understanding the resistance mechanisms, screening of diseases, and potential applications of secondary metabolites.

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