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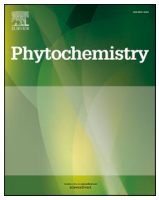

Interactions of fungi with non-isothiocyanate products of the plant glucosinolate pathway: A review on product formation, antifungal activity, mode of action and biotransformation

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ABSTRACT

The glucosinolate pathway, which is present in the order Brassicales, is one of the most researched defensive natural product biosynthesis pathways. Its core molecules, the glucosinolates are broken down upon pathogen challenge or tissue damage to yield an array of natural products that may help plants defend against the stressor. Though the most widely known glucosinolate decomposition products are the antimicrobial isothiocyanates, there is a wide range of other volatile and non-volatile natural products that arise from this biosynthetic pathway.

This review summarizes our current knowledge on the interaction of these much less examined, non-isothiocyanate products with fungi. It deals with compounds including (1) glucosinolates and their biosynthesis precursors; (2) glucosinolate-derived nitriles (e.g. derivatives of 1*H*-indole-3-acetonitrile), thiocyanates, epithionitriles and oxazolidine-2-thiones; (3) putative isothiocyanate downstream products such as raphanusamic acid, 1*H*-indole-3-methanol (= indole-3-carbinol) and its oligomers, 1*H*-indol-3-ylmethanamine and ascorbigen; (4) 1*H*-indole-3-acetonitrile downstream products such as 1*H*-indole-3-carbaldehyde (indole-3-carboxaldehyde), 1*H*-indole-3-carboxylic acid and their derivatives; and (5) indole phytoalexins including brassinin, cyclobrassinin and brassilexin.

Herein, a literature review on the following aspects is provided: their direct antifungal activity and the proposed mechanisms of antifungal action, increased biosynthesis after fungal challenge, as well as data on their biotransformation/detoxification by fungi, including but not limited to fungal myrosinase activity.

1. Introduction

Plants use metabolites as key tools in their interactions with their microbiome: specialized metabolite classes known to have such effects include coumarins, triterpenes, benzoxazinoids (Chen et al., 2021) as well as glucosinolate (GSL) decomposition products (Plaszkó et al., 2021).

The GSL pathway is one of the most well researched specialized metabolite biosynthetic pathways. The GSL pathway can be found in the plants of the order Brassicales (Pongrac et al., 2013; Sønderby et al., 2010). Bioactive compounds of the GSL pathway are stored *in planta* in the form of GSLs, with a glucosidase enzyme compartmentalized separately (Kissen et al., 2009). This elegant way enables the prevention of autotoxicity and the on-demand conversion of their precursors to compounds with high bioactivity at the same time (Rask et al., 2000).

Biosynthetically, GSLs originate from various amino acids, and are usually classified according to side chain chemistry (Blažević et al., 2020). The main groups are the aliphatic (from one of Ala, Leu, Ile, Val, Met or Glu), indole (from Trp) and benzenic (from Phe or Tyr) GSLs. The GSLs are thioglucosides of aldoxime sulfates (Fig. 1), and their biosynthesis is accomplished as a cooperation of various cytosolic and chloroplast enzymes, as recently reviewed (Chhajed et al., 2020; Harun et al., 2020). The buildup of their core structure can be preceded by an amino acid chain elongation in the chloroplast (Böttcher et al., 2014; Chhajed et al., 2020; Harun et al., 2020; Lahrmann et al., 2015; Sønderby et al., 2010) and can be followed by optional steps such as

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glycosylation or acylation (Blažević et al., 2020). The GSL-derived bioactive specialized metabolites include a chemically diverse array of compounds used against plant pathogens and in various other plant interactions.

The production of bioactive downstream products from GSLs starts with the enzymatic hydrolysis of the GSL thioglucosidic bond (Fig. 1), either by myrosinase (β-thioglucosidase glucohydrolase (EC 3.2.3.147), TGG in *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae)) during plant tissue damage or alternative ("non-typical") myrosinases in intact tissues. The latter include PEN2 (Bednarek et al., 2009) and likely other, currently unknown enzymes as well. Plant myrosinases are members of glucoside hydrolase family I, just like other glucosidases that play a role in plant defense (Wittstock et al., 2016). Plants usually express several isoforms that differ in spatial and temporal distribution and enzymatic properties. Though there is a report of a myrosinase with high specificity towards the plant's major GSL (Bernardi et al., 2003), research shows that plant myrosinase enzymes accept various GSL subclasses as substrates (Durham and Poulton, 1990; Li and Kushad, 2005). After hydrolysis, the unstable GSL aglycon (a thiohydroximate-*O*-sulfate) spontaneously rearranges to form various volatile decomposition products (Fig. 1). Examples with data regarding fungi are explicitly mentioned in this review and are listed in Table 1. The default rearrangement products are isothiocyanates (ITCs), but the process results in nitriles in the presence of nitrile specifier proteins (NSPs), at low pH (<5), or in the presence of Fe^{2+} ions (Wittstock et al., 2016). Breakdown mechanisms are well summarized by Hanschen et al. (2014).

The most widely researched GSL decomposition products are the ITCs. These compounds are reactive towards nucleophiles (Hanschen et al., 2014), resulting in a wide array of pharmacological effects. ITCs are also responsible for the health benefits of a diet rich in Brassicaceae plants: the risk of several diseases can be decreased by their consumption. This effect is achieved through modulation of inflammatory signaling and through modulation of liver xenobiotic metabolism, leading to chemoprevention (Eagles et al., 2020; Sturm and Wagner, 2017). As ITCs have potent antimicrobial activity, they have a significant effect on the soil microbiome and are used in a variety of agricultural and food industrial applications as well (Plaszkó et al., 2021).

Some ITCs, such as those from indole glucosinolates and ones with a hydroxylated side chain, are unstable and are readily converted to various downstream products (Figs. 1–2). The *in planta* PEN2-mediated breakdown of indole GSLs results in raphanusamic acid (**61**) and 1*H*-indole-3-methanamine (**62**) through several steps (Fig. 2) (Bednarek et al., 2009). In this case, the ITC is a biosynthetic intermediate. In the presence of ascorbic acid, ascorbigen (**69**) is generated from the unstable indole ITCs, but water can be a reaction partner as well, resulting in 1*H*-indole-3-methanol (**68**, = indole-3-carbinol). The latter can be a substrate of subsequent oxidative transformations (Fig. 3), resulting in 1*H*-indole-3-carboxaldehyde (**74**, = indole-3-carboxaldehyde) or 1*H*-indole-3-carboxylic acid (**75**) (Agerbirk et al., 2009), or spontaneous dimerization or oligomerization (Buskov et al., 2000; Hanschen et al., 2014).

1*H*-Indole-3-acetonitrile (**49**), which can be biosynthesized through indole GSLs or directly from IAOx (**65**, indole-3-acetaldoxime), is also a precursor to many downstream products (Fig. 3). Unfortunately, the exact proportion of these two routes is currently not adequately quantified. Downstream products of **49** include various aldehydes and acids, including **74** and **75** as well as their hydroxylated, methoxylated or glycosylated derivatives at positions 4, 5 and 6 (**77**–**89**).

The Brassicaceae phytoalexin brassinin (**67**) also arises from this pathway (Fig. 2) (Mucha et al., 2019) and is a starting point for the biosynthesis of various other phytoalexins (Fig. 4) (Blažević et al., 2020; Klein and Sattely, 2017; Pedras et al., 2002, 2004b, 2006b, 2007b). These biosynthesis routes contain several speculative assumptions, and further work will be required to fully elucidate them.

The literature on the above-mentioned additional GSL decomposition products is much smaller compared to that of the ITCs. This is in part due to the surprising chemical diversity of the alternative breakdown and downstream products, as detailed later. The ecological role of these compounds is therefore much less understood to date, but their significance in plant-microbe interactions is now beyond doubt.

Herein, we aim to provide a review on the interactions of fungi with the compounds of the GSL pathway, focusing on the less discussed groups: GSLs themselves, their precursors, non-default GSL decomposition products and various downstream products. We include studies that use whole plant systems as well as papers that used pure compounds to test antifungal activity of metabolism by fungal strains. We do not include the effects of isothiocyanates, as these have recently been reviewed from various aspects including (but not limited to) interactions with fungi (Plaszkó et al., 2021), anti-cancer effects (Dinh et al., 2021; Vanduchova et al., 2019) as well as pharmacokinetics (Lamy et al., 2011).

The current paper is based on articles found by searching Scopus and SciFinder^{n}. It aims to be exhaustive in coverage regarding the literature (up to 2021 July). Therefore, all known GSLs, their respective nitriles, epithionitriles, thiocyanates, possible oxazolidine-2-thiones and other rearrangement products, downstream GSL products and phytoalexins were searched on one of the platforms, either as a compound name in abstracts/titles in Scopus, or as chemical entities (CAS numbers) in SciFinder. The list of GSLs used to build other compounds include those in the review of Blažević et al. (2020)., including both tentative and conclusive ones. Search results were filtered to contain one of the text strings "fungus", "antifungal" or "fungistatic" in the titles or abstracts.

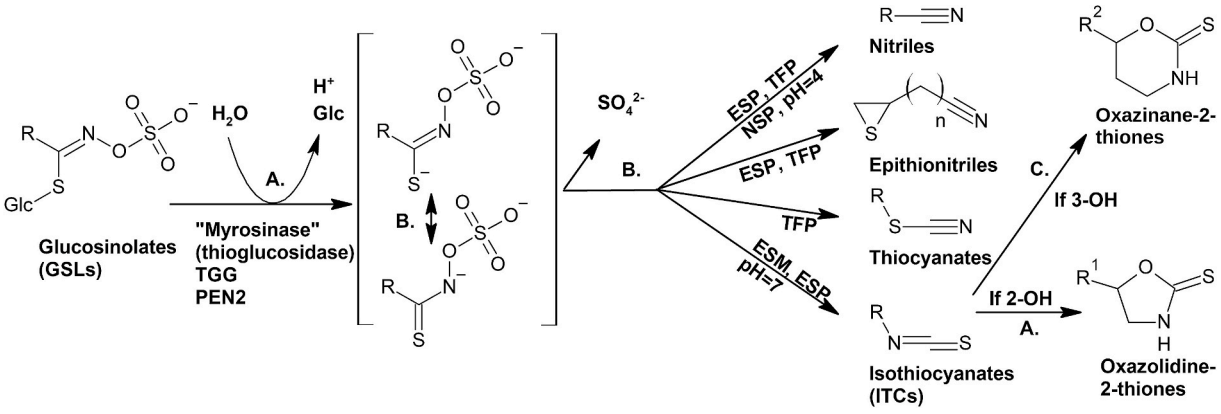

Fig. 1. Deglucosylation and subsequent spontaneous rearrangement of glucosinolates into various volatile natural products. Biosynthetic step references: A. Hanschen et al. (2014), B. Wittstock et al. (2016), C. Kjær et al. (1970). Abbreviation: ESP: epithiospecifier protein; TFP: thiocyanate-forming protein; ESM: epithiospecifier modifier protein; NSP: nitrile-specifier proteins.

Table 1
Glucosinolates and some of their respective decomposition products explicitly mentioned in the article. All possible theoretical decomposition products as well as their CAS numbers can be found in the supplementary materials. Abbreviations, GSL, glucosinolate; ITC, isothiocyanate.

GSL systematic name	GSL Trivial name	Corresponding nitrile	Corresponding other decomposition product
Aliphatic GSLs, hydrocarbon sidechain			
1, Methyl GSL	Glucocapparin	**2**, Acetonitrile	
3, Ethyl GSL	Glucolepidiin	**4**, Propanenitrile	
5, n-Propyl GSL	–	**6**, n-Butanenitrile	
7, Prop-2-enyl GSL	Sinigrin	**8**, 3-Butenenitrile (= Allyl cyanide)	**9**, 3-Isothiocyanato-1-propene (= Allyl ITC, AITC)[1]; **10**, 2-(Thiiran-2-yl) acetonitrile[2]; **11**, 3-thiocyanato-1-propene (= allyl thiocyanate)[3]
12, n-Butyl GSL	–		**13**, n-Butyl thiocyanate[3]
14, 1-Methylethyl GSL	Glucoputranjivin	**15**, 2-Methylpropanenitrile	
16, n-Pentyl GSL	–	**17**, Hexanenitrile	
Aliphatic GSLs, with side-chain heteroatoms			
18, 3-Hydroxypropyl GSL	–	**19**, 3-Hydroxy-butanenitrile	
20, 3-(Methylthio)propyl GSL	Glucoibervirin		
21, (2*R*)-2-Hydroxybut-3-enyl GSL	Progoitrin		**22**, Goitrin[4]
23, 4-Mercaptobutyl GSL	Glucosativin		**24**, Sativin[5,6]
25, 4-(Methylthio)butyl GSL	Glucoerucin		
26, 4-(Methylthio)butenyl GSL	Glucoraphasatin		**27**, 4-Methylthio-3-butenyl ITC; **28**, Methyl-4-methylthiobutyl dithiocarbamate;[5] **29**, Methyl-(Z)-4-methylthio-3-butenyl-dithiocarbamate;[5] **30**, Methyl-(E)-4-methylthio-3-butenyl-dithiocarbamate[5]
31, 8-Methylthiooctyl GSL	–		
Benzenic GSLs			
32, Phenyl GSL[7]	–	**33**, Benzonitrile	
34, Benzyl GSL	Glucotropaeolin	**35**, 2-Phenylacetonitrile	**36**, Benzyl thiocyanate[3]
37, 4-Hydroxybenzyl GSL	Sinalbin	**38**, 4-Hydroxy-2-phenylacetonitrile	
39, 3-Methoxybenzyl GSL	Glucolimnanthin		**40**, 3-Methoxybenzyl isothiocyanate[1]; **41**, 3-Methoxybenzyl thiocyanate[3]
42, 4-Methoxybenzyl GSL	Glucoaubrietin		**43**, 4-Methoxybenzyl thiocyanate[3]
44, 2-phenylethyl GSL	Gluconasturtiin	**45**, 3-Phenylpropanenitrile	**46**, 2-phenylethyl ITC (phenethyl ITC, PEITC)[1]
47, 2-(4-hydroxyphenyl) ethyl GSL	Homosinalbin		
Indolic GSLs			
48, Indol-3-yl methyl GSL	Glucobrassicin	**49**, 1*H*-Indole-3-acetonitrile	**50**, 1*H*-Indol-3-yl methyl ITC[1,8]
51, 4-hydroxyindol-3-yl methyl GSL	4-Hydroxyglucobrassicin		
52, 1-Methoxyindol-3-yl methyl GSL	Neoglucobrassicin	**53**, 1-Methoxy-1*H*-indole-3-acetonitrile (= caulilexin C)	
54, 4-Methoxyindol-3-yl methyl GSL	4-Methoxy-glucobrassicin	**55**, 4-Methoxy-1*H*-indole-3-acetonitrile (=arvelexin)	
56, 1,4-Dimethoxyindol-3-yl methyl GSL	1,4-Dimethoxy-glucobrassicin	**57**, 1,4-Dimethoxy-1*H*-indole-3-acetonitrile (= tenualexin)	

Notes. 1, isothiocyanate (primary GSL decomposition product); 2, epithionitrile (primary GSL decomposition product); 3, thiocyanate (primary GSL decomposition product); 4, oxazolidine-2-thione (ITC rearrangement product); 5, dithiocarbamate (ITC rearrangement product); 6, the hydrolysis product of glucosativin is 1,3-thiazepane-2-thione, but the the trivial name sativin can also refer to the isoflavonoid (41743-86-6); 7, phenyl GSL was discontinued according to (Blažević et al., 2020); 8, the isothiocyanate product is unstable and it is present as a biosynthetic intermediate only.

The complete list of queried compounds and their CAS numbers is available in the Supplementary Tables. The bibliographies from the two platforms were merged and filtered to remove duplicates in R (R Core Team, 2020). Despite the fact that several have relevance in Brassicaceae as pathogens, articles on oomycetes, Rhizaria and other non-fungal microorganisms were not included.

2. Interaction of glucosinolate precursors and fungi

Indole GSLs such as glucobrassicin (**48**), as well as Trp derived phytoalexins are biosynthesized from **65** (Fig. 2). Antifungal activity of **65** was only described in a few studies: A disc diffusion assay by Böhlendorf et al. (2006) found antifungal activity towards *Ustilago zeae* (Link) Unger (Ustilaginaceae), but not other tested fungi. In another study (Pedras and Montaut, 2003), the metabolism of **65** by fungi was examined. **65** was metabolized into **49** and 1*H*-indole-3-acetic acid (**95**) by pathogenic fungi, such as *Leptosphaeria maculans* Ces. & De Not. (Leptosphaeriaceae)/*Phoma lingam* (Tode) Desm. (o. Pleosporales), *Rhizoctonia solani* J.G. Kühn (Ceratobasidiaceae) and *Sclerotinia sclerotiorum* (Lib.) de Bary (Sclerotiniaceae) (Pedras and Montaut, 2003), mimicking plant biosynthetic routes (Fig. 2). The authors concluded that the enzymes responsible for this transformation are likely specific to crucifer-pathogens, as a non-phytopathogen metabolized the compounds through other compounds. As **65** and **49** also inhibited growth of the fungi to some extent, this can be viewed as a detoxification process. The ability to efficiently metabolize/detoxify host plant defensive compounds is a frequently found phenomenon in the literature of GSL pathway product-fungus interactions, as we will see later on.

The *Brassica* pathogen *S. sclerotiorum* contains an indoleacetaldoxime dehydratase (EC 4.99.1.6), as successfully characterized by Pedras et al. (2010). Compared to total synthetic analogue aldoximes, **65** was shown to be the best substrate of this enzyme, but it also accepted Tyr-based analogues. This flexibility is likely beneficial, as some plants – such as *Nasturtium officinale* R. Br. (Brassicaceae) – biosynthesize analogues of indole phytoalexins (Pedras and To, 2015). It is unknown whether this biotransformation contributes to the virulence of the fungus, as **65** does not accumulate in plants. On the other hand, presence of a specific biotransformation capability in a specialist pathogen suggests important functions—perhaps the prevention of the single-step conversion to **49**.

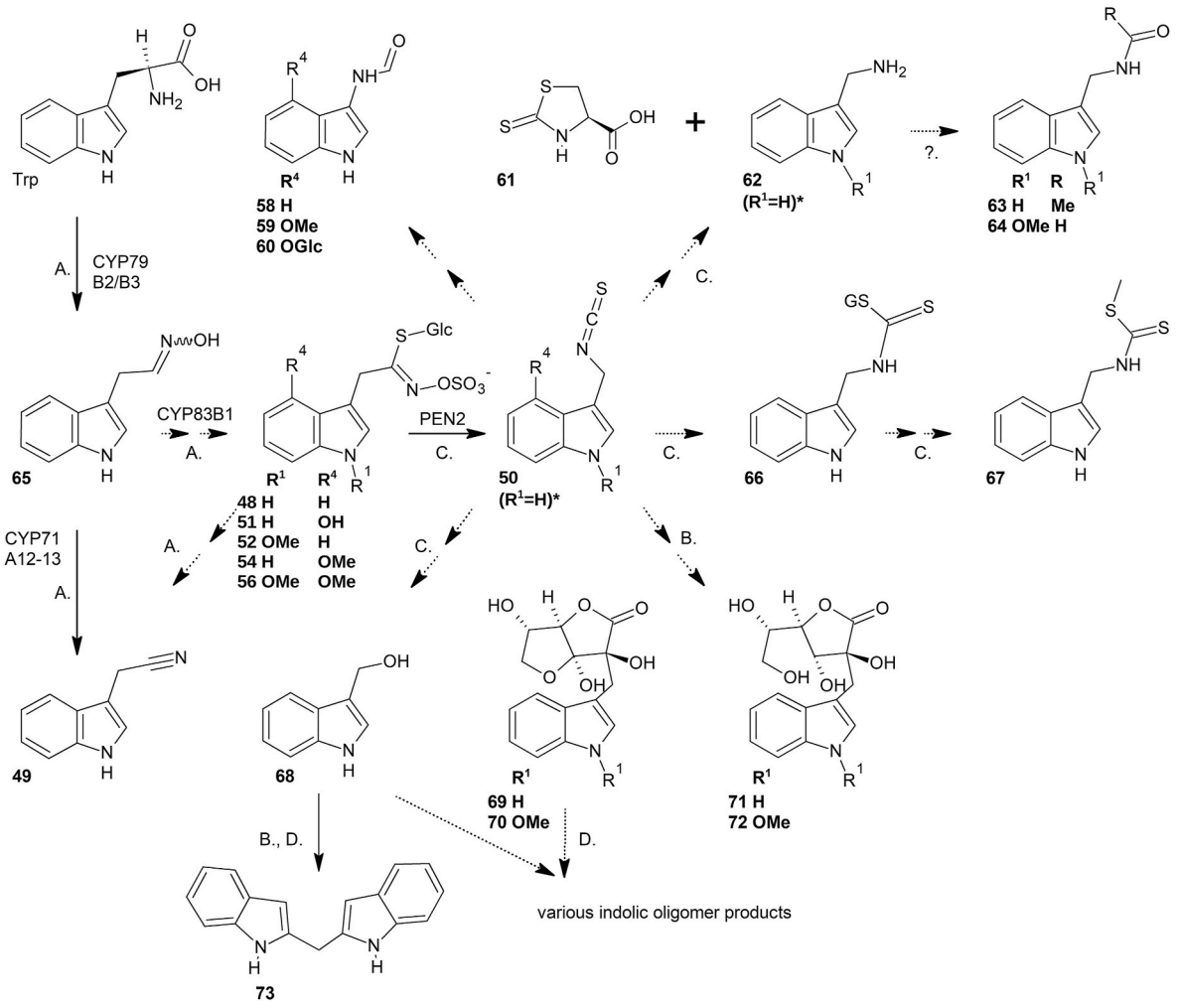

Fig. 2. Main indole glucosinolate isothiocyanate-derived downstream products. Biosynthetic step references: A. Böttcher et al. (2014), B. Agerbirk et al. (2009), C. Piślewska-Bednarek et al. (2018), D. Buskov et al. (2000). **Compound references: 48**, 1*H*-Indol-3-yl methyl glucosinolate (glucobrassicin); **49**, 1*H*-Indole-3-acetonitrile; **50**, 1*H*-Indol-3-yl methyl ITC; **51**, 4-Hydroxyindol-3-yl methyl GSL (4-Hydroxyglucobrassicin); **52**, 1-Methoxyindol-3-yl methyl GSL (Neoglucobrassicin); **54**, 4-Methoxyindol-3-yl methyl GSL (4-Methoxyglucobrassicin); **56**, 1,4-Dimethoxyindol-3-yl methyl GSL (1,4-Dimethoxyglucobrassicin); **58**, 1*H*-indol-3-yl formamide; **59**, N-(4-Methoxy-1*H*-indol-3-yl) formamide; **60**, N-(4-Glucosyloxy-1*H*-indol-3-yl) formamide; **61**, Raphanusamic acid; **62**, 1*H*-Indole-3-methanamine; **63**, N′-Acetyl-3-indolylmethanamine; **64**, N-(1-Methoxy-1*H*-indol-3-yl) methyl formamide (= caulilexin B); **65**, 1*H*-Indole-3-acetaldehyde oxime (IAOx); **66**, 1*H*-Indol-3-yl methyl isothiocyanate glutathione adduct; **67**, Brassinin; **68**, 1*H*-Indole-3-methanol; **69**, Ascorbigen; **70**, Neoascorbigen; **71**, Dihydroascorbigen; **72**, Dihydroneoascorbigen; **73**, 3,3′-Methylenebis-1*H*-Indole (diindoyl methane), **Trp**, Tryptophan. *, hydroxylation and methoxylation is biosynthetically done in GSL stage (**48, 51–52, 54, 56**), but is carried to downstream products.

3. Interaction of glucosinolates and fungi

3.1. Increased biosynthesis of glucosinolates during fungal challenge

In *in planta* studies, it is quite often concluded that different natural product classes are involved in defense against different fungal invaders. Numerous studies provide evidence that the GSL-derived compounds also have an important role in prevention of pathogenesis in planta, as detailed below. We should note that these papers cannot fully substitute research based on antifungal assays with pure compounds (the same is true vice versa). This is because plants deploy various other compounds for defense simultaneously, rendering the isolated study of efficacy of chemical entities a challenging task. In other words, the frequently shown increase in GSL concentrations after a fungal colonization attempt does not automatically render GSL pathway products the actual bioactive agents behind successful pathogen arrest. An example of the complexity of this issue is an NMR metabolomic study of *Brassica rapa* L. (Brassicaceae) (canola) which showed that inoculation with fungal pathogens resulted in an increase in concentrations of phenylpropanoids (sinapoyl malate, coumaroyl malate, caffeoyl malate), flavonoids (kaempferol, quercetin) and GSLs (**21**, **52**) as well (Abdel-Farid et al., 2009). Additionally, the biosynthesis of GSLs and downstream products is likely altered in other phenotypes, such as in mutants of *A. thaliana* with altered carbohydrate metabolism (Engelsdorf et al., 2016). In the latter case, despite altered biosynthesis of **54**, the authors concluded that penetration resistance to *Colletotrichum higginsianum* Sacc. (Glomerellaceae) is the result of altered carbohydrate availability.

In papers studying plant pathogen effects on GSL biosynthesis, the typical conclusion is that a higher basal GSL content, and/or the ability to respond to the fungal attack with a higher rate of GSL biosynthesis can result in decreased susceptibility to disease. An increased biosynthesis after fungal inoculation is described for various GSLs, including aliphatic, indole and other subclasses (Abdel-Farid et al., 2010; Ishimoto et al., 2004; Kuhn et al., 2017; Madloo et al., 2019; Novotny et al., 2018; Pedras and Zaharia, 2000; Robin et al., 2017; Sanchez-Vallet et al., 2010; Teng et al., 2021; Xu et al., 2016). However, the ratios of changes usually vary with the plant and/or cultivar (Abdel-Farid et al., 2010; Abuyusuf et al., 2018; Andini et al., 2019; Madloo et al., 2019; Robin

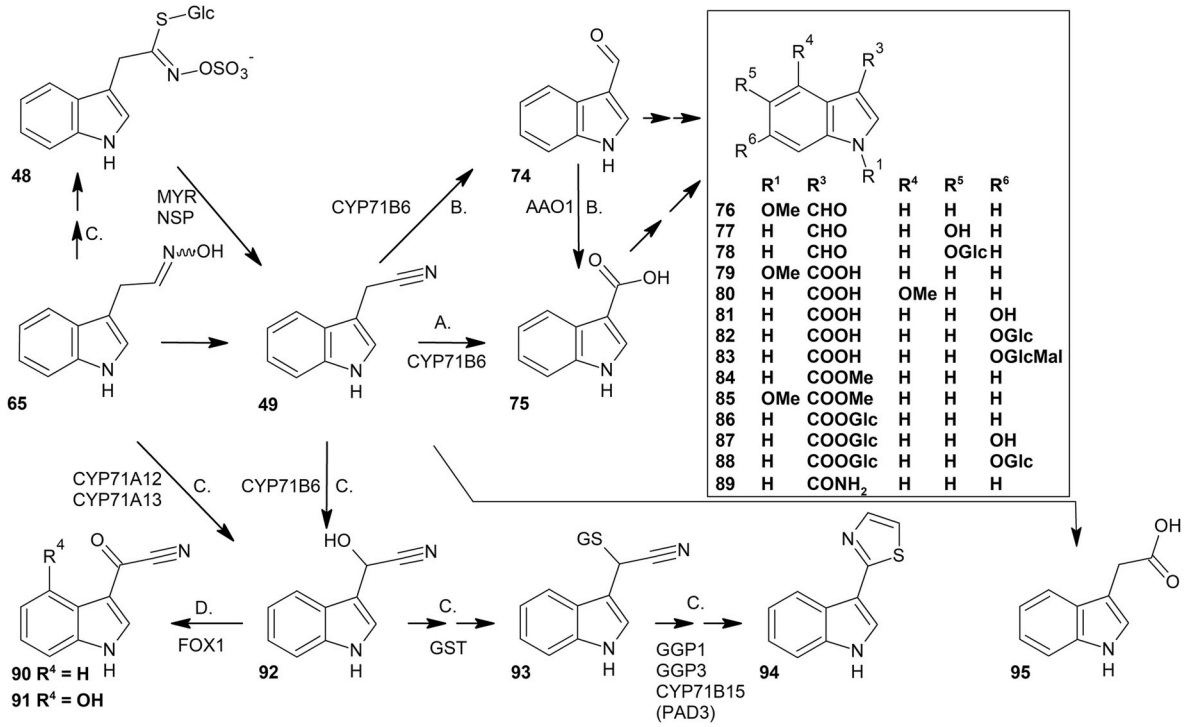

Fig. 3. Biosynthesis and main downstream products of 1*H*-indole-3-acetonitrile. Biosynthetic step references: A. Böttcher et al. (2014), B. Lahrmann et al. (2015), C. Mucha et al. (2019), D. Rajniak et al. (2015). **Compound references: 48**, 1*H*-Indol-3-yl methyl glucosinolate (glucobrassicin); **49**, 1*H*-Indole-3-acetonitrile; **65**, 1*H*-Indole-3-acetaldehyde oxime (IAOx); **74**, 1*H*-Indole-3-carboxaldehyde; **75**, 1*H*-Indole-3-carboxylic acid; **76**, 1-Methoxy-1*H*-indole-3-carboxaldehyde; **77**, 5-Hydroxy-1*H*-indole-3-carboxaldehyde; **78,** 5-Glucosyloxy-1*H*-indole-3-carboxaldehyde; **79**, 1-Methoxy-1*H*-indole-3-carboxylic acid; **80**, 4-Methoxy-1*H*-indole-3-carboxylic acid; **81**, 6-Hydroxy-1*H*-indole-3-carboxylic acid; **82**, 6-Glucosyloxy-1*H*-Indole-3-carboxylic acid; **83**, 6-Malonylglucosyloxy-1*H*-indole-3-carboxylic acid; **84**, 1*H*-Indole-3-carboxylic acid methyl ester; **85**, 1-Methoxy-1*H*-indole-3-carboxylic acid methyl ester; **86**, 1*H*-Indole-3-carboxylic acid glucose ester; **87**, 6-Hydroxy-1*H*-Indole-3-carboxylic acid glucose ester; **88**, 6-Glucosyloxy-1*H*-indole-3-carboxylic acid glucose ester; **89**, 1*H*-indole-3-carboxamide; **90**, 1*H*-Indole-3-carbonyl cyanide; **91**, 4-Hydroxy-1*H*-indole-3-carbonyl cyanide; **92**, α-Hydroxy-1*H*-indole-3-acetonitrile; **93**, α-Hydroxy-1*H*-indole-3-acetonitrile glutathione adduct; **94**, Camalexin; **95**, 1*H*-Indole-3-acetic acid (IAA).

et al., 2017, 2020), the plant organ (Lyons et al., 2015), the fungal strain (Abdel-Farid et al., 2010; Andini et al., 2019; Gallo et al., 2013; Robin et al., 2020) and the density of the fungal inoculum (Pétriacq et al., 2016). Additionally, a recent study even concluded that there is a significant diurnal variation in GSL response (Rupp et al., 2020). This renders the prediction of the outcome of an interaction a tough task.

The spatial distribution of the response within the plant was shown by a *Brassica napus* L. (Brassicaceae) infection model (Li et al., 1999), while local gene expressions were shown by an excellent study on YFP-transgenic plants with live cell microscopy (Hunziker et al., 2020), providing additional strong evidence to the role of these compounds in defense against fungi. The response (indole GSL biosynthesis and subsequent 4-methoxylation) took place in the epidermis of *A. thaliana* (Hunziker et al., 2020). These results highlight the need of analytical techniques capable of studying spatial distributions to study such phenomena, as these are not prone to dilutive effects of non-exposed cells or tissues.

3.2. Lack of direct antifungal effects of glucosinolates

As briefly addressed before, GSLs are only precursors to the compounds with actual bioactivity. Native GSLs themselves show no direct antifungal activity, as shown in the literature (Manici et al., 1997; Mithen et al., 1986; Pedras and Hossain, 2011; Shofran et al., 1998). Sinigrin (**7**) also did not influence the germination of blastospores of fungal entomopathogen *Paecilomyces fumosoroseus* (Wize) A.H.S. Br. & G. Sm. (Thermoascaceae) *in vitro*, up to a high concentration of 1 mg/mL (Vega et al., 1997). The desulfo-derivatives of **48** and **52** were also shown to be inactive in the study of Pedras and Hossain (2011).

3.3. Myrosinase activity and glucosinolate decomposition by fungi

The decomposition of GSLs by fungi was described as early as 1958 by Reese et al. showing that **7** is converted to allyl isothiocyanate (**9**) by an *Aspergillus sydowii* (Bainier & Sartory) Thom & Church (Aspergillaceae) strain. The purified *A. sydowii* myrosinase (thioglucoside glucohydrolase) was not activated by ascorbic acid, unlike that typical of myrosinases of plant origin (Ohtsuru et al., 1969a), suggesting a markedly different structure, possibly developed by convergent evolution. Later, the same property was shown for a myrosinase from *Aspergillus niger* Tiegh. (Aspergillaceae) (Ohtsuru and Hata, 1973).

To date, GSL decomposition by fungi has been described in several instances, suggesting a rather widespread utilization of thioglucosides throughout the fungal kingdom. Another article by the same group cited above also suggested that fungal myrosinases might be considered β-glucosidases (EC 3.2.1.21) highly specialized for the hydrolysis of GSLs (Ohtsuru et al., 1969b). This was later confirmed for plant myrosinases by biochemical, genetic and phylogenetic data: the review by Rask et al. (2000) concluded that myrosinases evolved from less specific O-β-glucosidases. These enzymes are responsible for the activation of cyanogenic glucosides—among other plant defensive precursors.

Detection of myrosinase activity in fungi is frequently accomplished through solid-state fermentation studies. In these papers, the seed meal of a Brassicaceae industrial crop is inoculated with fungi and the decrease in GSL concentration is detected with some sort of chromatographic technique. The activity itself can be directly measured via spectrophotometric or pH stat assays (Piekarska et al., 2013). Indirect detection by showing the presence of specific decomposition products is also a viable alternative. From the headspace of various soil fungi and

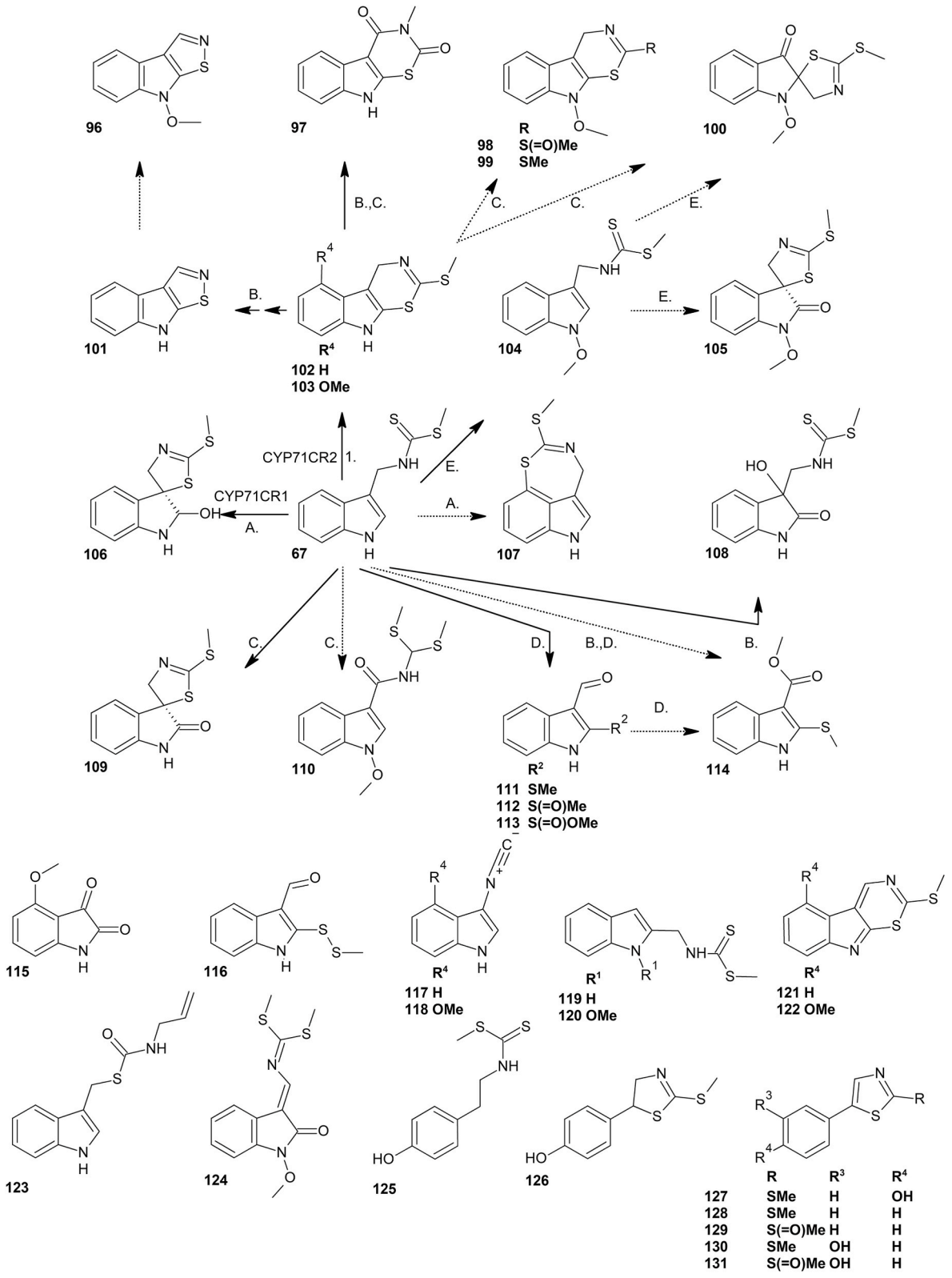

Fig. 4. Proposed biosynthetic connections of indole-derived Brassicaceae phytoalexins from brassinin. Biosynthetic step references: A. Blažević et al. (2020), B. Pedras et al. (2007b), C. Klein and Sattely (2017), D. Pedras et al. (2004b), E. Pedras et al. (2006b), Pedras et al. (2002). **Compound references: 67**, Brassinin; **96**, Sinalexin; **97**, Rutalexin; **98**, Sinalbin A; **99**, Sinalbin B; **100**, Erucalexin; **101**, Brassilexin; **102**, Cyclobrassinin; **103**, 4-Methoxy-cyclobrassinin; **104**; 1-Methoxy-brassinin; **105**, 1-Methoxy-spirobrassinin; **106**, Spirobrassinol; **107**, Cyclonasturlexin; **108**, Dioxybrassinin; **109**, Spirobrassinin; **110**, 1-Methoxy-brassenin B; **111**, Brassicanal A; **112**, Brassicanal A sulfoxide; **113**, Brassicanal C; **114**, Brassicanate A; **115**, Isalexin; **116**, Caulilexin A; **117**, 1*H*-Indole-3-isonitrile; **118**, 4-Methoxy-1*H*-indole-3-isonitrile (Isocyalexin A); **119**, Isobrassinin; **120**, 1-Methoxyisobrassinin; **121**, Dehydrocyclobrassinin; **122**, 4-Methoxy-dehydrocyclobrassinin; **123**, Brussalexin A; **124**, Wasalexin A; **125**, Nasturlexin A; **126**, Nasturlexin B; **127**, Tridentatol C; **128**, Nasturlexin C; **129**, Nasturlexin C sulfoxide; **130**, Nasturlexin D; **131**, Nasturlexin D sulfoxide.

endophytes growing on horseradish extract rich in GSLs, the specific decomposition products of **7**, allyl cyanide (**8**) and/or **9** could be detected (Plaszkó et al., 2020). Other approaches include screening studies that use media supplemented with a salt of Ba^{2+}, which produces a white precipitate upon SO_4^{2-} release from GSLs. This method enabled Sakorn et al. (2002) to show that three *Aspergillus* sp. strains from decayed mustard seeds have myrosinase activity. The specific enzymatic activity was also confirmed by running the assay with cell-free extracts of the fungus.

GSL decomposition by fungi is usually highly efficient. In solid-state fermentation of brown mustard seed meal, an *Aspergillus clavatus* Desm. (Aspergillaceae) and a *Fusarium oxysporum* Schltdl. (Nectriaceae) strain, as well as the endogenous flora, were capable of decomposing **7** and **47** within a few days (Smits et al., 1993). The same fungi decomposed 8 mM **7** within 12 h when incubated under non-growth conditions. *A. clavatus* decomposed **7** with the production of **8**, whereas *F. oxysporum* decomposed it with the release of no detectable volatile constituent. The cell-free extract of *A. clavatus* was also active, suggesting the presence of a specific decomposing enzyme. The importance of fungi in the decomposition of GSLs is apparent from the study of Morra and Borek (2010): the authors showed that GSLs can be preserved in Brassicaceae oilseed meals for as long as 30 months if the seed meal is protected from exposure to moisture, which promotes microbial growth. Otherwise, obvious fungal growth occurred, which coincided with decomposition of GSLs.

The myrosinase activity of various additional fungi was described, a complete list is shown in Table 2. The taxonomic variability is easily recognized, just like the abundance of *Aspergillus* spp. and *Fusarium* spp. The data suggest that it may not be a coincidence that *Fusarium* is one of the dominant fungal genera of the Brassicaceae rhizosphere, as shown by Ishimoto et al. (2000). Several fungi from the rhizosphere of *Eruca sativa* Mill. (Brassicaceae) and the endophytes of the same plant were also active according to Abdel-Fatah et al. (2021).

It's also worth noting that several of these fungi were isolated from a plant source, or the vicinity of a plant source that biosynthesizes GSLs: the fungi in the study of Szűcs et al. (2018) were isolated from roots of *Armoracia rusticana* G. Gaertn., B. Mey. & Scherb. (Brassicaceae), those of Sakorn et al. (2002) were from decayed mustard seed meal, the fungus used in the study of Ishimoto et al. (2004) was isolated from roots of a Brassicaceae plant. Moreover, the utilization of GSLs as a nutrient was also evidenced (Szűcs et al., 2018): several endophytic fungi were able to use **7** as a sole carbon source. Altogether, this suggest a specific adaptation of fungi to the utilization of GSLs.

Of course, studies also found lots of inactive strains, such as Chung et al. (2003), who could not detect any activity from *Aspergillus* sp., *Trichoderma* spp., and *Penicillium* spp.; or Szűcs et al. (2018), who found *Oidiodendron cerealis* (Thüm.) G.L. Barron (Myxotrichaceae) to be unable to decompose **7**, despite being an endophyte isolated from a Brassicaceae root. In the study of Sakorn et al. (2002), isolates of *Rhizopus* sp. and *Mucor* sp., isolated from decayed mustard seed, were also inactive.

As there is a considerable publication bias towards active strains, there are no exact data on the spread of thioglucoside hydrolases throughout the fungal kingdom. It would be quite interesting to systematically assess the spread of this activity either via systematic sampling and testing of fungi or through bioinformatics approaches.

During GSL breakdown, fungi probably use the glucose as a source of carbon and energy, but they have to cope with the toxicity of the released ITCs (Plaszkó et al., 2021), in case the default rearrangement of the aglycon takes place. The *Aspergillus* sp. in the paper by Butrindr et al. (2004) produced **9** but not **8**, while Chung et al. (2003), Galletti et al. (2008a) and Rakariyatham and Sakorn (2002) found only the less fungitoxic product **8**. Detection is not that straightforward, though: a significant proportion of the intracellularly released ITC can be in chemically bound form, as glutathione-conjugates, as concluded by Szűcs et al. (2018), who showed the presence of conjugates of **9** from *A. rusticana* extract metabolized by endophytes of the same plant, e.g., *Phoma radicina* (McAlpine) Boerema (o.Pleosporales) and *Setophoma terrestris* (H.N. Hansen) Gruyter, Aveskamp & Verkley (Phaeosphaeriaceae). In general, several examples show that avoiding ITC release is not the only strategy used by fungi during decomposition of GSLs in plant material. In fact, there are several studies on ITC detoxification by fungi, as reviewed by Plaszkó et al. (2021).

Table 2
Evidence of myrosinase activity in various fungal strains.

Fungal strains	Ref.
Aspergillus ficuum	Olukomaiya et al. (2020)
Aspergillus flavus	Galletti et al., (2008b)
Aspergillus niger	Jannathulla et al. (2017)
Aspergillus sojae	Olukomaiya et al. (2020)
Aspergillus sp.	Butrindr et al. (2004); Rakariyatham and Sakorn (2002)
Aspergillus terreus	Wang et al. (2012)
Aureobasidium pullulans	Croat et al. (2016)
Aureobasidium pullulans & *Neurospora crassa* co-culture	Alhomodi et al. (2021)
Curvularia sp.	Plaszkó et al. (2020)
Fusarium oxysporum	Chung et al. (2003); Szűcs et al. (2018)
Fusarium sp.	Ishimoto et al. (2004); Plaszkó et al. (2020)
Fusarium venenatum	Croat et al. (2017)
Lichtheimia sp.	Wang et al. (2012)
Macrophomina phaseolina	Szűcs et al. (2018)
Notophoma sp.	Plaszkó et al. (2020)
Paraphoma sp.	Plaszkó et al. (2020)
Phoma radicina	Szűcs et al. (2018)
Plectosphaerella sp.	Plaszkó et al. (2020)
Rhizopus sp.	Ishimoto et al. (2000)
Setophoma terrestris	Szűcs et al. (2018)
Trichosporon cutaneum	Huber et al. (1983)
Trichoderma reesei	Croat et al. (2017, 2016)

Authorities of fungal taxa: *Aspergillus ficuum* (Reichardt) Thom & Currie (Aspergillaceae), *Aspergillus flavus* Link (Aspergillaceae), *Aspergillus niger* Tiegh. (Aspergillaceae), *Aspergillus sojae* Sakag. & K. Yamada ex Murak. (Aspergillaceae), *Aspergillus terreus* Thom (Aspergillaceae), *Aureobasidium pullulans* (De Bary) G. Arnaud ex Cif., Ribaldi & Corte (Saccotheciaceae), *Fusarium venenatum* Nirenberg (Nectriaceae), *Fusarium oxysporum* Schltdl. (Nectriaceae), *Macrophomina phaseolina* (Tassi) Goid. (Botryosphaeriaceae), *Neurospora crassa* Shear & B.O. Dodge (Sordariaceae), *Phoma radicina* (McAlpine) Boerema (o. Pleosporales), *Setophoma terrestris* (H.N. Hansen) Gruyter, Aveskamp & Verkley (Phaeosphaeriaceae), *Trichosporon cutaneum* (Beurm., Gougerot & Vaucher bis) M. Ota (Trichosporonaceae), *Trichoderma reesei* E.G. Simmons (Hypocreaceae).

4. Evidences of *in vivo* glucosinolate decomposition

4.1. Increased biosynthesis of glucosinolate downstream products during fungal challenge

Although GSLs have no direct antifungal activity, their importance as the precursors of bioactive decomposition products in defense against fungi can be concluded from a large number of research papers.

Successful arrest of the colonizing fungi is typically accompanied by an increase in the concentrations of GSL downstream products, highlighting the relevance of *in vivo* GSL decomposition and its products in plant-fungus interactions. Most evidence comes from papers that compare *A. thaliana* mutants in their disease tolerance and defensive compound concentrations during colonization attempts of fungal pathogens.

The possible primary result of the GSL decomposition is summarized in Fig. 1. Among GSL decomposition products, the potent antifungal ITCs are the most frequently studied, but they are beyond the scope of this paper. While the reader interested in ITC-fungus interactions should consult a recent review such as that by Plaszkó et al. (2021), evidence on the direct antifungal activity of other GSL decomposition products are presented in detail herein, in sections 5-10.

The role of myrosinase in GSL decomposition and ITC release during

tissue disruption was recognized long ago. As GSLs themselves have no antifungal activity, the *in vivo* effects are also thought to be brought about by decomposition products. The inability to biosynthesize GSLs or indole compounds (Sanchez-Vallet et al., 2010) clearly results in an increase in sensitivity towards many types of fungal pathogens, but *in planta* GSL decomposition is currently not fully understood; recent papers concluded that currently unknown decomposition products may contribute to *in vivo* antifungal effects (Frerigmann et al., 2016; He et al., 2019; Kuhn et al., 2017; Yun et al., 2016). It is reasonable to think that our views in this field will change significantly in the coming years.

Besides the "classical" myrosinases (TGG in *A. thaliana*), there are additional enzymes possibly responsible for deglycosylation of GSLs. The study of Bednarek et al. (2009) on disease-resistant *A. thaliana* lines suggested that PEN2-dependent hydrolysis of indole GSLs does occur *in vivo* in living plant cells, localized at the cell periphery at fungal entry sites (Fuchs et al., 2016), resulting in indole ITCs that are converted to dithiocarbamates and further to the respective **62** derivatives and **61**. TGG was not shown to be required in the accumulation of extracellular indole GSL decomposition products according to Xu et al. (2016). Successful defense may also require CYP81F2 (responsible for 4-methoxylation of indole GSLs) and PEN3 (accumulating at the sites of fungal invasion) (Hiruma et al., 2010; Lipka et al., 2010). An indirect result pointing towards ITC release was published by Fukunaga et al. (2017), who suggested that Trp-derived metabolites and PEN2 both play a major role in the ROS-mediated cell death, as increased ROS generation was absent in *pen2* plants. Note that the GSL decomposition product ITCs are oxidative stressors, that act by consuming glutathione (GSH) (Bertóti et al., 2016).

On the other hand, although TGG is usually associated with ITC production upon tissue damage, its role in fungal defense can also be suspected based on the work of Agee et al. (2010). The authors found that the modified vacuole phenotype1-1 *A. thaliana* shows increased susceptibility towards *Alternaria brassicicola* (Schwein.) Wiltshire (Pleosporaceae). The affected MVP1 specifically interacts with TGG2. According to Buxdorf et al. (2013), the *tgg1/2* lines that are unable to biosynthesize ITCs from GSLs are also more sensitive to *A. brassicicola* and an adapted *Botrytis cinerea* Pers. (Sclerotiniaceae) isolate, but not a different *B. cinerea* isolate from grape. *In vitro* inhibition assays with ITCs against these fungi supported this hypothesis. Altogether, this strongly suggests some role of TGG-released ITCs in defense against fungi. The same study also found that epithiospecifier protein overexpressing plants – which mainly produce less potent epithionitriles – were also more sensitive to *A. brassicicola* than wild type accessions. However, the pattern of various Trp-derived (indole GSL-derived) metabolites strongly depends on the species examined, though the increased biosynthesis phenomenon seems to be rather generic, as shown in the study of Bednarek et al. (2011).

Altogether, our current knowledge suggests a complex, flexible system with several enzymes being responsible for in vivo GSL decomposition in different circumstances. This diversity of decomposing enzymes and the ability to generate various decomposition products together provide the foundation for the ability to respond to various types of microbial invaders. In this system, the type and behavior of the attacker likely determine the proportion of different defensive compounds. This flexibility is also apparent at the GSL class level, as detailed in section 4.3.

4.2. The role of glutathione in glucosinolate downstream product biosynthesis

The amount of *in situ* generated glutathione is also important for *in vivo* antifungal defense, as shown in the following publications. The plastid-localized γ-glutamylcysteine synthetase GSH1 (EC 6.3.2.2) is involved in the PEN2-based entry control pathway, as shown by Hiruma et al. (2013). In the absence of a functional GSH1, the amount of GSH, **61** and **62** was significantly lower, and invasive growth of the non-adapted hemibiotroph *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (Glomerellaceae) occurred in *A. thaliana*. The tau member glutathione-S-transferase GSTU13 (EC 2.5.1.18), which may catalyze the conjugation reaction of GSH with ITCs from indole GSLs, is also a critical component for the PEN2-mediated immune-reactions against several pathogens including *Erysiphe pisi* DC. (Erysiphaceae), *C. gloeosporoides* and *Plectosphaerella cucumerina* (Lindf.) W. Gams (Plectosphaerellaceae) in *A. thaliana* according to Piślewska-Bednarek et al. (2018). The gstu13 plants biosynthesized only reduced amounts of end products of the PEN2 pathway (such as **60**, **61** and **62**) and were much more susceptible to the tested pathogens. The phytochelatin synthase1 PCS1 (EC 2.3.2.15) – which is responsible for synthesis and decomposition of the GSH oligomer phytochelatin – also co-localizes with PEN2 myrosinase in the event of a pathogen attack (Hématy et al., 2020). Interestingly, in this case, the authors found that dysfunctional variants of PCS1 (incapable of phytochelatin synthesis or deglycination of glutathione conjugates) remain functional in the metabolism of indole GSLs.

These results enable us to draw two conclusions. First, GSH can be a possible mechanism for the prevention of autotoxicity by the plant cells, which is logical if we recall the oxidative stressor role of ITCs that Bertóti et al. (2016) and Plaszkó et al. (2021) previously mentioned. Second, these results support the hypothesis that the actual bioactive agents against fungi are indole GSL downstream products which are biosynthesized through ITCs. It is worth noting that several of these contain an additional sulfur, probably originating from a coupling reaction of an ITC with a thiol such as GSH or Cys (Hanschen et al., 2014) (Fig. 2). These compounds include **61**, **67** and **109** as well as different ITC conjugate dithiocarbamates, among others.

4.3. Significance of GSLs in plant - fungus interactions: an outlook on compatible interactions, mycorrhizae and pathogens

Plants respond to fungal challenge with a large number of co-expressed genes responsible for biosynthesis of GSLs and downstream products (Frerigmann et al., 2016; Iven et al., 2012; Kidd et al., 2011), and loss of the function of these usually results in increased susceptibility towards pathogens (Humphry et al., 2010). What is more, different GSL classes were shown to be regulated differentially (Castillo et al., 2019; Doughty et al., 1995; Kiddle et al., 1994), causing different responses among GSL subclasses after challenge with fungi with different strategies (Buxdorf et al., 2013; Castillo et al., 2019; Tewes and Müller, 2020) which in turn result in differences in the susceptibility of plant lines with different GSL responses. Additionally, a recent paper (Tewes and Müller, 2020) concluded that the specialist *Alternaria brassicae* (Berk.) Sacc. (Pleosporaceae) is more sensitive to the chemotypic variability of the host (*Bunias orientalis* L. (Brassicaceae)) than the generalist *B. cinerea*. From the plant side, Robin et al. (2017) have found that in cabbage, the ability to simultaneously increase biosynthesis of aliphatic GSLs (**20** and **25**) and indole GSLs (**48**, **52** and **51**) upon fungal challenge is associated with complete resistance, while the increase in concentration in one of the GSL groups only conferred partial resistance to *L. maculans*.

It is worth noting that the increase in the concentration of GSLs and downstream products does not automatically result in successful defense against the attacker (Madloo et al., 2019). Successful pathogens, such as *S. sclerotiorum,* can have the ability to prevent increased GSL biosynthesis which might contribute to their pathogenicity (J. Chen et al., 2020). Additionally, there are many studies which failed to detect any correlation between fungal disease susceptibility and GSL concentration (Andreasson et al., 2001; Giamoustaris and Mithen, 1997; Li et al., 1999; Sexton et al., 2009; Witzel et al., 2015; Wretblad and Dixelius, 2000). At first glance, the above papers appear to provide conflicting evidence to the conclusions drawn in the above sections. However, this actually reinforces the conclusion that (1) the actual fungus-plant pair strongly influences the outcome of the fungal challenge; (2) the pathogen

strategy seems to be different in specialists and generalists, suggesting some sort of adaptation to the GSLs and their decomposition products in the former and (3) that efficient generation of GSL downstream products is also key to fungal arrest. The second hypothesis will be further supported by the presence of specific phytoalexin decomposition enzymes detected in specialist pathogens, summarized in section 9 of our review.

The products of the GSL pathway are also important for establishment of beneficial plant - fungus interactions, which are some form of a balanced antagonistic coexistence (Schulz and Boyle, 2005). Beneficial interactions with the beneficial basidiomycota *Piriformospora indica* Sav. Verma, Aj. Varma, Rexer, G. Kost & P. Franken (cl. Basidiomycetes), the endophyte *Colletotrichum tofieldiae* (Pat.) Damm, P.F. Cannon & Crous (Glomerellaceae) and the beneficial *Trichoderma harzianum* Rifai (Hypocreaceae) (or T. parareesei Jaklitsch & Atanasova (Hypocreaceae)) all rely on a functional indole GSL biosynthesis apparatus (Hiruma et al., 2016; Nongbri et al., 2012; Poveda, 2021; Sherameti et al., 2008; Zimmermann et al., 2021). Several authors concluded that successful establishment of the beneficial interaction seems to rely on limiting the effects of antifungal compounds via down-regulation of GSL biosynthesis (Brotman et al., 2013; Gallo et al., 2013; Hunziker et al., 2020; Kottb et al., 2015). Interestingly though, this can coincide with priming against subsequent pathogen attacks (Gallo et al., 2013). An additional strategy can be hypothesized from the results of Poveda et al. (2019): a Kelch protein facilitating root colonization from *T. harzianum* might be responsible for *in planta* decomposition of GSLs.

Though the relevance of GSL downstream products in these interactions is beyond doubt, the actual chemical species responsible for the fungal arrest are not known. Though 1-, and 4-methoxylation of indole GSLs was found to be important in many instances (Brotman et al., 2013; Hunziker et al., 2020) and the results Poveda et al. (2019) suggest the involvement of an indole ITC-derived downstream product group, future research should clarify the identity of the actual antifungal agents.

GSL pattern was also shown to be correlated with arbuscular mycorrhizal status of a broader set of tested species including plants from Brassiceae, Resedaceae as well as Arabidaceae (Vierheilig et al., 2000). On the other hand, the authors concluded that GSLs themselves are not the sole factor for these plants' mycorrhizal status, which is supported by the findings of Ludwig-Müller et al. (2002). While Vierheilig et al. (2000) concluded that **46** (from the benzenic **44**) might be an agent that prevents AMF colonization, a recent study (Anthony et al., 2020) suggested indole GSLs as the most significant contributors: a BLAST search of indole GSL biosynthesis genes has revealed that indole GSL orthologs are lost in mycorrhizal Brassicales which are present in non-mycorrhizal species.

Fortunately, not only indirect evidences exist on the significance of these interactions: there are many studies done with isolated strains and pure GSL breakdown products. In the following, we summarize our current knowledge on various GSL decomposition products.

5. Interactions of glucosinolate-derived nitriles and fungi

The most studied non-default decomposition product class of GSLs is the nitriles (Fig. 1). Nevertheless, the volume of their scientific literature is dwarfed by that on the ITCs; therefore, their ecological role is not fully understood. The chemical milieu and/or presence of specifier proteins can deter the default rearrangement path from ITCs toward nitriles as briefly summarized in Fig. 1.

Several studies suggest a possible *in vivo* role during protection against fungal pathogenesis by showing increased biosynthesis during fungal colonization. Examples include the study of Pedras and Ahiahonu (2004) in which an increased production of **49** and **55** in *Erucastrum gallicum* O.E.Schulz (Brassicaceae) was observed after exposure to *S. sclerotiorum*. Other similar results were obtained for **49** (Hiruma et al., 2013) and **53** (Pedras and Zheng, 2010) as well.

In addition to possible direct effects of nitriles, their probable utilization as a biosynthetic intermediate (Figs. 2–3) must also be considered. Unfortunately, the typical proportion of the biosynthesis of indole-derived nitriles directly from **65** and through indole GSLs is not known. On the other hand, though much fewer results are available when compared to ITCs, GSL-derived nitriles showed direct antifungal activity in many studies.

5.1. Direct antifungal effects of glucosinolate-derived nitriles

There are several studies available that showed that nitrile-containing mixtures from plants (essential oils) have antifungal activity (Blažević et al., 2010a, 2010b; Dosoky et al., 2016; Javaid et al., 2018; Mastelić et al., 2010; Petrović et al., 2017; Popović et al., 2020; Radonić et al., 2011; Rodriguez et al., 2006; Zhang and Chen, 2017). But as these mixtures typically contain significant amounts of the much more potent ITCs, they cannot be considered direct evidence of the antifungal activity of nitriles.

Fortunately, various GSL-derived nitrile compounds were reported to inhibit fungal growth in pure form; these results are summarized in Table 3. In general, we can state that nitriles are much less efficient direct antifungal agents, compared to ITCs. This is also our personal research experience with various fungi (unpublished results). A dose of 0.5 mM **49** caused 65.0% median growth inhibition of various fungi (Table 3), while **45** was considerably weaker. As methodology significantly influences the results, direct comparison of the antifungal potency ITCs and nitriles merely by comparing literature data is likely prone to biases. The median IC_{50} value for **9** against fungi was 0.251 mM according to Taylor et al. (2014) who added the agent into molten agar plates, and 0.1084 mM according to Szűcs et al. (2018) who used liquid cultures. The method of Taylor et al. (2014) resembles that of the relevant studies in Table 3 better, but still, the ITCs seem to be more potent than the relatively strong antifungal **49**. Additionally, benzenic ITCs were typically more potent (Szűcs et al., 2018; Taylor et al., 2014). Due to additional methodical issues such as the dependence of the antifungal activity of ITCs on the amount of nucleophiles in the medium (Andini et al., 2020), side-by-side comparison studies provide the most information. A study (Shofran et al., 1998) on yeasts has shown that the nitrile **8** (from **7**) was not inhibitory at concentrations as high as 1 mg/mL, the respective ITC (**9**) was inhibitory at MIC 1–4 μg/mL. Another study by Zasada et al. (2012) also found that the ITC **40** is more potent than its respective nitrile.

As Table 3 shows, most of the results are on **49**, and only four studies (Ata et al., 2011; Radulović et al., 2012; Pedras et al., 2015; Popović et al., 2020) reported data on benzenic nitriles. Perhaps the lack of data on the most widespread **8** is due to both low activity and the publication bias that hinders publication of negative results, as well as its extreme volatility, which is a serious technical issue in antimicrobial testing (Houdkova and Kokoska, 2020). The tested fungi are all plant pathogen models, with a few exceptions. This lack of functional variability of fungi is especially prevalent when one compares the literature published on the inhibition of fungi by GSL-derived ITCs (Plaszkó et al., 2021).

Additional effects have also been described. Compound **49** not only inhibits the growth but also the biosynthesis of the mycotoxin 15-*O*-acetyl-4-deoxynivalenol (15-ADON) in the wheat pathogen *Fusarium graminearum* Schwabe. (Nectriaceae). Additional effects of **49** also includes an influence on the branching pattern of hyphae and delayed macroconidium germination in *F. graminearum* (Luo et al., 2016). This effect was also shown for the biotransformation product **95**. The inhibitory effect on biofilm formation in *Candida albicans* (C.P. Robin) Berkhout (o. Saccharomycetales) was also reported (Oh et al., 2012), probably through strong stimulation of the transcription of NRG1, a transcription factor that influences filamentation and biofilm formation.

To better understand of the significance of nitriles in the plant-fungal interactions, other functional groups of fungi and nitriles from other GSL classes will need to be included in these tests in the future. Alternatively, comparison of the fungal microbiome of nitrile-dominant genotypes

Table 3
Antifungal activity of glucosinolate-derived nitriles. Additional details about the compounds can be found in Table S1.

ID	Source of agent	Fungal Strains	Fungus function	Activity against	Bioactive concentration	Ref.
Benzenic nitriles						
35	synthesized	*Aspergillus fumigatus*, *Candida albicans*	environmental human related	mycelial growth	MIC: 0.15 mg/mL MIC: 0.60 mg/mL	Radulović et al. (2012)
38	*Drypetes gossweileri*	*Candida albicans*	not indicated	mycelial growth	MIC: 16 μg/mL	Ata et al. (2011)
45	*Barbarea vulgaris* or synthesized	*Alternaria brassicicola*, *Leptosphaeria maculans*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	14% inhib. at 0.5 mM 9% inhib. at 0.5 mM 12% inhib. at 0.5 mM	Pedras et al. (2015)
45	standard	*Aspergillus niger*, *Candida albicans*, *Penicillium notatum*	environmental/ food spoilage	mycelial growth	MIC: 31.25 μg/mL MIC: 125 μg/mL MIC: 31.25 μg/mL	Popović et al. (2020)
Indole nitriles						
49	standard	*Fusarium oxysporum*	plant pathogen	mycelial growth	100% inhib. at 800 ppm	Hessayon (1952)
49	standard	*Sclerotinia trifoliorum*	plant pathogen	mycelial growth	50% inhib. at 0.1 mg/mL	Koivistoinen et al. (1959)
49	*Pseudomonas* sp. ST4	*Sporisorium scitamineum*	plant pathogen	hyphal growth	MIC: 0.6 mM	Liu et al. (2020)
49	standard	*Fusarium graminearum*	mycotoxigenic	mycelial growth, mycotoxin production	1 mM (semiquantitative)	Luo et al. (2016)
49	standard	*Leptosphaeria maculans*	plant pathogen	mycelial growth	15% inhib. at 0.4 mM	Mithen et al. (1986)
49	standard	*Candida albicans*	nematode pathogen	biofilm formation	1 mM (semiquantitative)	Oh et al. (2012)
49	standard	*Alternaria brassicicola*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	27% inhibition at 0.5 mM 41% inhib. at 0.5 mM 27% inhib. at 0.5 mM	Pedras and Hossain (2011)
49	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	25% inhib. at 0.5 mM	Pedras and Snitynsky (2010)
49	*Brassica juncea*	*Phoma lingam*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	100% inhib. at 0.5 mM 86% inhib. at 0.5 mM 38% inhin. at 0.5 mM	Pedras et al. (2002)
49	standard	*Phoma lingam*, *Rhizoctonia solani*	plant pathogen	mycelial growth	70% inhib. at 0.5 mM 71% inhib. at 0.5 mM	Pedras and Montaut (2003)
49	standard	*Leptosphaeria maculans*	plant pathogen	mycelial growth	100% inhib. at 0.5 mM	Pedras et al. (2003)
49	synthesized	*Alternaria brassicicola*	plant pathogen	mycelial growth	32% inhib. at 0.5 mM	Pedras et al., (2009a)
49	*Isatis tinctoria* or synthesized	*Cladosporium cucumerinum*, *Coniophora puteana*	plant pathogen	mycelial growth	TLC assay	Seifert and Unger (1994)
49	*Brassica oleracea*	*Penicillium chrysogenum*	not indicated	mycelial growth	100% inhib. at 0.20 mg/g medium	Smissman et al. (1961)
49	standard	*Bipolaris oryzae*	plant pathogen	mycelial growth	TLC assay	Ueno et al., (2005a)
49	standard	*Magnaporthe grisea*	plant pathogen	spore germination	100 μg/mL (semiquantitative)	Ueno et al., (2005b)
49	synthesized	*Botrytis cinerea*	plant pathogen	mycelial growth	65% inhib. at 0.5 mM	Pedras et al., (2011a)
49	standard	*Alternaria brassicicola*	plant pathogen	mycelial growth	100% inhib. at 0.5 mM	Pedras and Abdoli (2013)
53	synthesized	*Alternaria brassicicola*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	43% inhibition at 0.5 mM 100% inhib. at 0.5 mM 54% inhib. at 0.5 mM	Pedras and Hossain (2011)
53	*Erucastrum canariense*	*Alternaria brassicicola*, *Leptosphaeria maculans*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	30% inhib. at 0.5 mM 35% inhib. at 0.5 mM 79% inhib. at 0.5 mM	Pedras and To (2017)
53	synthesized	*Leptosphaeria maculans*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	77% inhib. at 0.5 mM 100% inhib. at 0.5 mM 30% inhib. at 0.5 mM	Pedras et al., (2006a)
55	synthesized	*Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	66% inhib. at 0.5 mM 46% inhib. at 0.5 mM	Pedras and Ahiahonu (2004)
55	synthesized	*Alternaria brassicicola*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	59% inhib. at 0.5 mM 70% inhib. at 0.5 mM 77% inhib. at 0.5 mM	Pedras and Hossain (2011)
55	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	73% inhib. at 0.5 mM	Pedras et al. (2003)
55	standard	*Leptosphaeria maculans*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	59% inhib. at 0.5 mM 70% inhib. at 0.5 mM 37% inhib. at 0.5 mM	Pedras et al., (2006a)
57	synthesized	*Alternaria brassicicola*, *Leptosphaeria maculans*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	100% inhib. at 0.5 mM 69% inhib. at 0.5 mM 100% inhib. at 0.5 mM 100% inhib. at 0.5 mM	Pedras and Yaya (2014)

Authorities of botanical taxa: *Barbarea vulgaris* W.T.Aiton (Brassicaceae), *Brassica juncea* (L.) Czern. (Brassicaceae), *Brassica oleracea* L. (Brassicaceae), *Drypetes gossweileri* S.Moore (Euphorbiaceae), *Isatis tinctora* L. (Brassicaceae).
Authorities of fungal taxa: *Alternaria brassicicola* (Schwein.) Wiltshire (Pleosporaceae), *Aspergillus fumigatus* Fresen. (Aspergillaceae), *Aspergillus niger* Tiegh. (Aspergillaceae), *Bipolaris oryzae* (Breda de Haan) Shoemaker (Pleosporaceae), *Botrytis cinerea* Pers. (Sclerotiniaceae), *Candida albicans* (C.P. Robin) Berkhout (o. Saccharomycetales), *Cladosporium cucumerinum* Ellis & Arthur (Cladosporiaceae), *Coniophora puteana* (Schumach.) P. Karst. (Coniophoraceae), *Fusarium graminearum* Schwabe (Nectriaceae), *Fusarium oxysporum* Schltdl. (Nectriaceae), *Leptosphaeria maculans* Ces. & De Not. (Leptosphaeriaceae), *Magnaporthe grisea* (T.T. Hebert) M.E. Barr (Magnaporthaceae), *Penicillium chrysogenum* Thom (Aspergillaceae), Penicillium notatum Westling (Aspergillaceae), *Phoma lingam* (Tode) Desm. (o. Pleosporales), *Rhizoctonia solani* J.G. Kühn (Ceratobasidiaceae), *Sclerotinia sclerotiorum* (Lib.) de Bary (Sclerotiniaceae), *Sclerotinia trifoliorum* Erikss. (Sclerotiniaceae), *Sporisorium scitamineum* (Syd.) M. Piepenbr., M. Stoll & Oberw. (Ustilaginaceae).
Compound references: 35, 2-Phenylacetonitrile; **38**, 4-Hydroxy-2-phenylacetonitrile; **45**, 3-Phenylpropanenitrile; **49**, 1*H*-Indole-3-acetonitrile; **53**, 1-Methoxy-1*H*-indole-3-acetonitrile (= caulilexin C); **55**, 4-Methoxy-1*H*-indole-3-acetonitrile (=arvelexin); **57,** 1,4-Dimethoxy-1*H*-indole-3-acetonitrile (= tenualexin).

with ITC-dominant genotypes (similar to the genotypes used in the studies by Buxdorf et al. (2013) and Witzel et al. (2015)) could also make a significant contribution to our knowledge.

5.2. Biosynthesis of nitriles, identical to glucosinolate decomposition products, by fungi

The complexity of possible interactions is further complicated by the fact that several fungi have the ability to produce **49** or other nitriles as well as several other defensive products in plants (Böttcher et al., 2014; Schmidt et al., 2020). This suggests additional possible interactions between plants and fungi, as **49** may act as a precursor of e.g. derivatives of **75**. These derivatives are also thought to play a role in plant-fungus interactions, yet proof of them being phytoalexins is lacking to date (Schmidt et al., 2020). Fungi shown to be capable of biosynthesizing **49** or other nitriles have been summarized in Table 4. The considerable taxonomic and functional variability is easily recognized: the list includes aliphatic, benzenic and indole nitriles. These results typically come from studies that grew fungi in typical microbiological media, followed by purification of compounds and structure elucidation. Therefore, the *in planta* relevance of these biosynthetic capabilities is currently unknown. The relative simplicity of the biosynthesis of these nitriles from the respective amino acids should prevent us from drawing the simple, prejudicative conclusion that these fungi interfere with the GSL system via their nitriles. However, it would be interesting to see what substrates are used to generate these compounds if *in planta* biosynthesis could occur.

Table 4
Evidences of biosynthesis of nitriles identical to glucosinolate decomposition products, by fungi. Additional details about the compounds can be found in Table S1.

ID	Strain	Fungus function	Ref.
Aliphatic nitriles			
15	*Penicillium aurantiogriseum*	plant pathogen	García-Gómez et al. (2019)
17	Mixture of: *Aspergillus versicolor, Fusarium culmorum, Penicillium chrysogenum, Ulocladium botrytis, Wallemia sebi*	not indicated	Claeson et al. (2002)
19	*Aspergillus* sp.	*Melia azedarach* endophyte	Xiao et al. (2014)
Benzenic nitriles			
33	*Eremothecium cymbalariae*	not indicated	Ravasio et al. (2014)
33	*Gliocladium roseum*	*Eucryphia cordifolia* endophyte	Strobel et al. (2008)
33	*Talaromyces stipitatus*	marine-derived	Agrawal et al. (2021)
35	*Dichotomomyces cejpii*	marine-derived	Barra et al. (2017)
35	*Penicillium expansum*	environmental	Kim et al. (2019)
38	*Penicillium chrysogenum*	marine-derived	Qu et al. (2012)
45	*Aspergillus terreus*	marine-derived	Agrawal et al. (2021)
Indole nitriles			
49	*Aureobasidium pullulans*	phylloplane fungi	Buckley and Pugh (1971)
49	*Cantharellus cibarius*	ectomycorrhizal	Strzelczyk et al. (1997)
49	*Cladosporium herbarum*	moss related	Valadon and Lodge (1970)
49	*Epicoccum nigrum*	phylloplane fungi	Buckley and Pugh (1971)
53	*Verticillium dahliae*	plant pathogen	Zhang et al. (2016)

Authorities of botanical taxa: *Eucryphia cordifolia* Cav. (Eucryphiaceae), *Melia azedarach* L. (Meliaceae).
Authorities of fungal taxa: *Aspergillus terreus* Thom (Aspergillaceae), *Aspergillus versicolor* (Vuill.) Tirab. (Aspergillaceae), *Aureobasidium pullulans* (De Bary) G. Arnaud ex Cif., Ribaldi & Corte (Saccotheciaceae), *Cantharellus cibarius* Fr. (Hydnaceae), *Cladosporium herbarum* (Pers.) Link (Cladosporiaceae), *Dichotomomyces cejpii* (Milko) D.B. Scott (Trichocomaceae), *Epicoccum nigrum* Link (Didymellaceae), *Eremothecium cymbalariae* Borzí (Eremotheciaceae), *Fusarium culmorum* (Wm.G. Sm.) Sacc. (Nectriaceae), *Gliocladium roseum* Bainier (Hypocreaceae), *Penicillium aurantiogriseum* Dierckx (Aspergillaceae), *Penicillium chrysogenum* Thom (Aspergillaceae), *Penicillium expansum* Link (Aspergillaceae), *Talaromyces stipitatus* (Thom) C.R. Benj. (Trichocomaceae), *Ulocladium botrytis* Preuss (Pleosporaceae), *Verticillium dahliae* Kleb. (Plectosphaerellaceae), *Wallemia sebi* (Fr.) Arx (Wallemiaceae).
Compound references: 15, 2-Methylpropanenitrile; **17**, Hexanenitrile; **19**, 3-Hydroxybutanenitrile; **33**, Benzonitrile; **35**, 2-Phenylacetonitrile; **38**, 4-Hydroxy-2-phenylacetonitrile; **45**, 3-Phenylpropanenitrile; **49**, 1*H*-Indole-3-acetonitrile; **53**, 1-Methoxy-1*H*-indole-3-acetonitrile (= caulilexin C).

5.3. Metabolism/detoxification of nitriles by fungi

Though much less toxic than the ITCs, high concentrations of nitriles have inhibitory effects on fungi. Therefore, these organisms have to detoxify these plant compounds. There are several manuscripts on metabolism of GSL-derived nitriles by fungi, or on fungal nitrilases that can theoretically accept GSL-derived nitriles as substrates. Fungi that were shown to be able to metabolize **49** include *P. lingam (L. maculans)* (Pedras et al., 2002), *F. graminearum* (Luo et al., 2016), *Beauveria bassiana* (Bals.-Criv.) Vuill. (Cordycipitaceae) (Boaventura et al., 2004), *S. sclerotiorum* (Pedras et al., 2010; Pedras and Hossain, 2011), *R.* solani and *A. brassicicola* (Pedras and Hossain, 2011) and the endophyte *Cyanodermella asteris* L. Jahn & Ludw.-Mull. (Stictidaceae) (Jahn et al., 2021). In Pedras and Hossain (2011), the tested fungi also decomposed the nitriles **53** and **55** into their respective carboxylic acid derivatives. The products **75** and **80** were also much less inhibitory to the fungi than their respective nitrile precursors: the median inhibitory effect of **75** compared to **49** at 0.5 mM was 24.4% and 65%, respectively (Tables 3 and 6). Hence, this is an effective detoxification route, whereas the biotransformation of the 1-methoxy derivative **53** did not reduce overall antifungal activity. The insect pathogen *B. bassiana* uses alternative biotransformation routes (Boaventura et al., 2004; Pedras and Montaut, 2003).

Biotransformation of nitriles usually takes place via nitrilases (EC 3.5.5.1)—enzymes that hydrolyze nitriles to the corresponding carboxylic acids—which are present in various fungi but are absent in most of the plant kingdom (Harper, 1977). An interesting example are the fungal nitrilases from *Trametes versicolor* (L.) Lloyd (Polyporaceae) and *Agaricus bisporus* (J.E. Lange) Imbach (Agaricaceae), which were shown to be closer homologues to plant nitrilase NIT4 than to NIT1/2/3 based on their amino acid sequences (Rucká et al., 2020). These fungal enzymes were able to biotransform **45**, a cyanogenic glycoside and other nitriles as well. Additional fungi that can decompose benzenic nitriles (e.g. **33** and **35**) include *Gibberella moniliformis* Wineland (Nectriaceae), *Neurospora crassa* Shear & B.O. Dodge (Sordariaceae) and *A. niger* (Kaplan et al., 2011), *Gibberella intermedia* (authority unverifiable) (Y. Wu et al., 2013), *A. sydowii* (de Oliveira et al., 2014), *Fusarium solani* (Mart.) Sacc. (Nectriaceae) and *Penicillium multicolor* Grig.-Man. & Porad. (Aspergillaceae) (Kaplan et al., 2006a) *Fusarium lateritium* Nees (Nectriaceae) (Nolan et al., 2003) and a yeast *Cryptococcus* strain (Rezende et al., 2000). Since the resulting decomposition product is **95** (Luo et al., 2016), the wheat pathogen *F. graminearum* likely also utilizes a nitrilase.

Additionally, several fungi were shown to be able to use various nitriles as sole sources of carbon and nitrogen, as shown in the case of **33** (Harper, 1977), **2**, **4**, **6**, or dinitriles unsimilar to those originating from

GSLs (Kuwahara et al., 1980). Nitriles as the sole nitrogen source were also accepted by various yeasts from a Brazilian gold mine including *Aureobasidium* (Rezende et al., 1999), the yeast *Exophiala oligosperma* Calendron ex De Hoog & Tintelnot (Herpotrichiellaceae) (Rustler and Stolz, 2007), *F. oxysporum* (Gong et al., 2011), the marine fungi *A. sydowii*, *Bionectra* sp., *Penicillium decaturense* S.W. Peterson, E.M. Bayer & Wicklow (Aspergillaceae) and *Penicillium raistrickii* G. Sm. (Aspergillaceae) (de Oliveira et al., 2013), and Kaplan et al. (2006a) also used a medium containing pyridine-3-carbonitrile as the sole source of nitrogen to enrich *F. solani*.

Several fungal nitrilases accept a wide range of substrates, as shown by Petříčková et al. (2012) and Yusuf et al. (2015). However, selectivity studies show that different nitrilases in fungi of Agaricomycotina have different affinities towards substrates **33** and **45** (Rucká et al., 2019). The nitrilase is usually induced by the presence of its substrates (Kaplan et al., 2006b). The enzymes could be classified into several subclasses by Veselá et al. (2016): aromatic nitrilases (from *Aspergillus kawachii* (authority unverifiable)), arylacetonitrilases (EC 3.5.5.5) (from *A. kawachii* and *Macrophomina phaseolina* (Tassi) Goid. (Botryosphaeriaceae)), broad substrate specificity nitrilases (from *Trichoderma virens* (J.H. Mill., Giddens & A.A. Foster) Arx (Hypocreaceae)) as well as nitrile hydratases (EC 4.2.1.84) (from *Botryotinia fuckeliana* (de Bary) Whetzel (Sclerotiniaceae), *Pyrenophora teres* Drechsler (Pleosporaceae)). Interestingly, the nitrilase from a *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg (Nectriaceae) isolate was capable of working at high efficacy in water-methanol and water-ethanol mixtures (~75%, ~60%) (Yusuf et al., 2015).

Overall, it seems that a wide range of fungi have specific enzymes to deal with the GSL-derived or other nitriles. Moreover, unlike with ITCs, this does not seem to be restricted to pathogens of Brassicaceae. ITCs show antifungal activity against a wide range of fungi, yet, detoxifying enzymes are not at all widespread: a specific enzyme capable of detoxification of ITCs was only recently described from a Brassicaceae pathogen (Jingyuan Chen et al., 2020).

Nevertheless, we must add that data on the detoxification capacity of several GSL-derived nitriles are still lacking, since nitrilases are usually detected using less expensive model nitriles, as in the examples above. In addition, the contribution of nitrilases to the virulence of the fungal pathogens of Brassicaceae plants is not yet known, let alone the *in planta* expression of possible fungal nitrilases tailored against GSL-derived nitriles. From the plant side, the wide availability of fungal nitrilases has likely been a significant driving force of further evolution for the GSL-derived compounds, resulting in more advanced downstream products, such as those dealt with in sections 7-8.

6. Interactions of fungi with other primary glucosinolate decomposition products

6.1. Interactions of fungi with oxazolidine-2-thiones and other isothiocyanate rearrangement products

When an ITC side chain contains a functional group that is reactive towards the carbon of the isothiocyanate group and the compound is flexible enough to enable the reaction, a rapid and spontaneous rearrangement takes place. One of the more widely known compounds arising via this scheme are the oxazolidine-2-thiones which are formed from 2-hydroxy-ITCs (Fig. 1). Theoretically several of the GSLs can form ITCs prone to this rearrangement, but only a subset were described from plant origin so far (Table S4). In some species, however, this compound group is one of the dominant GSL decomposition product groups, for example **24** in rocket (*E. sativa*) (Fechner et al., 2018), or **22** in *B. rapa* (Abdel-Farid et al., 2010). In a few instances, spontaneous rearrangements of 3-hydroxy-ITCs into 1,3-oxazinane-2-thiones was also described (Kjær et al., 1958, 1970) (Fig. 1).

In a study with various plant hydrolysates containing ITCs and oxazolidine-2-thiones, antifungal activity of the plant pellets was proven against *R. solani* (Lazzeri et al., 2004), but one cannot assess the contribution of oxazolidine-2-thiones to the activity from the applied experimental design. More informative are the studies by Manici et al. (2000) and Peterka and Schlosser (1989), as in these, the oxazolidine-2-thiones were compared to ITCs, by *in situ* generation from GSLs using myrosinases. The antifungal activity of these compounds was in general found to be much weaker compared to the ITCs.

The antifungal potency of ITC-derived dithiocarbamate rearrangement products is also much lower compared to the ITCs, according to Matsuoka et al. (1997) (Table 5). The rearrangement products of **27**, including **28**, **29** and **30** showed a MIC of 100–200 μg/mL for molds like *Aspergillus fumigatus* Fresen. (Aspergillaceae), and yeasts like *C. albicans* and *S. pombe* Lindner (Schizosaccharomycetaceae). Additionally, dithiocarbamates can be formed from ITCs with a free thiol in the side chain (Fig. 1). Though also a dithiocarbamate, **61** is dealt with among the indolic ITC downstream products (Fig. 2), as it is not a single-step conversion product of a GSL-derived ITC. The same is true for phytoalexins bearing a dithiocarbamate group (**67**, **104**, **108**).

Two additional further rearrangements of the thiones were summarized by Blažević et al. (2020), resulting in oxazolidinones and thiazolidinones. However, no data on the interaction of these compounds and fungi were found during our literature search. In fact, the information on their distribution in Brassicales and *in planta* concentrations is also very limited.

Altogether, data on direct effects of these compounds on fungi are very limited, even when compared to that of the nitriles.

6.2. Interaction of fungi with epithionitriles

Another non-default GSL decomposition product group are the epithionitriles. As these compounds are quite unstable, the number of studies are very limited. A study (Shofran et al., 1998) examined direct effect of epithionitriles on fungi. It was shown that the epithionitrile **10** was not inhibitory at concentrations as high as 1 mg/mL, while the respective ITC **9** was inhibitory at MIC 1–4 μg/mL.

Based on the work of Witzel et al. (2011), the role of epithionitriles in fungal defense against *Verticillium longisporum* (C. Stark) Karapapa, Bainbr. & Heale (Plectosphaerellaceae) seems to be less important than that of nitriles and the ITCs. During fungal challenge of various *A. thaliana* lines with *V. longisporum*, the epithionitrile-dominant plant line also responded with increased biosynthesis of **31** decomposition products (both ITC and nitrile) but epithionitrile levels were not changed.

6.3. Interaction of fungi with thiocyanates

Though synthetic thiocyanates were shown to be antifungal, data on GSL-derived thiocyanates are very limited (Table 5). Reports on antifungal mixtures of plant origin are available (Mannai et al., 2021; Ossowicki et al., 2017), but these cannot be used to assess the contribution of the thiocyanates to the overall activity. Thiocyanates, identical to those from GSL decomposition, also appear as volatiles of bacteria and also *Tricholoma matsutake* (S. Ito & S. Imai) Singer (Tricholomataceae) (pine-mushroom) (Cho et al., 2008).

Significant antifungal activity was shown for phenyl thiocyanate by Zsolnai (1966), but the corresponding GSL **32** was discontinued according to Blažević et al. (2020) due to insufficient evidence on the structure. Data on antifungal activity are available on **11** and **36** (Zsolnai, 1966), as well as **13** (Foley et al., 1958). More recent data on **36**, **41** were published (Radulović et al., 2012), where the authors found an activity comparable to **35** against *C. albicans* and *A. fumigatus* (MIC 0.15–1.25 mg/mL). The study of Shofran et al. (1998) has shown that **11** was active towards some yeast strains (MIC 50 μg/mL) but was still much less potent than the respective ITC **9** (MIC 1–4 μg/mL). The antifungal activity of these compounds is also thought to rely on reactivity with biomolecules, just like in the case of isothiocyanates (Morley

Table 5
Antifungal activity of dithiocarbamates and thiocyanates. Additional details about the compounds can be found in Fig. 2 and Table S1 and S4.

ID	Source of agent	Fungal Strains	Fungus function	Activity against	Bioactive concentration	Ref.
Dithiocarbamates						
28	*Raphanus sativus*	*Aspergillus fumigatus,* *Cladosporium colocasiae,* *Eurotium chevalieri,* *Schizosaccharomyces pombe*	environmental	mycelial growth	MIC: 200 μg/mL MIC: 200 μg/mL MIC: 200 μg/mL MIC: 200 μg/mL	Matsuoka et al. (1997)
29	*Raphanus sativus*	*Aspergillus fumigatus,* *Cladosporium colocasiae,* *Eurotium chevalieri,* *Schizosaccharomyces pombe*	environmental	mycelial growth	MIC: 200 μg/mL MIC: 100 μg/mL MIC: 100 μg/mL MIC: 200 μg/mL	Matsuoka et al. (1997)
30	*Raphanus sativus*	*Aspergillus fumigatus,* *Cladosporium colocasiae,* *Eurotium chevalieri,* *Mucor racemosus,* *Candida albicans,* *Schizosaccharomyces pombe*	environmental	mycelial growth	MIC: 200 μg/mL MIC: 100 μg/mL MIC: 100 μg/mL MIC: 200 μg/mL MIC: 200 μg/mL MIC: 200 μg/mL	Matsuoka et al. (1997)
Thiocyanates						
13	standard	*Candida albicans,* *Neurospora crassa*	not indicated	mycelial growth	50% inhibition at 0.1 mg/mL 50% inhib. at 0.01 mg/mL	Foley et al. (1958)
36	synthesized	*Aspergillus fumigatus,* *Candida albicans*	environmental human related	mycelial growth	MIC: 0.15 mg/mL MIC: 1.25 mg/mL	Radulović et al. (2012)
41	synthesized	*Aspergillus fumigatus,* *Candida albicans*	environmental human related	mycelial growth	MIC: 0.30 mg/mL MIC: 0.60 mg/mL	Radulović et al. (2012)
43	synthesized	*Aspergillus fumigatus,* *Candida albicans*	environmental human related	mycelial growth	MIC: 0.15 mg/mL MIC: 0.60 mg/mL	Radulović et al. (2012)

Authorities of botanical taxa: *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae), *Raphanus sativus* L. (Brassicaceae).
Authorities of fungal taxa: *Aspergillus fumigatus* Fresen. (Aspergillaceae), *Candida albicans* (C.P. Robin) Berkhout (o. Saccharomycetales), *Candida parapsilosis* (Ashford) Langeron & Talice (o. Saccharomycetales), *Cladosporium colocasiae* Sawada (Cladosporiaceae), *Eurotium chevalieri* L. Mangin (Aspergillaceae), *Mucor racemosus* Bull. (Mucoraceae), *Neurospora crassa* Shear & B.O. Dodge (Sordariaceae), *Schizosaccharomyces pombe* Lindner (Schizosaccharomycetaceae).
Compound references: 13, n-Butyl thiocyanate; **28**, Methyl 4-methylthiobutyldithiocarbamate; **29**, Methyl (*Z*)-4-methylthio-3-butenyldithiocarbamate; **30**, Methyl (*E*)-4-methylthio-3-butenyldithiocarbamate; **36**, Benzyl thiocyanate; **41**, 3-Methoxybenzyl thiocyanate; **43**, 4-Methoxybenzyl thiocyanate.

and Naji, 1995).

7. Interactions of indole isothiocyanate downstream products and fungi

The myrosinase reaction of indole GSLs results in compounds that readily rearrange to form non-ITC products, such as **68** (Wittstock et al., 2016) as well as several other downstream products (Fig. 2), depending on the reaction partner with the indole carbocation intermediate (Hanschen et al., 2014). The increased biosynthesis of these downstream products strongly suggests in vivo GSL decomposition and their importance in plant-fungal interactions.

The biosynthesis of **61** was increased by fungal challenge according to a large number of studies (Bednarek et al., 2011; Floerl et al., 2012; Frerigmann et al., 2016; Fukunaga et al., 2017; Gamir et al., 2014; Hématy et al., 2020; Humphry et al., 2010; König et al., 2012; Kuhn et al., 2017; Sanchez-Vallet et al., 2010) in various models of *A. thaliana* and other plants. In contrast to **62**, indole GSL and methoxylated indole GSLs, **61** was also found at basal levels in almost all tested Brassicacae plants by (Bednarek et al., 2011), including *Olimarabidopsis cabulica* (Hook.f. & Thomson) Al-Shehbaz, O'Kane & R.A.Price (Brassicaceae), *Crucihimalaya lasiocarpa* (Hook.f. & Thomson) Al-Shehbaz, O'Kane & R. A. Price (Brassicaceae), *Sisymbrium irio* L. (Brassicaceae), *Arabis alpina* L. (Brassicaceae) and *Thellungiella halophila* O.E. Schulz (Brassicaceae). Most of these species also showed increased biosynthesis of **61** after fungal challenge. Induction of **61** depends on functional PEN2 and GSH generation, as shown by (Piślewska-Bednarek et al., 2018).

The biosynthesis of **62** was also elicited by fungal challenge in *A. thaliana* models (Fukunaga et al., 2017; Hématy et al., 2020; Hiruma et al., 2013; Humphry et al., 2010; Kuhn et al., 2017). The accumulation of the compound is not as universal as that of **61** (Bednarek et al., 2011): only two of 11 tested Brassicaceae species showed impressive increases in biosynthesis, and the levels were order(s) of magnitude lower compared to that of **61** and derivatives of **75**. According to (Piślewska-Bednarek et al., 2018), biosynthesis shows the same dependencies as that of **61**: a functional PEN2, and GSH generation is required. Impairment of GSH biosynthesis results in higher fungal entry rate and lower amounts of the compound (Hiruma et al., 2013), suggesting a role in fungal arrest.

The biosynthesis of **60** was also shown to be dependent on a functional PEN2 and GSTU13, just like in the case of **61** and **62** (Piślewska-Bednarek et al., 2018). The compound also showed significant induction after fungal challenge in (Fukunaga et al., 2017; Piślewska-Bednarek et al., 2018).

The N-acetyl derivative of **62** was elicited by *A. brassicicola* in *Brassica juncea* (L.) Czern (Brassicaceae) (Pedras et al., 2009a). We note that in this case a very significant reduction of **49**, **53** and **55** was observed at the same time. Whether this is the result of competing biochemical pathways or **49** being a precursor is not known. In either case, there seems to be a shift towards more downstream products upon fungal inoculation in the plant.

Neoascorbigen (**70**) and a dihydroneoascorbigen (**72**) glucoside also showed induction by fungal colonization in the crucifer *Thellungiella salsuginea* O.E.Schulz (Brassicaceae) (Pedras and Zheng, 2010).

7.1. Antifungal activity and mechanisms of indole isothiocyanate downstream products

As summarized in Table 6, **68** was shown to be antifungal against various fungi. As in the case of nitriles, we observe that these data were gathered almost exclusively on plant pathogens. The literature dealing with pharmacological targets of non-ITC GSL decomposition products is also quite limited despite the fact that the significance of these compounds is now widely recognized.

Though the compound is relatively potent among the GSL downstream products discussed herein, its direct activity is significantly weaker compared to that of the ITCs: while 0.5 mM **68** resulted in a median inhibition of 48% (four fungi, Table 6), the EC_{50} medians for **9** and **46** from the study of Taylor et al. (2014) were 0.251 and 0.169 mM, respectively. It is worth to note that Taylor et al. (2014) used a similar

Table 6
Antifungal activity of simple 1*H*-indole-3-acetonitrile and 1*H*-indol-3-yl methyl isothiocyanate derivatives. Additional details about the compounds can be found in Fig. 3 and Table S2.

ID	Source of agent	Fungal Strains	Fungus function	Activity against	Bioactive concentration	Ref.
1H-Indole-3-carboxaldehyde and derivatives						
74	salamander symbiont *Janthinobacterium lividum*	*Batrachochytrium dendrobatidis*	amphibian pathogen	mycelial growth	MIC: 68.89 μM	Brucker et al. (2008)
74	*Halichondria* sp. (sponge)	*Mortierella ramannianus**	not indicated	mycelial growth	TLC assay	Li et al. (1994)
74	*Pseudomonas* sp. ST4	*Sporisorium scitamineum*	plant pathogen	fungal mating, hyphal growth	MIC: 0.6 mM (mating), 3 mM (growth)	Liu et al. (2020)
74	standard	*Fulgensia bracteata*	mycobiont	spore germination	$>10^{-6}$ M (semiquantitative)	Meeβen et al. (2013)
74	standard	*Alternaria brassicicola,* *Rhizoctonia solani,* *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	29% inhib. at 0.5 mM 32% inhib. at 0.5 mM 16% inhib. at 0.5 mM	Pedras and Hossain. (2011)
74	synthesized	*Phoma lingam*	plant pathogen	spore germination	65% inhib. at 1 mM	Pedras and Khan. (1996)
74	synthesized	*Alternaria alternata,* *Alternaria brassicae,* *Bipolaris sorokinianum**, *Fusarium graminearum,* *Fusarium oxysporum,* *Pyricularia oryzae*	plant pathogen	mycelial growth	35.0% inhib. at 100 μg/mL 36.8% inhib. at 100 μg/mL 69.5% inhib. at 100 μg/mL 13.1% inhib. at 100 μg/mL 40.0% inhib. at 100 μg/mL 23.4% inhib. at 100 μg/mL	Xu et al. (2011)
74	*Epichloë festucae*	*Cryphonectria parasitica*	plant pathogen	mycelial growth	>12.5 μg (semiquantitative)	Yue et al. (2000)
76	*Brassica napus*	*Alternaria brassicicola,* *Rhizoctonia solani,* *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	74% inhibition at 0.5 mM 74% inhib. at 0.5 mM 49% inhib. at 0.5 mM	Pedras et al. (2008)
1H-Indole-3-carbonyl cyanide and derivatives						
90	standard	*Alternaria brassicicola,* *Botrytis cinerea*	plant pathogen	mycelial growth	IC50: 50 μM IC50: 25 μM	Rajniak et al. (2015)
91	standard	*Alternaria brassicicola,* *Botrytis cinerea*	plant pathogen	mycelial growth	100 μM (semiquantitative)	Rajniak et al. (2015)
1H-Indole-3-carboxylic acid and derivatives						
75	*Staphylococcus* sp.	*Schizophyllum commune*	not indicated	mycelial growth	100 μg (semiquantitative)	Abdelhameed et al. (2019)
75	standard	*Fusarium graminearum*	plant pathogen	mycelial growth	IC50: >10 mM	Cuperlovic-Culf et al. (2016)
75	*Streptomyces* sp.	*Aspergillus flavus,* *Aspergillus niger,* *Alternaria alternata,* *Curvularia maculans,* *Curvularia lunata,* *Fusarium oxysporum,* *Penicillium citrinum*	not indicated	mycelial growth	MIC: 40 μg/mL MIC: 20 μg/mL MIC: 150 μg/mL MIC: 125 μg/mL MIC: 80 μg/mL MIC: 15 μg/mL MIC: 20 μg/mL	Kavitha et al. (2010)
75	*Pseudomonas* sp. ST4	*Sporisorium scitamineum*	plant pathogen	fungal mating	MIC: 0.8 mM	Liu et al. (2020)
75	standard	*Alternaria brassicicola,* *Rhizoctonia solani,* *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	22% inhib. at 0.5 mM 9% inhib. at 0.5 mM 34% inhib. at 0.5 mM	Pedras and Hossain (2011)
75	synthesized	*Botrytis cinerea*	plant pathogen	mycelial growth	31% inhib. at 0.5 mM	Pedras et al., (2011a)
75	standard	*Alternaria brassicicola*	plant pathogen	mycelial growth	26% inhib. at 0.5 mM	Pedras and Abdoli (2013)
75	synthesized	*Rhizoctonia solani*	plant pathogen	mycelial growth	20.18% inhib. at 50 μg/mL	Zeng et al. (2020)
79	synthesized	*Alternaria brassicicola,* *Rhizoctonia solani,* *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	48% inhib. at 0.5 mM 14% inhib. at 0.5 mM 67% inhib. at 0.5 mM	Pedras and Hossain (2011)
80	synthesized	*Alternaria brassicicola,* *Rhizoctonia solani,* *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	18% inhib. at 0.5 mM 14% inhib. at 0.5 mM 11% inhib. at 0.5 mM	Pedras and Hossain (2011)
84	*Epichloë festucae*	*Cryphonectria parasitica*	plant pathogen	mycelial growth	>12.5 μg (semiquantitative)	Yue et al. (2000)
85	synthesized	*Phoma lingam,* *Phoma wasabiae*	plant pathogen	spore germination	100% inhib. at 0.5 mM	Pedras and Sorensen (1998)
89	synthesized	*Alternaria brassicicola*	plant pathogen	mycelial growth	68% inhib. at 0.5 mM	Pedras and Abdoli (2013)
1H-Indole-3-methanol and downstream compounds						
68	standard	*Candida albicans*	human related	mycelial growth	MIC: 0.125 mg/mL	Ko et al. (2016)
68	*Pseudomonas* sp. ST4	*Sporisorium scitamineum*	plant pathogen	fungal mating, hyphal growth	MIC: 0.6 mM (mating), 0.4 mM (growth)	Liu et al. (2020)
68	standard	*Leptosphaeria maculans*	plant pathogen	mycelial growth	92.1% inhib. at 0.4 mM	Mithen et al. (1986)
68	standard	*Alternaria brassicicola,* *Rhizoctonia solani,* *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	43% inhib. at 0.5 mM 44% inhib. at 0.5 mM 73% inhib. at 0.5 mM	Pedras and Hossain (2011)
68	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	52% inhib. at 0.5 mM	Pedras and Sarma-Mamillapalle, (2012b)
68	standard	*Alternaria brassicae,* *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	semiquantitative assay	Sotelo et al. (2015)
68	standard	*Candida albicans*	not indicated	mycelial growth	MIC: 60 μM	Sung and Lee (2007)
68	standard	*Candida albicans,* *Saccharomyces cerevisiae,* *Trichosporon beigelii*	not indicated	mycelial growth	MIC: 10 μg/mL MIC: 10 μg/mL MIC: 20 μg/mL	Sung and Lee (2007)
68	synthesized	*Rhizoctonia solani*	plant pathogen	mycelial growth	30.67% inhib. at 50 μg/mL	Zeng et al. (2020)
73	standard	*Puccinia graminis*	plant pathogen	mycelial growth	6×10^{-6} M (semiquantitative)	Grambow and Tücks (1979)

(*continued on next page*)

Table 6 (*continued*)

ID	Source of agent	Fungal Strains	Fungus function	Activity against	Bioactive concentration	Ref.
73	standard	*Leptosphaeria maculans*	plant pathogen	mycelial growth	28% inhib. at 0.4 mM	Mithen et al. (1986)
73	*Aspergillus nidulans* from deep-sea sediment	*Colletotrichum gloeosporioides*	plant pathogen	mycelial growth	MIC: 64 μg/mL	Lü et al. (2020)
73	synthesized	*Alternaria brassicicola*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	100% inhib. at 0.5 mM 100% inhib. at 0.5 mM 100% inhib. at 0.5 mM	Pedras and Hossain (2011)
Indole isothiocyanate downstream products						
58	synthesized	*Alternaria brassicicola*, *Leptosphaeria maculans*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	42% inhib. at 0.5 mM 31% inhib. at 0.5 mM 15% inhib. at 0.5 mM 25% inhib. at 0.5 mM	Pedras and Yaya (2012)
59	synthesized	*Alternaria brassicicola*, *Leptosphaeria maculans*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	21% inhib. at 0.5 mM 27% inhib. at 0.5 mM 29% inhib. at 0.5 mM 32% inhib. at 0.5 mM	Pedras and Yaya (2012)
61	*Arabidopsis thaliana*	*Plectosphaerella cucumerina*	plant pathogen	spore germination	EC_{50}: 367 μM	Sanchez-Vallet et al. (2010)
62	synthesized	*Alternaria brassicicola*	plant pathogen	mycelial growth	16% inhib. at 0.5 mM	Pedras et al., (2009a)
62	synthesized	*Botrytis cinerea*	plant pathogen	mycelial growth	38% inhib. at 0.5 mM	Pedras et al., (2011a)
62	*Arabidopsis thaliana*	*Plectosphaerella cucumerina*	plant pathogen	spore germination	EC_{50}: 183 μM	Sanchez-Vallet et al. (2010)
64	synthesized	*Leptosphaeria maculans*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	31% inhib. at 0.5 mM 18% inhib. at 0.5 mM 31% inhib. at 0.5 mM	Pedras et al., (2006a)
69	synthesized	*Alternaria brassicicola*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	36% inhib. at 0.5 mM 12% inhib. at 0.5 mM 10% inhib. at 0.5 mM	Pedras and Hossain (2011)
69	standard	*Leptosphaeria maculans*	plant pathogen	mycelial growth	28% inhib. at 0.4 mM	Mithen et al. (1986)
69	*Brassica napus*	*Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	24% inhib. at 0.5 mM 42% inhib. at 0.5 mM	Pedras et al. (2008)
70	*Brassica napus*	*Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	14% inhib. at 0.5 mM 34% inhib. at 0.5 mM	Pedras et al. (2008)
71	*Brassica napus*	*Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	59% inhib. at 0.5 mM 24% inhib. at 0.5 mM	Pedras et al. (2008)
72	*Brassica napus*	*Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	18% inhib. at 0.5 mM 22% inhib. at 0.5 mM	Pedras et al. (2008)
117	synthesized	*Alternaria brassicicola*, *Leptosphaeria maculans*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	42% inhib. at 0.5 mM 5% inhib. at 0.5 mM 100% inhib. at 0.5 mM 60% inhib. at 0.5 mM	Pedras and Yaya (2012)
1H-Indole-3-acetaldehyde oxime						
65	*Archangium gephyra* fermentation	Ustilago zeae	not indicated	mycelial growth	40 μg (semiquantitative)	Böhlendorf et al. (2006)

Authorities of algal and botanical taxa: *Janthinobacterium lividum* Eisenberg (Oxalobacteraceae), *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae), *Brassica napus* L. (Brassicaceae).

Authorities of fungal taxa: *Alternaria alternata* (Fr.) Keissl. (Pleosporaceae), *Alternaria brassicae* (Berk.) Sacc. (Pleosporaceae), *Alternaria brassicicola* (Schwein.) Wiltshire (Pleosporaceae), *Aspergillus flavus* Link (Aspergillaceae), *Aspergillus nidulans* (Eidam) G. Winter (Aspergillaceae), *Aspergillus niger* Tiegh. (Aspergillaceae), *Batrachochytrium dendrobatidis* Longcore, Pessier & D.K. Nichols, (o. Rhizophydiales), *Botrytis cinerea* Pers. (Sclerotiniaceae), *Candida albicans* (C.P. Robin) Berkhout (o. Saccharomycetales), *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (Glomerellaceae), *Cryphonectria parasitica* (Murrill) M.E. Barr (Valsaceae), *Curvularia lunata* (Wakker) Boedijn (Pleosporaceae), *Curvularia maculans* (C.K. Bancr.) Boedijn (Pleosporaceae), *Epichloë festucae* Leuchtm., Schardl & M.R. Siegel (Clavicipitaceae), *Fulgensia bracteata* (Hoffm.) Räsänen (Teloschistaceae), *Fusarium graminearum* Schwabe (Nectriaceae), *Fusarium oxysporum* Schltdl. (Nectriaceae), *Leptosphaeria maculans* Ces. & De Not. (Leptosphaeriaceae), *Penicillium citrinum* Thom (Aspergillaceae), *Phoma lingam* (Tode) Desm. (o. Pleosporales), *Phoma wasabiae* Yokogi (o. Pleosporales), *Plectosphaerella cucumerina* (Lindf.) W. Gams (Plectosphaerellaceae), *Puccinia graminis* Pers. (Pucciniaceae), *Pyricularia oryzae* Cavara (Pyriculariaceae), *Rhizoctonia solani* J.G. Kühn (Ceratobasidiaceae), *Saccharomyces cerevisiae* (Desm.) Meyen (Saccharomycetaceae), *Schizophyllum commune* Fr. (Schizophyllaceae), *Sclerotinia sclerotiorum* (Lib.) de Bary (Sclerotiniaceae), *Sporisorium scitamineum* (Syd.) M. Piepenbr., M. Stoll & Oberw. (Ustilaginaceae), *Trichosporon beigelii* (Küchenm. & Rabenh.) Vuill. (Trichosporonaceae), *Ustilago zeae* (Link) Unger (Ustilaginaceae).

* Authority couldn't be verified neither by the original manuscript nor the relevant databases.

Compound references: 58, 1*H*-indol-3-yl formamide; **59**, N-(4-Methoxy-1*H*-indol-3-yl) formamide; **61**, Raphanusamic acid; **62**, 1*H*-Indole-3-methanamine; **65**, 1*H*-Indole-3-acetaldehyde oxime (IAOx); **68**, 1*H*-Indole-3-methanol; **69**, Ascorbigen; **70**, Neoascorbigen; **71**, Dihydroascorbigen; **72**, Dihydroneoascorbigen; **73**, 3,3′-Methylenebis-1*H*-Indole (diindoyl methane); **74**, 1*H*-Indole-3-carboxaldehyde; **75**, 1*H*-Indole-3-carboxylic acid; **76**, 1-Methoxy-1*H*-indole-3-carboxaldehyde; **79**, 1-Methoxy-1*H*-indole-3-carboxylic acid; **80**, 4-Methoxy-1*H*-indole-3-carboxylic acid; **84**, 1*H*-Indole-3-carboxylic acid methyl ester; **85**, 1-Methoxy-1*H*-indole-3-carboxylic acid methyl ester; **89**, 1*H*-indole-3-carboxamide; **90**, 1*H*-Indole-3-carbonyl cyanide; **91**, 4-Hydroxy-1*H*-indole-3-carbonyl cyanide; **117**, 1*H*-indole-3-isonitrile.

methodology to the relevant papers in Table 6 and that this relative weakness compared to ITCs was also shown against several bacteria by Aires et al. (2009).

The arrest of the cell cycle at the G2/M phase in a *C. albicans* model by **68**, and a possible mechanism of antifungal activity was suggested to be membrane function disruption (W.S. Sung and Lee, 2007). The compound was shown to generate reactive oxygen species and induced apoptosis in a *C. albicans* model as shown by metacaspase-activation, cytochrome *c* release, DNA and nuclear fragmentation as well as direct measurement of ROS (Hwang et al., 2011). Additionally, the study of Woo Sang Sung and Lee (2007) showed that **68** binds to DNA.

3,3′-methylenebis-1*H*-indole (**73**), which can be spontaneously generated by a dimerization reaction from **68** (Hanschen et al., 2014), showed a stimulatory effect on growth and germ tube to mycelial transition of stimulated mycelial growth of the rust fungus *Puccinia graminis* f. *tritici* Erikss. & Henning (Pucciniaceae). This effect was

antagonized by **74** or **95**, while **75** showed a very weak antagonistic effect (Grambow and Tücks, 1979). According to Pedras and Hossain (2011), spontaneous conversion of **68** into **73** also took place in the culture media of *S. sclerotiorum*. Furthermore, **73** was found to be more inhibitory than its precursor. The dimerization reaction can proceed further to form indol-3-ylmethyl oligomers of 3–5 monomers, linked by 2-2′, 2–3′ or 3-3′ linkages (Buskov et al., 2000). Additionally, 3,3′, 3″-methylidynetris-1*H*-Indole (**132**, = 1,1,1-tris(3-indolyl)methane) and oligomer adducts with **69** were also described to form spontaneously (Buskov et al., 2000). Unfortunately, the only information on the antifungal activity of these compounds is from the study of Veluri et al. (2003) who concluded that **132** was inactive against the fungi *Mucor miehei* Cooney & R. Emers. (Mucoraceae) and *C. albicans*. Data are also lacking on *in planta* levels and whether concentration change after fungal challenge.

Ascorbigen (**69**) and its methylated derivative successfully reduced disease symptoms in non-Brassicaceae models, according to a few papers (Kátay et al., 2011; Kátay and Tyihák, 1998). On the other hand, the results of the comparative studies on various plant pathogens in several excellent papers of Pedras et al. (Pedras et al., 1999, 2008, 2009a, 2011a; Pedras and Hossain, 2011) show that **69**, **70**, their dihydro derivatives as well as **62** have relatively low antifungal potency compared not only to ITCs but also nitriles (Tables 3 and 6). The median growth inhibition at 0.5 mM was 27% and 24% for derivatives of **62** and **69**, respectively (Table 6). Various strains showed very different sensitivities. In the same studies, **68** had relatively strong antifungal potency (44% median inhibition at 0.5 mM), while **73** showed activity comparable to that of **94** (100% median inhibition at 0.5 mM). On the other hand, the fungal metabolite N′-acetyl-3-indolylmethanamine (**63**) showed similar activity to that of **62**. **58** and its derivatives were also studied (Pedras and Yaya, 2012). The compounds were found to be of low antifungal potency (26% median inhibition at 0.5 mM), depending on the plant pathogen tested. The relatively weak antimicrobial activity of **69** was also detected against bacteria by Aires et al. (2009): its potency was similar to that of **45**, lower than that of **49** and **68** and much lower than that of the tested ITCs. Contrary evidence was found by Mithen et al. (1986), who found that **68** is a much stronger antifungal agent against *L. maculans* than **69** and **73**. Additional data on wider sets of fungal strains are strongly warranted.

Unfortunately, there is only one paper presenting direct antifungal data of **61** (Sanchez-Vallet et al., 2010). The EC_{50} value was found to be 366.7 μM against *P. cucumerina*, which is about two times higher compared to the value of relatively weak antifungal agent **75** (183.0 μM). We might speculate that since it is a dithiocarbamate, it can rearrange to ITC under certain conditions (Hanschen et al., 2014), but one should recall that dithiocarbamate rearrangement products can be much less potent antifungal agents when directly compared to their precursor ITCs (Matsuoka et al., 1997).

The study of Bednarek et al. (2011) has shown that various Brassicaceae species biosynthesize different mixtures of the above (and several other) compounds, suspected to be the causative agents against fungal challenge. Furthermore, the concentrations of the bioactive agents can differ by a few orders of magnitude. Since different compounds may be suitable against different invaders, the actual concentrations are also likely to be significantly affected by the type of attacker. The lack of data leaves additional questions to be addressed in further research. These include the actual bioactivity under *in vivo* conditions; the experiments cited above were typically run on fungi growing on minimal media. Data are also lacking on the possible synergistic interactions of these compounds regarding antifungal activity, though synergistic activity can sometimes be observed between plant metabolite mixtures (Pina-Vaz et al., 2004). Moreover, it is possible that additional downstream products will be discovered.

7.2. Metabolism/detoxification of indole isothiocyanate downstream products by fungi

Although there are some data on direct antifungal activities of indole isothiocyanate downstream products, metabolism has been less studied to date.

The metabolism of **68** was only described in a few studies. The compound was metabolized by *A. brassicicola* and *R. solani* to less effective antifungal agents **74** and **75** (Pedras and Hossain, 2011).

Interesting studies on the endophytic fungus *Daldinia eschscholzii* (Ehrenb.) Rehm (Hypoxylaceae) suggest that some fungi may accept GSL decomposition products as biosynthetic building blocks. *D. eschscholzii* metabolized **68** to oxygen-containing novel natural products (alkaloids) consisting of di- and trimeric indole structures (Lin and Tan, 2018). The compounds were named dalesindoloid A, dalesindoloid B and 3-(1*H*-indole-3ylmethyl)-2-oxindole. *D. eschscholzii* also metabolized **68** into polyketide-indole hybrids, named indolchromins A and B (Lin et al., 2019).

8. Interactions of 1*H*-indole-3-acetonitrile downstream products and fungi

Though 1*H*-indole-3-carboxaldehyde (**74**) and 1*H*-indole-3-carboxylic acid (**75**) are theoretically only a few oxidative steps away from **68**, these compounds are rather thought to be generated from **49** (Böttcher et al., 2014; Kuhn et al., 2017; Müller et al., 2019), with a non-GL pathway likely being dominant (Frerigmann et al., 2016; Pastorczyk et al., 2020) (Fig. 3). Additional evidence supporting this includes results are also available (Pastorczyk et al., 2020; Piślewska-Bednarek et al., 2018): Unlike **61** and **62**, the biosynthesis of **75** and derivatives did not rely on a functional GSTU13 according to Piślewska-Bednarek et al. (2018), while induction of **82** seemed not to rely on PEN2-mediated IG hydrolysis (Kosaka et al., 2021). Unfortunately, the exact ratio of the carbon flow on the two routes (Fig. 3) remains to be measured and is probably a subject of fine-tuning as well as genotype-to-genotype variability.

The role of **74**, **75** and their derivatives is suggested by several experimental results showing increased biosynthesis after fungal colonization in various organisms. An increase in **74** levels in a *A. thaliana* line primed for salicylic acid-dependent responses was shown after fungal challenge (Gamir et al., 2014).

Elicitation response to fungal inoculation or a pathogen-associated molecular pattern flg22 was shown in various *A. thaliana* models and other species for various derivatives of **75**: **75** (König et al., 2012; Kuhn et al., 2017; Sanchez-Vallet et al., 2010), **86** (Bednarek et al., 2011; Frerigmann et al., 2016; Fukunaga et al., 2017; Pastorczyk et al., 2020; Piślewska-Bednarek et al., 2018), **87** (Bednarek et al., 2011), **82** (Bednarek et al., 2011; Frerigmann et al., 2016; Fukunaga et al., 2017; König et al., 2012; Kosaka et al., 2021; Pastorczyk et al., 2020; Piślewska-Bednarek et al., 2018) as well as **84** (Gamir et al., 2014) and **85** (Pedras and Sorensen, 1998). Some of these compounds are induced in many Brassicaceae species (Bednarek et al., 2011).

Compound **75** may be important via other mechanisms in plant-fungus interactions. It is suspected to be involved in callose priming (an important component in defense against fungi) in *A. thaliana* (Gamir et al., 2018; Pastor-Fernández et al., 2019). Tolerance against *V. longisporum* correlates with higher levels of **75**, **61** and salicylic acid according to König et al. (2012). In *Arabidopsis*, the CYP71A12-mediated synthesis of **75** and its derivatives is required for postinvasive immunity against *A. brassicicola*: a significant induction of **82** was observed after colonization (Kosaka et al., 2021). The study of Kavitha et al. (2010) has even showed in vivo inhibition of *Fusarium* wilt by **75** in sorghum plants, while Oukala et al. (2021) found that **75** protects tomato plants against *B. cinerea*. These activities may rely in part on modulation of plant physiology rather than direct antifungal activity. Compound **74** was also reported to have an in vivo effect in a non-Brassicaceae plant: the

inhibition of corn smut (*Ustilago maydis* (DC.) Corda (Ustilaginaceae)) infection on sugarcane was observed (Liu et al., 2020).

A role in endophyte-plant interactions can also be attributed to **74**. The endophytic fungus *Harpophora oryzae* (syn. Falciphora oryzae Z.L. Yuan, C.L. Zhang & F.C. Lin ex J. Luo & N. Zhang (Magnaporthaceae)) promotes lateral root growth and inhibited primary root growth, and the effects are accompanied by a significant increase in **74** (Sun et al., 2020). Interestingly, **74** also has a role in the pre-contact state between lichen photobiont and mycobiont members; it is secreted by the photobionts from several lichens tested by Meeβen et al. (2013), though a clear role could not be determined.

8.1. Antifungal activity and mechanisms of 1H-indole-3-acetonitrile downstream products

Limited data has been published on the direct antifungal activity of this product group. Furthermore, direct activity seems to be even weaker compared to previous groups: at 0.5 mM, median growth inhibition against fungi was only 26% and 29% on average for **75** and **74**, respectively (Table 6). This questions direct antifungal roles in planta roles to some extent. Some substituent patterns seem to be important to achieve acceptable antifungal activity, which means the compounds **75** and **74** are more likely to be precursors to the actual bioactive agents.

Compound **74** showed direct antifungal activity, as reported by Pedras and Hossain (2011). As reported by Liu et al. (2020), **74** also interfered with fungal sexual mating and hyphal growth of *Sporisorium scitamineum* (Syd.) M. Piepenbr., M. Stoll & Oberw. (Ustilaginaceae). Two 5-hydroxy-derivatives of **74** and **84** also inhibited *C. albicans* isocitrate lyase (EC 4.1.3.1) but did not show antifungal activity according to Lee et al. (2009). However, the 1-methoxy derivative of **74** showed a stronger antifungal potency (Pedras et al., 2008) comparable to that of the **49** (Tables 3 and 6).

Compound **75** showed weak direct antifungal activity according to several studies (Pedras et al., 2011a; Pedras and Abdoli, 2013; Pedras and Hossain, 2011). Compound **75** also inhibited the mating activity but not the growth of *S. scitamineum* (Liu et al., 2020). Compared to **75**, a somewhat stronger activity was shown for the 1-methoxy derivative, while the 4-methoxy derivative was considerably weaker (Pedras and Hossain, 2011).

The methoxylated ester **85** was also active against plant pathogens (Pedras and Sorensen, 1998). Moreover, the same study concluded that, interestingly, it showed a stronger effect against the virulent isolates of *P. lingam* than against the weakly virulent isolate. The compound also inhibited the germination of *Phoma* pathogens (Pedras et al., 1999). The 5-hydroxy derivative inhibited *C. albicans* isocitrate lyase, but did not show antifungal activity according to Lee et al. (2009).

During infection the conversion of **65** to indole GSLs is decreased and conversion of **65** to **49** biosynthesis intensified according to Mucha et al. (2019). Though the biosynthesis of **90** and its derivatives is thought to be dominated by direct biosynthesis from **65** and not the GSL pathway is, it is unclear whether the GSL-derived **49** can be fed to this route. Regarding fungi, the potency of the carbonyl nitriles **90** and **91** against two fungal strains was comparable to that of **94** according to Rajniak et al. (2015).

We can draw a similar conclusion to that found in the case of nitriles. In particular, additional testing of inhibitory effects against additional functional classes of fungi and investigation of the effects of **74**, **75** and derivatives on the plant microbiome are warranted. Moreover, additional targeted comparison QSAR studies on these derivatives with/without various modifications (hydroxylation, methylation, methoxylation and glucosylation pattern) would add much to our understanding on how the chemical mixture might actually work in planta. Due to the lack of better options, we are currently extrapolating the antifungal activity data obtained from *in vitro* minimal media to *in planta* conditions.

8.2. Biosynthesis of 1H-indole-3-carboxaldehyde and 1H-indole-3-carboxylic acid by fungi

Biosynthesis of simple indole compounds, identical to those derived from **49**, were presented from many fungal strains; these results are summarized in Table 7. Though the taxonomic and functional diversity is striking at first glance, one should bear in mind, that these compound are quite simple and are a few steps away from the core metabolic pathways. Additionally, most of the studies in Table 7 detected these compounds from microbiological media, leaving the question on *in planta* production rates open. On the other hand, the role in plant-fungus interactions seems likely from another perspective: of 46 fungi, 25 are endophytes and 4 are ectomycorrhizae. There are typical endophytes in the list, including *Epicoccum nigrum* Link (Didymellaceae) (Dzoyem et al., 2017) and *Epichloë festucae* Leuchtm., Schardl & M.R. Siegel (Clavicipitaceae) (Yue et al., 2000). Since many of these fungi are not associated with Brassicaceae plants, we should be careful not to overestimate the possibility that the fungi interfere with the GSL pathway specifically via these compounds. However, because these compounds have bioactivity in other plants as well, they can be important agents in plant-fungus interactions.

9. Interactions of fungi with glucosinolate-derived phytoalexins

Indole ITCs can be the starting point for a wide variety of downstream phytoalexins, mostly characterized by the group of M. S. C. Pedras. These compounds are chemical defensive agents, biosynthesized on-demand in response to a challenge by a pathogen. Unfortunately, the pathways of the conversion from **67** to various other downstream phytoalexins have only been partially characterized to date (Fig. 4). This fact along with the chemical diversity of these compounds renders attempts at classification a difficult task. Their antifungal activity shows high variability (30–100% inhibition at 0.5 mM), the median value is similar to that of **49**, hence, lower than that of ITCs (Table 8).

Frequent structural elements include a heterocyclic ring fused to the indole (**98, 101, 107**), a spiro-carbon atom (**100, 106, 109**), and an oxidized heterocyclic ring, either on the indole ring as in **108**, **106**, **109**, **100**, **124** or as an additional ring (**97**) (Fig. 4).

Like in the case of other compounds discussed above, the in vivo role of these compounds is supported by their increased biosynthesis after fungal inoculation. This phenomenon was reported for **105** (Pedras and Ahiahonu, 2004), **124** and its Z isomer, as well as **96** (Pedras and Smith, 1997), as well as nasturlexins (Pedras et al., 2015). The accumulation of **102** in *B. juncea*, *B. napus*, *B. rapa* during challenge with non-aggressive or aggressive isolates of *L. maculans* (Dahiya and Rimmer, 1988, 1989) has shown that resistant plants accumulated more phytoalexins than the susceptible ones. Recall that the ability to respond to pathogen challenge with an increased GSL biosynthesis frequently results in tolerance or resistance to the pathogen (section 4.1.). Compounds **123, 100** and wasalexins may also act as phytoalexins in plants (Pedras et al., 1999, 2006b, 2007c).

The actual biosynthetic routes of these compounds involve several unresolved steps, that are to be experimentally determined in the future. Fortunately, most of them were reported to have direct antifungal activity in their pure form as detailed below.

9.1. Direct antifungal activity and antifungal mechanisms of glucosinolate-derived phytoalexins

Several of the compounds shown in Fig. 4 show potent antifungal activity, as summarized in Table 8. The literature highly favored Brassicaceae pathogens, but it may also prove interesting to include non-adapted species in tests for antifungal activity in the future. Since they are highly specialized, these compounds could be excellent candidates in an attempt to quantify adaptation of fungi to the plant defensive metabolome if a comparison on Brassicaceae pathogens, endophytes and

Table 7
Biosynthesis of simple indole derivatives, equivalent to glucosinolate downstream products, by fungi. Additional details about the compounds can be found in Fig. 3 and Table S2.

ID	Strain	Fungus function	Ref.
1H-Indole-3-carboxaldehyde			
74	*Acremonium implicatum*	*Lycopersicon esculentum* endophyte	Yu-heng et al., (2016)
74	*Aspergillus* sp.	*Solanum nigrum* endophyte	El-Hawary et al. (2017)
74	*Aspergillus versicolor*	endolichenic	Li et al. (2015)
74	*Aspergillus versicolor*	isolated from *Codium fragile*	Liu et al. (2012)
74	*Aureobasidium pullulans*	*Aloe vera* endophyte	El-Amrani et al. (2016)
74	*Ceriporia lacerate**	*Huperzia serrata* endophyte	Ying et al. (2014)
74	*Clitocybe nuda*	edible mushroom	Chen et al. (2012)
74	*Colletotrichum aotearoa*	*Huperzia serrata* endophyte	Wu et al. (2019)
74	*Cordyceps militaris* in presence of tryptophan	environmental	Höregott (1973)
74	*Epichloë festucae*	*Festuca* sp. endophyte	Yue et al. (2000)
74	*Hebeloma hiemale*	ectomycorrhizal	Rouillon et al. (1986)
74	*Hypoxylon investiens*	*Litsea akoensis* endophyte	Chang et al. (2014)
74	*Inocybe geophylla*	poisonous mushroom	Reinoso et al. (2013)
74	*Irpex lacteus*	*Distylium chinense* endophyte	Duan et al. (2019)
74	*Lactarius subplinthogalus*	environmental	Wang et al. (2004)
74	*Lasiodiplodia* sp.	*Viscum coloratum* endophyte	Qian et al. (2014)
74	*Malbranchea albolutea*	environmental	Díaz-Rojas et al. (2021)
74	*Ophiocordyceps sobolifera*	*Ilex formosana* endophyte	Feng et al. (2019)
74	*Paxillus involutus*	ectomycorrhizal	Rudawska and Kieliszewska-Rokicka (1997)
74	*Penicillium* sp.	marine-derived	Shaala and Youssef (2015)
74	*Penicillium vinaceum*	marine-derived	Asiri et al. (2015)
74	*Scopulariopsis* sp.	marine-derived	Elnaggar et al. (2017)
74	unidentified	environmental	Shimizu et al. (2003)
1H-Indole-3-carboxylic acid			
75	*Amauroderma amoiensis**	environmental	Zhang et al. (2013)
75	*Annulohypoxylon ilanen*	*Cinnamomum* sp. endophyte	M.-D. Wu et al. (2013)
75	*Aspergillus* sp.	*Solanum nigrum* endophyte	El-Hawary et al. (2017)
75	*Aspergillus versicolor*	*Codium fragile* endophyte	Liu et al. (2012)
75	*Aureobasidium pullulans*	*Aloe vera* endophyte	El-Amrani et al. (2016)
75	*Chaetomium* sp.	*Otanthus maritimus* endophyte	Aly et al. (2009)
75	*Chaetomium gracile*	not indicated	Bai et al. (2015)
75	*Colletotrichum gloeosporioides*	*Sabicea cinerea* endophyte	André et al. (2017)
75	*Cordyceps militaris* in presence of tryptophan	environmental	Höregott (1973)
75	*Epicoccum nigrum*	*Entada abyssinica* endophyte	Dzoyem et al. (2017)
75	*Fusarium kuroshium*	plant pathogen	Gutiérrez-Sánchez et al. (2021)
75	*Ganoderma tropicum*	environmental	Ma et al. (2013)
75	*Hebeloma hiemale*	ectomycorrhizal	Rouillon et al. (1986)
75	*Hypocreales* sp.	isolated from *Symphyocladia latiuscula*	Li et al. (2008)
75	*Irpex lacteus*	*Distylium chinense* endophyte	Duan et al. (2019)
75	*Lasiodiplodia* sp.	*Viscum coloratum* endophyte	Qian et al. (2014)
75	*Paxillus involutus*	ectomycorrhizal	Rudawska and Kieliszewska-Rokicka (1997)
75	*Pleurotus ostreatus*	edible mushroom	Papaspyridi et al. (2011)
75	*Pseudallescheria ellipsoidea*	marine-derived	Liu et al. (2015)
75	*Scopulariopsis* sp.	marine-derived	Elnaggar et al. (2017)
75	*Wallemia sebi*	*Excoecaria agallocha* epiphyte	Peng et al. (2011)
75	unidentified	*Cephalotaxus hainanensis* endophyte	Dai et al. (2009)
75	unidentified	isolated from *Polysiphonia urceolata*	Zhao et al. (2009)
Other			
73	*Aspergillus nidulans*	marine-derived	Lü et al. (2020)

Authorities of algal and botanical taxa: *Aloe vera* L. (Aloaceae), *Cephalotaxus hainanensis* H.L.Li (Cephalotaxaceae), *Codium fragile* (Suringar) Hariot (Codiaceae), *Distylium chinense* Hemsl. (Hamamelidaceae), *Entada abyssinica* Steud. (Leguminosae), *Excoecaria agallocha* L. (Euphorbiaceae), *Huperzia serrata* (Thunb.) Tervis. (Lycopodiaceae), *Ilex formosana* Maxim. (Aquifoliaceae), *Lycopersicon esculentum* Mill. (Solanaceae), *Otanthus maritimus* L. (Asteraceae), *Polysiphonia urceolata* (Lightfoot ex Dillwyn) Greville (Rhodomelaceae), *Sabicea cinerea* Aubl. (Rubiaceae), *Solanum nigrum* L. (Solanaceae), *Symphyocladia latiuscula* (Harvey) Yamada (Rhodomelaceae), *Viscum coloratum* Nakai (Viscaceae), *Vochysia divergens* Pohl (Vochysiaceae).
Authorities of fungal taxa: *Acremonium implicatum* (J.C. Gilman & E.V. Abbott) W. Gams (o. Hypocreales), *Annulohypoxylon ilanen* (Y.M. Ju & J.D. Rogers) Y.M. Ju, J.D. Rogers & H.M. Hsieh (Hypoxylaceae), *Aspergillus nidulans* (Eidam) G. Winter (Aspergillaceae), *Aspergillus versicolor* (Vuill.) Tirab. (Aspergillaceae), *Aureobasidium pullulans* (De Bary) G. Arnaud ex Cif., Ribaldi & Corte (Saccotheciaceae), *Chaetomium gracile* Udagawa (Chaetomiaceae), *Clitocybe nuda* (Bull.) H.E. Bigelow & A.H. Sm. (Tricholomataceae), *Colletotrichum aotearoa* B. Weir & P.R. Johnst. (Glomerellaceae), *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc (Glomerellaceae), *Cordyceps militaris* (L.). Fr. (Cordycipitaceae), *Epichloë festucae* Leuchtm., Schardl & M.R. Siegel (Clavicipitaceae), *Epicoccum nigrum* Link (Didymellaceae), *Fusarium kuroshium* (F. Na, J.D. Carrillo & A. Eskalen ex Sand.-Denis & Crous) O'Donnell, Geiser, Kasson & T. Aoki (Nectriaceae), *Ganoderma tropicum* (Jungh.) Bres. (Polyporaceae), *Hebeloma hiemale* Bres. (Strophariaceae), *Hypoxylon investiens* (Schweinitz) M.A. Curtis (Hypoxylaceae), *Inocybe geophylla* (Bull.) P. Kumm. (Inocybaceae), *Irpex lacteus* (Fr.) Fr. (Irpicaceae), *Lactarius subplinthogalus* Coker (Russulaceae), *Malbranchea albolutea* Sigler & J.W. Carmich. (Myxotrichaceae), *Ophiocordyceps sobolifera* (Hill ex Watson) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora (Ophiocordycipitaceae), *Paxillus involutus* (Batsch) Fr. (Paxillaceae), *Penicillium vinaceum* J.C. Gilman & E.V. Abbott (Aspergillaceae), *Pleurotus ostreatus* (Jacq.) P. Kumm. (Pleurotaceae), *Pseudallescheria ellipsoidea* (Arx & Fassat.) McGinnis, A. A. Padhye & Ajello (Microascaceae), *Wallemia sebi* (Fr.) Arx (Wallemiaceae).
* Authority couldn't be verified neither by the original manuscript nor the relevant databases.
Compound references: 73, 3,3′-Methylenebis-1*H*-indole; **74,** 1*H*-Indole-3-carboxaldehyde; **75,** 1*H*-Indole-3-carboxylic acid.

strains from other plants were conducted. A similar approach successfully showed some adaptation to ITCs by Brassicaceae endophytes compared to soil fungi (Szűcs et al., 2018).

The high diversity of this group prevents drawing general conclusions about the structural requirements of potent activity.

Some phytoalexins with a dithiocarbamate group show antifungal activity comparable to that of **49** (Tables 3 and 8). Based on the possible ITC release (Hanschen et al., 2014) and thiol reactivity (Kaul et al., 2021) it would be an attractive interpretation that the dithiocarbamate phytoalexin is a precursor that is taken up by the fungus, where it is metabolized to release the potent antifungal ITCs. We note that dithiocarbamates are a class of commercial fungicide used in plant protection at the same time (Kaul et al., 2021). However, presence of the dithiocarbamate group alone is not sufficient for the activity: **108** is a

Table 8
Antifungal activity of indole glucosinolate-derived phytoalexins. Additional details about the compounds can be found in Fig. 4 and Table S9.

ID	Source of agent	Fungal Strains	Fungus function	Activity against	Bioactive concentration	Ref.
67	transgenic *Nicotiana benthamiana*	*Alternaria brassicicola*, *Botrytis cinerea*	plant pathogen	mycelial growth	IC50: 1 mM IC50: 75 μM	Calgaro-Kozina et al. (2020)
67	synthesized	*Alternaria brassicicola*	plant pathogen	mycelial growth	40% inhib. at 100 μM	Colou et al. (2019)
67	synthesized	*Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	100% inhib. at 0.3 mM	Pedras and Hossain (2007)
67	synthesized	*Phoma lingam*	plant pathogen	mycelial growth	34% inhib. at 0.5 mM (virulent) 100% inhib. at 0.5 mM (avirulent)	Pedras and Okanga (2000)
67	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	55% inhib. at 0.5 mM	Pedras and Sarma-Mamillapalle, (2012a)
67	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	37% inhib. at 0.5 mM	Pedras and Snitynsky (2010)
67	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	38% inhib. at 0.5 mM	Pedras et al., (2007a)
67	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	100% inhib. at 0.5 mM	Pedras et al., (2007b)
67	synthesized	*Alternaria brassicicola*	plant pathogen	mycelial growth	39% inhib. at 0.5 mM	Pedras et al., (2009a)
67	synthesized	*Botrytis cinerea*	plant pathogen	mycelial growth	100% inhib. at 0.5 mM	Pedras et al., (2011a)
67	synthesized	*Alternaria brassicicola*	plant pathogen	mycelial growth	59% inhib. at 0.5 mM	Pedras et al. (2012)
67	synthesized	*Alternaria brassicicola*, *Alternaria brassicae*	plant pathogen	mycelial growth	EC50: 3–13 mM EC50: 3 mM	Sellam et al. (2007b)
67	standard	*Alternaria brassicicola*	plant pathogen	mycelial growth	EC50: 2 mM	Srivastava et al. (2013)
67	synthesized	*Bipolaris leersiae*	not indicated	mycelial growth	0.05 mM (semiquantitative)	Suchy et al. (2001)
96	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	61% inhib. at 0.5 mM	Pedras and Snitynsky (2010)
96	synthesized	*Alternaria brassicae*, *Phoma lingam*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	100% inhib. at 0.5 mM 60% inhib. at 0.5 mM 100% inhib. at 0.5 mM 100% inhib. at 0.5 mM	Pedras and Zaharia (2001)
97	synthesized	*Alternaria brassicicola*	plant pathogen	mycelial growth	20% inhib. at 0.1 mM	Pedras and Abdoli (2017)
98	synthesized	*Phoma lingam*	plant pathogen	spore germination	100% inhib. at 0.5 mM	Pedras and Zaharia (2000)
99	synthesized	*Phoma lingam*	plant pathogen	spore germination	30% inhib. at 0.5 mM	Pedras and Zaharia (2000)
100	*Erucastrum canariense*	*Alternaria brassicicola*, *Leptosphaeria maculans*, *Sclerotinia sclerotiorum*				Pedras and To (2017)
100	synthesized	*Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	100% inhib. at 0.5 mM 40% inhib. at 0.5 mM	Pedras et al., (2006b)
100	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	40% inhib. at 0.5 mM	Pedras and Sarma-Mamillapalle, (2012b)
101	synthesized	*Phoma lingam*	plant pathogen	mycelial growth	100% inhib. at 0.5 mM	Pedras and Okanga (1999)
101	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	48% inhib. at 0.5 mM	Pedras and Snitynsky (2010)
101	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	100% inhib. at 0.5 mM	Pedras and Suchy, (2006b)
101	synthesized	*Alternaria brassicae*, *Phoma lingam*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	100% inhib. at 0.5 mM 100% inhib. at 0.5 mM 100% inhib. at 0.5 mM 100% inhib. at 0.5 mM	Pedras and Zaharia (2001)
101	synthesized	*Alternaria brassicicola*	plant pathogen	mycelial growth	85% inhib. at 0.5 mM	Pedras et al., (2009a)
101	synthesized	*Botrytis cinerea*	plant pathogen	mycelial growth	100% inhib. at 0.5 mM	Pedras et al., (2011a)
102	synthesized	*Phoma lingam*, *Rhizoctonia solani*	plant pathogen	mycelial growth	30% inhib. at 0.5 mM 27% inhib. at 0.5 mM	Pedras and Okanga (1999)
102	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	22% inhib. at 0.5 mM	Pedras and Snitynsky (2010)
102	synthesized	*Alternaria brassicicola*	plant pathogen	mycelial growth	61% inhib. at 0.5 mM	Pedras et al., (2009a)
102	synthesized	*Botrytis cinerea*	plant pathogen	mycelial growth	100% inhib. at 0.5 mM	Pedras et al., (2011a)
103	*Brassica napus*					Pedras et al. (2008)

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Table 8 (*continued*)

ID	Source of agent	Fungal Strains	Fungus function	Activity against	Bioactive concentration	Ref.
		Rhizoctonia solani,	plant	mycelial	56% inhib. at 0.5 mM	
		Sclerotinia sclerotiorum	pathogen	growth	82% inhib. at 0.5 mM	
104	synthesized	*Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	100% inhib. at 0.3 mM	Pedras et al., (2004a)
105	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	40% inhib. at 0.5 mM	Pedras and Snitynsky (2010)
105	*Erucastrum gallicum*	*Rhizoctonia solani,*	plant	mycelial	68% inhib. at 0.5 mM	Pedras and Ahiahonu (2004)
		Sclerotinia sclerotiorum	pathogen	growth	53% inhib. at 0.5 mM	
105	*Erucastrum canariense*	*Alternaria brassicicola,*	plant	mycelial	36% inhib. at 0.5 mM	Pedras and To (2017)
		Leptosphaeria maculans,	pathogen	growth	44% inhib. at 0.5 mM	
		Sclerotinia sclerotiorum			77% inhib. at 0.5 mM	
105	synthesized	*Rhizoctonia solani,*	plant	mycelial	68% inhib. at 0.5 mM	Pedras et al., (2006b)
		Sclerotinia sclerotiorum	pathogen	growth	53% inhib. at 0.5 mM	
107	synthesized	*Alternaria brassicicola*	plant pathogen	mycelial growth	87% inhib. at 0.15 mM	Pedras and To (2016)
108	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	inactive at 0.5 mM	Pedras and Snitynsky (2010)
108	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	inactive at 0.5 mM	Pedras and Okanga (1999)
109	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	28% inhib. at 0.5 mM	Pedras and Snitynsky (2010)
109	synthesized	*Alternaria brassicicola*	plant pathogen	mycelial growth	54% inhib. at 0.5 mM	Pedras et al., (2009a)
109	synthesized	*Bipolaris leersiae*	not indicated	mycelial growth	1 mM (semiquantitative)	Suchy et al. (2001)
109	*Raphanus sativus*	*Pyricularia oryzae*	not indicated	mycelial growth	100% inhib. at 400 ppm	Takasugi et al. (1987)
111	synthesized	*Phoma lingam*	plant pathogen	spore germination	92% inhib. at 1 mM	Pedras and Khan (1996)
111	synthesized	*Rhizoctonia solani*	plant pathogen	mycelial growth	14% inhib. at 0.5 mM	Pedras and Okanga (1999)
111	synthesized	*Cladosporium cucumerinum,*	plant	mycelial	100% inhib. at 1 μM	Pedras et al., (2004b)
		Phoma lingam,	pathogen	growth	100% inhib. at 0.5 mM	
		Rhizoctonia solani,			28% inhib. at 0.5 mM	
		Sclerotinia sclerotiorum			80% inhib. at 0.5 mM	
111	standard	*Leptosphaeria maculans,*	plant	mycelial	70% inhib. at 0.5 mM	Pedras et al., (2006a)
		Rhizoctonia solani,	pathogen	growth	53% inhib. at 0.5 mM	
		Sclerotinia sclerotiorum			33% inhib. at 0.5 mM	
112	synthesized	*Phoma lingam*	plant pathogen	spore germination	75% inhib. at 1 mM	Pedras and Khan (1996)
113	standard	*Leptosphaeria maculans,*	plant	mycelial	70% inhib. at 0.5 mM	Pedras et al., (2006a)
		Rhizoctonia solani,	pathogen	growth	53% inhib. at 0.5 mM	
		Sclerotinia sclerotiorum			33% inhib. at 0.5 mM	
114	synthesized	*Alternaria brassicicola*	plant pathogen	mycelial growth	100% inhib. at 0.5 mM	Pedras and Abdoli (2017)
114	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	54% inhib. at 0.5 mM	Pedras and Snitynsky (2010)
114	synthesized	*Cladosporium cucumerinum,*	plant	mycelial	100% inhib. at 0.01 μM	Pedras et al., (2004b)
		Phoma lingam,	pathogen	growth	100% inhib. at 0.5 mM	
		Rhizoctonia solani,			100% inhib. at 0.5 mM	
		Sclerotinia sclerotiorum			100% inhib. at 0.5 mM	
115	synthesized	*Alternaria brassicicola*	plant pathogen	mycelial growth	40% inhib. at 0.5 mM	Pedras and Abdoli (2017)
115	synthesized	*Cladosporium cucumerinum,*	plant	mycelial	100% inhib. at 1 μM	Pedras et al., (2004b)
		Phoma lingam	pathogen	growth	48% inhib. at 0.5 mM	
116	synthesized	*Leptosphaeria maculans,*	plant	mycelial	55% inhib. at 0.5 mM	Pedras et al., (2006a)
		Rhizoctonia solani,	pathogen	growth	100% inhib. at 0.5 mM	
		Sclerotinia sclerotiorum			100% inhib. at 0.5 mM	
118	synthesized	*Alternaria brassicicola,*	plant	mycelial	47% inhib. at 0.5 mM	Pedras and Yaya (2012)
		Leptosphaeria maculans,	pathogen	growth	29% inhib. at 0.5 mM	
		Rhizoctonia solani,			100% inhib. at 0.5 mM	
		Sclerotinia sclerotiorum			65% inhib. at 0.5 mM	
119	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	43% inhib. at 0.5 mM	Pedras and Sarma-Mamillapalle, (2012a)
120	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	34% inhibition at 0.5 mM	Pedras and Sarma-Mamillapalle, (2012a)
121	*Brassica napus*	*Rhizoctonia solani,*	plant	mycelial	39% inhib. at 0.5 mM	Pedras et al. (2008)
		Sclerotinia sclerotiorum	pathogen	growth	27% inhib. at 0.5 mM	
122	*Brassica napus*	*Rhizoctonia solani,*	plant	mycelial	42% inhib. at 0.5 mM	Pedras et al. (2008)
		Sclerotinia sclerotiorum	pathogen	growth	27% inhib. at 0.5 mM	
123	synthesized	*Alternaria brassicicola,*	plant	mycelial	56% inhib. at 0.5 mM	Pedras et al., (2007c)
		Leptosphaeria maculans,	pathogen	growth	47% inhib. at 0.5 mM	
		Rhizoctonia solani,			54% inhib. at 0.5 mM	
		Sclerotinia sclerotiorum			89% inhib. at 0.5 mM	
123	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	43% inhib. at 0.5 mM	Pedras and Sarma-Mamillapalle, (2012b)

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Table 8 (*continued*)

ID	Source of agent	Fungal Strains	Fungus function	Activity against	Bioactive concentration	Ref.
124	synthesized	*Phoma lingam*	plant pathogen	spore germination	concentration not indicated	Pedras et al. (1999)
124	synthesized	*Leptosphaeria maculans*	plant pathogen	mycelial growth	42% inhib. at 0.5 mM	Pedras et al. (2003)
125	synthesized	*Alternaria brassicicola,* *Leptosphaeria maculans,* *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	69% inhib. at 0.5 mM 100% inhib. at 0.5 mM 100% inhib. at 0.5 mM	Pedras and To (2015)
126	synthesized	*Alternaria brassicicola,* *Leptosphaeria maculans,* *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	76% inhib. at 0.5 mM 94% inhib. at 0.5 mM 59% inhib. at 0.5 mM	Pedras and To (2015)
127	synthesized	*Alternaria brassicicola,* *Leptosphaeria maculans,* *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	100% inhib. at 0.5 mM 100% inhib. at 0.5 mM 100% inhib. at 0.5 mM	Pedras and To (2015)
128	*Barbarea vulgaris, Barbarea verna* or synthesized	*Alternaria brassicicola, Leptosphaeria maculans, Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	74% inhib. at 0.5 mM 63% inhib. at 0.5 mM 83% inhib. at 0.5 mM	Pedras et al. (2015)
129	*Barbarea vulgaris, Barbarea verna* or synthesized	*Alternaria brassicicola,* *Leptosphaeria maculans*	plant pathogen	mycelial growth	50% inhib. at 0.5 mM 46% inhib. at 0.5 mM	Pedras et al. (2015)
130	*Barbarea vulgaris, Barbarea verna* or synthesized	*Alternaria brassicicola,* *Leptosphaeria maculans,* *Sclerotinia sclerotiorum*	plant pathogen	mycelial growth	100% inhib. at 0.5 mM 100% inhib. at 0.5 mM 100% inhib. at 0.5 mM	Pedras et al. (2015)
131	*Barbarea vulgaris, Barbarea verna* or synthesized	*Alternaria brassicicola,* *Leptosphaeria maculans*	plant pathogen	mycelial growth	47% inhib. at 0.5 mM 26% inhib. at 0.5 mM	Pedras et al. (2015)

Authorities of botanical taxa: *Barbarea verna* (Mill.) Asch. (Brassicaceae), *Barbarea vulgaris* W.T.Aiton (Brassicaceae), *Brassica napus* L. (Brassicaceae), *Erucastrum canariense* Webb & Berthel. (Brassicaceae), *Erucastrum gallicum* O.E.Schulz (Brassicaceae), *Nicotiana benthamiana* Domin (Solanaceae), *Raphanus sativus* L. (Brassicaceae).

Authorities of fungal taxa: *Alternaria brassicae* (Berk.) Sacc. (Pleosporaceae), *Alternaria brassicicola* (Schwein.) Wiltshire (Pleosporaceae), *Bipolaris leersiae* (G.F. Atk.) Shoemaker (Pleosporaceae), *Botrytis cinerea* Pers. (Sclerotiniaceae), *Cladosporium cucumerinum* Ellis & Arthur (Cladosporiaceae), *Leptosphaeria maculans* Ces. & De Not. (Leptosphaeriaceae), *Phoma lingam* (Tode) Desm. (o. Pleosporales), *Pyricularia oryzae* Cavara (Pyriculariaceae), *Rhizoctonia solani* J.G. Kühn (Ceratobasidiaceae), *Sclerotinia sclerotiorum* (Lib.) de Bary (Sclerotiniaceae).

Compound references: 67, Brassinin; **96**, Sinalexin; **97**, Rutalexin; **98**, Sinalbin A; **99**, Sinalbin B; **100**, Erucalexin; **101**, Brassilexin; **102**, Cyclobrassinin; **103**, 4-Methoxy-cyclobrassinin; **104**; 1-Methoxy-brassinin; **105**, 1-Methoxy-spirobrassinin; **107**, Cyclonasturlexin; **108**, Dioxybrassinin; **109**, Spirobrassinin; **111**, Brassicanal A; **112**, Brassicanal A sulfoxide; **113**, Brassicanal C; **114**, Brassicanate A; **115**, Isalexin; **116**, Caulilexin A; **118**, 4-Methoxy-1*H*-indole-3-isonitrile (Isocyalexin A); **119**, Isobrassinin; **120**, 1-Methoxyisobrassinin; **121**, Dehydrocyclobrassinin; **122**, 4-Methoxy-dehydrocyclobrassinin; **123**, Brussalexin A; **124**, Wasalexin A; **125**, Nasturlexin A; **126**, Nasturlexin B; **127**, Tridentatol C; **128**, Nasturlexin C; **129**, Nasturlexin C sulfoxide; **130**, Nasturlexin D; **131**, Nasturlexin D sulfoxide.

derivative of **67** with an intact dithiocarbamate moiety, yet it is inactive against *L. maculans* (Pedras and Snitynsky, 2010). Additionally, Matsuoka et al. (1997) have reported that dithiocarbamates are relatively weak antifungal agents, compared to their ITC precursors. There are some data published on the antifungal mechanism of **67**; some of these data support the ITC release hypothesis, others do not. Analysis of the proteome of *A. brassicicola* treated with **67** by Pedras and Minic (2012) revealed many differentially expressed proteins, including ones involved in metabolism, processing, synthesis, as well as several heat shock proteins (HSPs). A low dose of **67** (0.1 mM) resulted in a net upregulation of metabolism, chaperone and co-chaperone proteins, while a high dose (0.5 mM) resulted in a more complex effect on metabolism as well as an upregulation of proteins involved in protein processing. In a study (Cho et al., 2014), a gene expression profile comparison was carried out between the wild type and Δbdtf1 *A. brassicicola*, where the mutant could not metabolize **67** making it highly susceptible to the compound. A small set of 15 genes were upregulated both under exposure of **67** and plant infection, suggesting a possible role in the metabolism of **67**. The genes encoded diverse enzymes, including a gene with low similarity to glutathione-S-transferase and two transporters. In contrast, when studying the transcriptional response of *A. brassicicola* exposed to an ITC (**9**), the study by Sellam et al. (2007a,b) found that the majority of the differentially expressed genes belong to ones involved in oxidative stress management and membrane transporters. Though the study of Cho et al. (2014) might suggest possible ITC release from the compound, this was not proven, and other studies show a much more complex response during exposure, suggesting that additional modes of action contribute to the antifungal effects of **67**.

Unfortunately, there are very few studies on possible mechanisms of other phytoalexins. For example, brassinin oxidase, a specific detoxification enzyme of **67**, was competitively inhibited by **102** (M. S. C. Pedras et al., 2008), suggesting a possible *in vivo* role of the compound.

Metabolism routes of various phytoalexins by fungal pathogens has been reviewed by Pedras and Abdoli (2017). Therefore, only short summaries are given in the following sections, focusing on specific detoxification enzymes.

9.2. Metabolism/detoxification of brassinin and dithiocarbamate phytoalexins by fungi

Brassinin (**67**) is a relatively simple dithiocarbamate adduct of **50** and methyl thiol (Fig. 4) which was described to be detoxified by specific fungal enzymes. The inability to metabolize **67** (Bdtf-mutant) resulted in decreased virulence of *A. brassicicola* on *Brassica*, but not *Arabidopsis* according to Srivastava et al. (2013), which suggests the importance of the detoxification of **67** in virulence of the fungus. Biotransformation is usually done via glycosylation or reductive/oxidative modifications, as detailed below.

The compound is either biotransformed in fungi by glucosylation, or a cleavage of the side chain. The enzyme brassinin glucosyltransferase 1 (SsBGT1) from the plant-pathogenic *S. sclerotiorum*, for example, is able to detoxify **67** and two other phytoalexins via glucosylation (Sexton et al., 2009). The enzyme is induced in response to **94**, **102**, **101**, **67** and an analogue of **94**. The tested fungus was shown to be able to completely metabolize **67** as well as **104** into their corresponding glucosyl derivates within 12–24 h (Pedras et al., 2004a). The glucosylated compounds showed no antifungal activity.

In other instances, fungal detoxification was found to use side chain cleavage. The virulence of *L. maculans*, a fungal pathogen of *Brassica*, correlated with its ability to metabolize **67** according to Pedras et al. (1997). It was later discovered that *L. maculans* detoxifies **67** by a specific enzyme, brassinin oxidase (Pedras et al., 2005), with **74** being the

product of the enzymatic reaction (Pedras et al., 2011b). Another isolate of *L. maculans* biotransformed the compound to **63** through **62** (Pedras et al., 2007a), while other dithiocarbamate analogues were biotransformed into respective amines and N-Ac-amines in an analogous manner (Pedras and Sarma-Mamillapalle, 2012a).

Another specific enzyme, brassinin hydrolase from *L. maculans* and *A. brassicicola*, catalyzes the hydrolysis of the dithiocarbamate group, resulting in **62** (Pedras et al., 2009b), ethane thiol and carbonyl sulfide (Pedras et al., 2012). The enzymes from both fungi were purified and their sequence suggested that they belong to the amidases (EC 3.5.1.4) (Pedras et al., 2009b). **62** was further biotransformed into **63** (Pedras et al., 2009a). While **62** was much less active against *A. brassicicola* than **67**, **63** lacked antifungal activity (Pedras et al., 2009a). Similar products were also obtained during biotransformation of **67** by a canola-virulent isolate of *L. maculans* (Pedras and Sarma-Mamillapalle, 2012a).

In *B. cinerea*, **67** was also metabolized to **62** which was slowly biotransformed further into **63**, **74** and **75** as shown by Pedras et al. (2011a). Again, the metabolites were much less toxic than **67**, suggesting that this route can be used as an efficient detoxification mechanism.

An analogue of **67** with an additional methylene group in the side-chain is detoxified by both non-adapted and adapted strains of fungal pathogens, using different detoxification routes according to Pedras and Okanga (2000): the virulent *P. lingam* used transformation routes to modify (oxidize, or fuse) the ring system, but also metabolized it into compounds with the ring system intact and the dithiocarbamate moiety removed (to **95**). All biotransformations led to a significant loss of antifungal potency against both virulent and avirulent strains.

The biotransformation of **108** resulted in a spiro thiocarbamoic lactone compound, with an apparent elimination of one of the sulfur atoms (Pedras and Snitynsky, 2010).

The presence of specific decomposing enzymes against **67** are likely the result of an efficient co-evolution of fungi to defend against GSL decomposition products. Even though the specificity for **67** is there, these enzymes are probably independent developments, as suggested by the different chemical reactions they catalyze. Specific **67** detoxifying enzymes include a glucosyl transferase, a hydrolase acting on the dithiocarbamate, and an oxidase showing specificity towards indole compounds. Unfortunately, the metabolism of other phytoalexins is not characterized to this extent to date.

9.3. Metabolism/detoxification of carbamodithioic acid derivatives by fungi

There is also evidence that the carbamodithioic acid derivatives **109** and **105** are detoxified by fungi: the biotransformations resulted in loss of antifungal potency. The two compounds were metabolized via a non-glucosylative pathway in *S. sclerotiorum* (Pedras and Hossain, 2006), while *L. maculans* metabolized them into the cyclic dithiocarbamates spirothiazolidinethione and its 1-methoxy derivative, respectively (Pedras and Snitynsky, 2010).

The compounds with a carbamodithioic acid derivative in the side chain **124** and its Z isomer were metabolized by two virulent isolates of *L. maculans* according to Pedras and Suchy (2006a). The biotransformation was a side chain modifications either by conversion to a carbamothioic acid derivative or by cleavage.

9.4. Metabolism/detoxification of cyclobrassinin by fungi

There are several fungal biotransformation routes described for **102**. Fungi either detoxify the compounds by opening the 1,3-thiazine ring, or by glucosylation at the indole N (Pedras and Abdoli, 2017). Interestingly, other phytoalexins were obtained as transformation products, such as **111** in the case of *R. solani* (Pedras and Okanga, 1999) and **101** in *B. cinerea* (Pedras et al., 2011a). Moreover, virulent and avirulent *P. lingam* isolates differed in the biotransformation activity towards **102**: the avirulent strain produced the antifungal **101**, while the virulent strain produced the inactive **108** as the main end product (Pedras and Snitynsky, 2010).

A specific fungal enzyme responsible for biotransformation of **102**, cyclobrassinin hydrolase, was also characterized (Pedras and Minic, 2014). The enzyme from *A. brassicicola* converts **102** into a carbamothioate by opening the 1,3-thiazine ring. It is inhibited by various phytoalexins: **101** and its 1-methyl derivative were found to be noncompetitive inhibitors, **94** was found to be a competitive inhibitor, while **108** inhibits it through a mixed mechanism. Compounds **96** and **111** were also inhibitory (the type of inhibition was not determined), but **67**, 1-methyl derivative of **67** and **109** lacked effects on the enzyme.

9.5. Metabolism/detoxification of thiomethyl-indole phytoalexins by fungi

There are also a few studies dealing with metabolism of the thiomethyl-indole compounds **111** and **114** (Fig. 4). The fungus *L. maculans* was shown to biotransform the compound via oxidation of the -SMe and reduction of the –CHO group into $–CH_2OH$ or $–CH_3$ (Pedras et al., 1997; Pedras and Khan, 1996). The reduction of the –CHO resulted in complete loss of activity regarding inhibition of spore germination. In the study by Pedras and Khan (1996), an avirulent strain showed much less biotransformation potential compared to virulent ones. During the biotransformation in *A. brassicicola* (Pedras and Abdoli, 2017), the thiomethyl group remained intact. Instead, the hydrolysis of the methyl ester is followed by decarboxylation, resulting in 2-methylsulfanediyl-1*H*-indole.

9.6. Metabolism/detoxification of 1,2-thiazole phytoalexins by fungi

The phytoalexins **101** and **96** were biotransformed by glycosylation by *S. sclerotiorum* (Pedras and Hossain, 2006). On the other hand, the biotransformation route was cleavage of the 1,2-thiazole ring, resulting in 3-aminomethylene-indole-2-thione in both *L. maculans* and *B. cinerea* (Pedras et al., 2011a; Pedras and Snitynsky, 2010). Interestingly, the product was shown to oxidize slowly and spontaneously to produce **101** again.

In another study on metabolism of **101** by *L. maculans* (Pedras and Suchy, 2005), biotransformation continued to 2-thio-derivatives of **74**. The sulfur was in various oxidation states from –SH to $–SO_3H$ in different metabolites. The methoxy derivative **96** suffered opening of the ring in the same manner. Both cases resulted in a decrease in antifungal potency.

9.7. Metabolism/detoxification of other phytoalexins by fungi

Rutalexin (**97**) has an oxidized 1,3-thiazine ring fused to the indole moiety (Fig. 4). During biotransformation by *A. brassicicola* this ring is opened, and the product is either oxidized spontaneously to a sulfonic acid through dimerization and subsequent cleavage, or is fused with phomapyrone G (an intermediate of polyketide origin) to form a hybrid metabolite rutapyrone (Pedras and Abdoli, 2017).

The study by Pedras and Abdoli (2017) found that **115** was reduced via *A. brassicicola* to its 3-hydroxy derivative, which spontaneously undergoes oxidation to form **115** again.

10. Interactions of other, putative glucosinolate downstream products and fungi

There are scarce reports of additional compounds, hypothesized to be originated from GSLs, reviewed by Blažević et al. (2020). These additional, previously unmentioned putative GSL downstream products include (1) raphanusanin isomers; (2) the putative epithionitrile downstream products petiolatamide and alliarinoside as well as (3) maca thiohydantoins. There is a lack of publications regarding the

interactions of these compounds with fungi. The allelopathic effects of plants that produce alliarinoside is clearly linked to GSL decomposition products altering mycorrhizal communities (Cipollini and Cipollini, 2016), the contribution of these unique glycosides to the effects is not clear due to the presence of the strongly antifungal ITCs. Maca hydantoins, on the other hand have been tested for antifungal activity under laboratory conditions against *A. fumigatus*, *Candida parapsilosis* (Ashford) Langeron & Talice (o. Saccharomycetales) and *C. albicans*, yet proven to be inactive (Yu et al., 2017). Clearly, we are far from understanding of the role of these compounds in the life of their producers. The many unknowns regarding their biosynthesis are also to be addressed in future work.

11. Conclusions

The great diversity of Brassicaceae plants used for human consumption or as industrial crops underlines the significance of this plant group to mankind, not to mention results that suggest possible disease prevention capabilities if consumed on a regular basis. Without doubt, the potency of the natural products from the glucosinolate pathway has significantly contributed to Brassicales becoming a successful group of plants. However, most of the published literature is focused on the default decomposition products of glucosinolates, the isothiocyanates, especially when antimicrobial activity or possible applications are to be considered. This review aimed to fill this gap by summarizing our current knowledge on the interaction of fungi with other volatile and non-volatile compounds that arise from this pathway.

Our understanding of the role of glucosinolate-derived compounds in defense against pathogens has grown considerably in the last decade. Studies comparing fungal pathogenesis on knockout *A. thaliana* lines have been particularly useful in contributing to our knowledge, but several studies on important agricultural crops have also been published. There is considerable evidence that biosynthesis and in vivo decomposition of glucosinolates is an important step in arresting fungal pathogenesis, and this system also plays a role in balancing mutualistic interactions with fungi. Almost all reviewed compounds were shown to be elicited during fungal challenge in various plants: an increased biosynthesis capacity is frequently found, and this capacity is frequently (but not always) correlated with disease resistance/tolerance.

The biosynthesis of mixtures of various glucosinolate subclasses enables these plants to use an array of highly diverse degradation products that can be used against pathogens with various strategies. The importance of these glucosinolate downstream products is in many instances supported by data showing direct antifungal activity.

However, the whole picture is complicated by the fact that fungi can detoxify the defensive compounds, not to mention use them as carbon and/or nitrogen sources. This is exemplified by the widespread utilization capacity of glucosinolates through fungal myrosinase activity or using nitriles via nitrilases. Fungal metabolism is quite versatile, and several specific enzymes could be characterized that are capable of specifically detoxifying Brassicaceae phytoalexins or other glucosinolate downstream products. Examples include brassinin oxidase, brassinin glucosyltransferase 1 and brassinin hydrolase.

To make the situation even more complicated, several compounds, including 1*H*-indole-3-acetonitrile, 1*H*-indole-3-carboxylic acid and 1*H*-indole-3-carboxaldehyde, were shown to be biosynthesized by a wide range of filamentous fungi. However, the extent of this activity during plant colonization is unknown.

Altogether, despite the importance of the glucosinolate-derived compounds, many questions remain open. The exact composition of the *in vivo* glucosinolate-derived mixture of antifungal agents is not fully characterized, and it likely contains additional, currently unidentified natural products. The antifungal mechanisms of these compounds are also far from being understood. Then there is always the possibility of finding other additional ecological roles of volatile and non-volatile natural products. These and additional points should be addressed in further research to gain a better understanding of the interaction reviewed herein. A deeper understanding of this system will not only have an impact on agricultural practice, but also increase our understanding of strategies of pathogenesis by fungi and defense against pathogenesis in plants.

Author contributions

Conceptualization, S.G.; Writing – original draft preparation, S.G., T. P., Z.Sz.; critical revision, G.V.; funding, S.G. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

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

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Appendix A. Supplementary data

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