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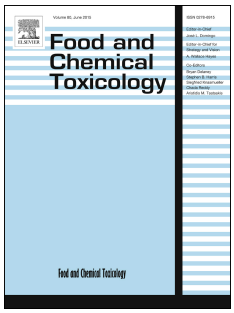

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PII: S0278-6915(17)30795-0

DOI: 10.1016/j.fct.2017.12.047

Reference: FCT 9492

To appear in: *Food and Chemical Toxicology*

Received Date: 30 September 2017

Revised Date: 21 December 2017

Accepted Date: 22 December 2017

Please cite this article as: Petrov, V., Qureshi, M.K., Hille, J., Gechev, T., Occurrence, biochemistry and biological effects of host-selective plant mycotoxins, *Food and Chemical Toxicology* (2018), doi: 10.1016/j.fct.2017.12.047.

Occurrence, biochemistry and biological effects of host-selective plant mycotoxins

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Key words

host-selective toxins, programmed cell death, reactive oxygen species, pathogens, necrotrophs, antitumor activity

Highlights

- Some fungal necrotrophs induce plant diseases by secreting specific host-selective toxins (HSTs).
- HSTs are either secondary metabolites or proteinaceous compounds.
- HSTs provoke cell death in sensitive plant tissues by different mechanisms, often involving reactive oxygen species (ROS).
- There are examples of HSTs having cytotoxic or cytostatic biological activities in animal and human cells.

List of abbreviations

EDA	9,10-epoxy-8-hydroxy-9-methyl-decatrienoic acid
HDAC	histone deacetylase
HDACI	histone deacetylase inhibitor
HR	hypersensitive response
HST	host selective toxin
PCD	programmed cell death
PKS	polyketide synthase
ROS	reactive oxygen species
SAM	sphinganine-analogue mycotoxins

1. Introduction

Some plant pathogenic fungi are able to synthesize toxic chemicals that are detrimental to plant growth and are related to the development of diseases. Normally they are classified either as pathogenicity factors, responsible for the onset of infections, or as virulence factors that determine the severity of the condition (Tsuge et al., 2013). Most of these plant mycotoxins are nonspecific since they can affect a wide range of species irrespectively of whether they are a host for the pathogen or not. However, a small group of so called host selective toxins (HSTs) are damaging only to the species that serve as hosts to the respective fungi (Wolpert et al., 2002). So far more than 20 HSTs have been identified, a lot of which are secondary metabolites and the rest are proteins (Lu et al., 2015). Interestingly, the production of HSTs is taxonomically restricted, with the bulk of such toxins produced by just two genera, *Alternaria* and *Cochliobolus*, and only several other species like *Pyrenophora tritici-repentis*, *Periconia circinata* and *Pyronellaea zeae-maydis*. The exact reason for this lack of diversity is still unknown but one speculation is that many HSTs remain undiscovered (Walton and Panaccione, 1993).

HSTs are regarded mainly as pathogenicity factors, since they are necessary for the induction of a disease. On one hand, fungal isolates that are unable to synthesize them generally cannot damage their host. On the other hand, the condition for plants to be susceptible to the corresponding pathogen infection is their sensitivity to the respective HST (Howlett, 2006; Yoder, 1980). Therefore, HSTs are defined as “compatibility agents”. In addition, HSTs are an example of molecular species that determine fungal physiological races able to affect different target species and present the opportunity to study the specificity of plant-parasite interactions. In principle, the outcome of the contact between an HST-producing fungus and a possible host is

defined by the presence of a single plant gene. However, unlike in other cases that follow the gene-for-gene principle developed by Flor (Flor, 1971), which postulates interaction between one avirulence determinant (*Avr*) in the pathogen and one plant resistance (*R*) gene, several fungal genes are required for HST production (Markham and Hille, 2001). Moreover, the HST target alleles in the plant genome actually confer susceptibility rather than resistance, which is known as the inverse gene-for-gene model (Stergiopoulos et al., 2013). The characteristics of the sensitivity of plants to HSTs can vary from dominant and semi-dominant to recessive or even cytoplasmically inherited (Walton and Panaccione, 1993).

Decades after the discovery of the first HSTs and the diseases associated to them (Meehan and Murphy, 1947; Tanaka, 1933), this group of chemicals is still a topic of rigorous research and as a result has contributed to the understanding of mechanisms of susceptibility and resistance, host-range determination, pathogen races, fungal genetics, secondary metabolism and evolution. Interestingly, although by definition HSTs influence only certain plant species, cases in which they exert harmful or beneficial effects on animal and human cells have also been documented. This, however, is still a relatively unexplored field and reports in the scientific literature on the topic are not abundant. Additional research on HSTs will throw light on their potential toxicological impact *in vivo* and contribution to mycotoxicoses in animals and humans or their possible applications as biologically active compounds in pharmacology, medicine and agriculture.

The purpose of this review is not only to provide concise information on the nature, mode of action and biochemistry of HSTs, but also on the current knowledge of the responses they induce in plant, animal and human cells.

2. Occurrence, targets and caused diseases

HSTs have been in the focus of scientific interest for many years not only due to their contribution to fundamental science, especially concerning elucidating the mechanisms of plant-pathogen interactions, but also because of their practical significance. All of them lead to plant diseases of considerable economic importance (Table 1) and some major outbreaks resulting in severe yield losses, like the epidemics of Victoria blight in oats and Southern corn leaf blight in the USA or black spot of pears in Japan, were caused by HST-producing fungi (Meehan and Murphy, 1947; Nishimura et al., 1978; Ullstrup, 1972).

2.1. *Alernaria* derived HSTs - The necrotrophic filamentous fungus *Alternaria alternata* is known to have several pathogenic variants (pathotypes) which are characterized with morphological similarity but pathological differences. These pathotypes produce one of the largest groups of individual HSTs by a single species and affect a variety of crops (Table 1). Up to date six such *Alternaria alternata* HSTs have been identified (Takaoka et al., 2014). Four of the *A. alternata* HSTs are synthesized by pathotypes that were originally considered to be unique biological species. The first of them is the causative agent of the black spot of Japanese pear, designated as AK-toxin. The involvement of a host-selective fungal metabolite in the development of black spot was suggested as early as the 1930s (Tanaka, 1933) and this is the first report in which an HST was associated with a plant disease. At the time this HST attracted the attention of the Japanese scientific community due to the threat it represents to farmers growing the newly introduced cultivar Nijisseiki (Tsuge et al., 2013). These pears are very susceptible to the pathogen, which was initially identified as a separate species - *Alternaria kikuchiana*. When later the biologically active phytotoxic substance was isolated, it was named AK-toxin. Another *Alternaria alternata* pathotype, originally designated as *Alternaria mali*,

inflicts the Alternaria blotch disease in apples, which is important mostly in countries of Southeastern Asia like Japan, South Korea and China, but has caused issues even in the USA. After a major outbreak in Japan in 1956 (Sawamura, 1972) the economic importance of the disease was recognized and research on its mechanism intensified, which ultimately led to the isolation and characterization of the toxin, at first termed alternaliolide and now known as AM-toxin (Okuno et al., 1974). Two other diseases – brown spot of tangerine and leaf spot of rough lemon, are provoked by the ACT- and ACR-toxins, respectively, which were originally attributed to a species called *Alternaria citri* (Gardner et al., 1985a; Kohmoto et al., 1993). The HST AF-toxin, secreted by the strawberry pathotype of *Alternaria alternata*, induces the Alternaria black spot of strawberry. Intriguingly, the strawberry and tangerine pathotypes were shown to be pathogenic also to the pear cultivars that are susceptible to the AK-toxin (Ito et al., 2004; Takaoka et al., 2014). Moreover, each of these pathotypes appears to synthesize two related compounds – AF-toxins I and II, and ACT-toxins I and II. AF-toxin I and ACM-toxin I damage strawberry and tangerines, respectively, as well as pear. AF-toxin II elicits a response only in pear while ACT-toxin II affects mostly pear, but is mildly toxic also to citrus plants (Kohmoto et al., 1993; Maekawa et al., 1984). And lastly, the sixth HST in the arsenal of *Alternaria alternata*, named AAL-toxin, is characteristic for the pathotype *lycopersici.* AAL-toxin exists in two forms, AAL T_a and AAL T_b, of which AAL-toxin T_a is the major one. These chemicals cause the stem canker disease in tomatoes and are able to induce severe necrosis in susceptible cultivars even at nanomolar concentrations (Witsenboer et al., 1992).

Another representative of the genus *Alternaria*, *Alternaria longipes*, a very close relative of *A. alternata*, provokes the brown spot in tobacco (Kodama et al., 1990). This is one of the most damaging leaf spot conditions across crop species and in the past century has caused losses

ranging from several to dozens of millions of dollars annually only in the USA (Lucas, 1975). The responsible HST in this case is AT-toxin. There has been a long debate in the scientific community concerning the species identity of this fungal pathogen. Some authors defended the argument that *Alternaria longipes* and *Alternaria alternata* are separate species, based on morphological, molecular and chemical distinctions (Andersen et al., 2001). Others, however, recommended interpreting such subtle differences, especially the morphological ones, as intraspecific variability (Tsuge et al., 2013; Wolpert et al., 2002). A very recent comparative genomics paper may have at last resolved this dispute (Hou et al., 2016). The results of the phylogenetic analyses published in this study reveal that *A. alternata* and *A. longipes* diverged only around 3 million years ago, which supports the hypothesis that *A. longipes* is a young, but unique species. It must be noted, however, that in previous reviews on the topic of Alternaria derived HSTs, AT-toxin is considered a product of *Alternaria alternata* (Tsuge et al., 2013; Wolpert et al., 2002). Finally, a third species from the Alternaria genus, *A. brassicicola*, leads to the black spot disease in Brassica plants, again through a mechanism involving an HST toxin. It is termed brassicicolin A and it has been demonstrated that the substance is more toxic to the sensitive brown mustard than to the tolerant canola (Pedras et al., 2009).

Table 1. A summary of the variety of fungal pathogens synthesizing HSTs, their HST products and diseases provoked in specific hosts. The species names previously given to some of the *Alternaria alternata* pathotypes are shown in parenthesis.

Fungal pathogen (previous name)	Toxin	Associated disease	Host
Alternaria alternata (*A. kikuchiana*)	AK-toxin	black spot	Japanese pear
Alternaria alternata (*A. mali*)	AM-toxin	alternaria blotch	apple
Alternaria alternata	AF-toxin	alternaria black spot	strawberry
Alternaria alternata (*A. citri*)	ACT-toxin	brown spot	tangerine
Alternaria alternata (*A. citri*)	ACR-toxin	leaf spot	rough lemon
Alternaria alternata	AAL-toxin	stem canker	tomato
Alternaria longipes	AT-toxin	brown spot	tobacco
Alternaria brassicicola	brassicicolin A	black spot disease	Brassica plants
Cochliobolus carbonum	HC-toxin	Northern leaf spot disease	maize
Cochliobolus heterostrophus	T-toxin	Southern leaf blight disease	*cms-T* maize
Cochliobolus heterostrophus	ChToxA		maize
Cochliobolus victoriae	victorin	Victoria blight disease	oats
Bipolaris sacchari	HS-toxin	eyespot disease	sugarcane
Pyronellaea zeae-maydis	PM-toxin	yellow leaf blight	maize
Periconia circinata	peritoxins (PC-toxins)	milo disease	sorghum
Pyrenophora tritici-repentis	Ptr ToxA, Ptr ToxB, Ptr ToxC	tan spot	wheat
Stagonospora nodorum	SnToxA, SnTox1-7	glume blotch	wheat
Stemphylium vesicarium	SV-toxins	brown spot	European pears
Leptosphaeria maculans	phomalide	blackleg disease	canola, rapeseed
Leptosphaeria maculans	depsilairdin		brown mustard
Leptosphaeria maculans	maculansins		brown mustard
Corynespora cassiicola	cassiicolin	*Corynespora* leaf fall disease	numerous species

2.2. HSTs of *Cochliobolus sp.* - Four other HSTs are produced by members of the genus *Cochliobolus* (anamorph *Bipolaris*/*Curvularia*). These are filamentous ascomycete fungi, some of which are highly aggressive pathogens of monocotyledonous plants (Condon et al., 2013). Recently it was suggested that the phytotoxic members of the genus had a common ancestor, which gave rise to the individual pathogenic species later than 20 million years ago (Ohm et al., 2012). One of them is *Cochliobolus carbonum*, a super virulent pathogen which is the causal agent of the Northern leaf spot disease in maize (Walton, 2006). Several races of the species are known, of which race 1 manifests the extreme pathogenicity that leads to the development of large necrotic lesions on susceptible plants. The reason is the release of a specific HST, designated HC-toxin (Kawai et al., 1983). This HC-toxin was one of the first pathogen metabolites demonstrated to have host-selectivity (Scheffer and Ullstrup, 1965) and was therefore instrumental as a model for further research on HSTs. The second *Cochliobolus*-derived HST is T-toxin and it is characteristic for *Cochliobolus heterostrophus*, which is responsible for the Southern corn leaf blight disease (SCLB) in maize carrying Texas cytoplasm for male sterility (*cms-T*) (Wise et al., 1999). Previously, the terms HMT- or BMT-toxin were also utilized for this HST (Stergiopoulos et al., 2013). The production of T-toxin greatly enhances the virulence of the corresponding *Cochliobolus heterostrophus* race, but is not absolutely necessary for pathogenicity since mutants unable to synthesize the toxin are still capable of inducing disease symptoms (Walton, 1996). Historically, SCLB is associated with one of the most economically devastating crop epidemics of the 20th century which was compromising the maize yield in the USA during the 1950s and 1960s (Ullstrup, 1972). Recently, another maize-specific HST, named ChToxA, was isolated from the same pathogen

(Lu et al., 2015). Next, the Victoria blight disease in oats is provoked by the fungus *Cochliobolus victoriae* that secretes a toxin called victorin. Victoria blight provides a very good illustration of the disease-inducing properties of HSTs since the pathogenicity of *Cochliobolus victoriae* is strictly dependent on its ability to produce victorin. On one hand isolates that cannot synthetize this compound are nonpathogenic (Scheffer et al., 1967) while on the other hand application of the toxin alone on sensitive oats plants reproduces the characteristic disease symptoms (Navarre and Wolpert, 1999). The last representative of this group of HSTs is the HS-toxin, also known as helminthosporoside, of *Bipolaris sacchari* (or *Helminthosporium sacchari*), a fungal species which is responsible for the eyespot disease in sugarcane (Schroter et al., 1985). As in the previous examples, the degree of susceptibility to the pathogen correlates with the sensitivity to the toxin, although it has been proposed that other factors may also influence the disease progression (Bournival et al., 1994). Interestingly, *B. sacchari* produces analogous toxins (toxoids) with similar biochemical structure, but lower molecular weight. They antagonize the effects of the HS-toxin and hence their biological function is still unclear (Stergiopoulos et al., 2013).

2.3. Other HSTs - The rest of the HSTs are synthesized by fungal species dispersed in different genera. The PM-toxin, for example, is a metabolite of *Pyronellaea zeae-maydis* (formerly known as *Mycosphaerella zeae-maydis)*, the causal agent of yellow leaf blight of maize (Aveskamp et al., 2010). This HST is very similar in structure, affected maize genotypes, and role in pathogenicity, to the T-toxin of *Cochliobolus heterostrophus* described above. However, unlike the T-toxin, PM-toxin is absolutely required for the induction of the disease on susceptible plants.

Another necrotrophic fungal pathogen, *Periconia circinata*, is responsible for the milo disease, characterized with root and crown rot of sorghum. This species produces a couple of HSTs with similar toxic properties, designated as peritoxin A and peritoxin B (also PC-toxins), that are selectively toxic to the host even when applied alone (Churchill et al., 2001).

Pyrenophora tritici-repentis provokes another disease of considerable economic importance – the tan spot of wheat, by means of the HST toxins Ptr ToxA, Ptr ToxB and Ptr ToxC (Ciuffetti et al., 2010; Effertz et al., 2002). Based on their ability to secrete one or a combination of these HSTs, different races of the pathogen have been identified, currently nominated as races from 1 to 8 (Lamari et al., 2003).

An even larger group of HSTs is utilized by *Stagonospora nodorum* (also *Parastagonospora nodorum*), which causes the glume blotch (Stagonospora nodorum blotch, SNB) on wheat. Currently, eight *S. nodorum* HSTs: SnToxA, and SnTox1 to SnTox7, have been isolated (Friesen et al., 2012; Friesen et al., 2008; Gao et al., 2015; Shi et al., 2015).

In turn, the HSTs SV-toxins I and II are related to the development of brown spot on European pears triggered by *Stemphylium vesicarium*. The fungus has a wide range of hosts, but the infection of susceptible pear cultivars is dependent on its ability to synthesize SV-toxins (Singh et al., 1999).

A very interesting mixture of HSTs of various chemical nature is in the arsenal of *Leptosphaeria maculans* (anamorph *Phoma lingam*), the pathogen leading to blackleg disease on the oilseed crops canola and rapeseed. These include phomalide, depsilairdin and maculansins A and B (Stergiopoulos et al., 2013). The *L. maculans* HST associated with the blackleg disease is phomalide, which induces symptoms on the susceptible canola and rapeseed but does not affect other related Brassica species like brown and white mustard (Ward et al., 1999). Conversely,

depsilairdin and the maculansins demonstrate inverse toxicity and damage brown mustard leaves, but not canola (Pedras and Chumala, 2011; Pedras et al., 2004).

Finally, *Corynespora cassiicola*, a cosmopolitan necrotroph with a range of over 70 hosts has been shown to produce the HST substance cassiicolin. It is involved in the *Corynespora* leaf fall disease (CLFD) which is most devastating to rubber trees (*Hevea brasiliensis*) but affects also numerous other plants like tobacco, tomato, soybean, cotton, etc. (Barthe et al., 2007).

3. Chemical nature

Despite their common functions in mediating pathogenicity, the described HSTs are quite various in their chemical composition (Table 2). The majority of them are secondary metabolites that are distributed in several biochemical classes (Figure 1), but a few are ribosomally produced peptides with much higher molecular weight.

3.1. Secondary metabolites – Typically for secondary metabolites, the HSTs are not important for growth, development and reproduction, but confer ecological advantages to the organisms that synthesize them.

Table 2. A summary of the chemical identities of all of the HSTs described in the text and studies in which these HSTs were isolated and structurally characterized.

Toxin	Chemical nature	Reference
AK-toxin	EDA-derivative	(Nakashima, et al., 1985)
AM-toxin	depsipeptide	(Okuno et al., 1974)
AF-toxin	EDA-derivative	(Nakatsuka et al., 1986)
ACT-toxin	EDA-derivative	(Kohmoto, et al., 1993)
ACR-toxin	polyketide	(Gardner et al., 1985b)
AAL-toxin	polyketide	(Bottini and Gilchrist, 1981)
AT-toxin	not resolved	
brassicicolin A	mannitol-derivative	(Gloer et al, 1988)
HC-toxin	cyclic peptide	(Walton et al., 1982)
T-toxin	polyketide	(Kono et al., 1985a)
ChToxA	protein	(Lu et al., 2015)
victorin	cyclic peptide	(Wolpert et al., 1985)
HS-toxin	sesquiterpene + sugar residues	(Macko et al., 1983)
PM-toxin	polyketide	(Kono et al., 1985a)
peritoxins (PC-toxins)	polyketide + cyclic peptide	(Macko et al., 1992)
Ptr ToxA	protein	(Balance et al., 1989)
Ptr ToxB	protein	(Strelkov et al., 1999)
Ptr ToxC	nonionic, polar molecule	(Effertz et al., 2002)
SnToxA	protein	(Friesen et al., 2006)
SnTox1	protein	(Liu et al., 2004)
SnTox2	protein	(Friesen et al., 2007)
SnTox3	protein	(Liu et al., 2009)
SnTox4	protein	(Abeysekara et al., 2009)
SnTox5	protein	(Friesen et al., 2012)
SnTox6	protein	(Gao et al., 2015)
SnTox7	protein	(Shi et al., 2015)
SV-toxins	not resolved	
phomalide	depsipeptide	(Ward et al., 1999)
depsilairdin	depsipeptide	(Pedras et al., 2004)
maculansins	mannitol-derivative	(Pedras and Yu, 2008)
cassiicolin	protein	(Barthe et al., 2007)

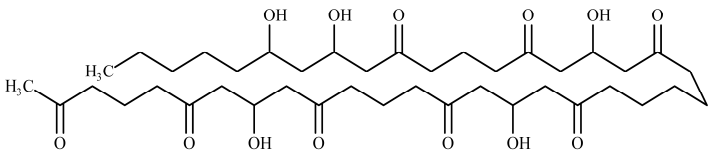
The T-toxin, PM-toxin, peritoxins, as well as *A. alternata* AAL- and ACR-toxins are examples of polyketides, a structurally diverse group of highly oxygenated compounds containing alternating carbonyl and methylene groups. They are biosynthesized by a Claisen mechanism that includes repetitive decarboxylative condensation of acetate units derived from malonyl-CoA. The process is reminiscent of fatty-acid synthesis and is catalyzed by a large family of multidomain enzymes or enzyme complexes called polyketide synthases (PKS) (Huffman et al., 2010). T-toxin comprises a group of related linear polyketides, produced by a type I PKS, whose chain length varies between 35 and 49 carbon atoms, with the major component being the C_{41} derivative (Kono et al., 1985a; Inderbitzin et al., 2010) (Figure 1). As mentioned above, PM-toxin is structurally analogous to T-toxin, but with a carbon chain of 33 to 35 C atoms (Kono et al., 1985a; Wolpert et al., 2002). Much more intriguing is the biochemistry of peritoxins, since they are hybrid molecules that consist of a cyclized peptide and a chlorinated polyketide (Macko et al., 1992). In addition, some biologically inactive peritoxin intermediates like *N*-3-(*E*-pentenyl)-glutaroyl-aspartate, circinatin, and 7-chlorocircinatin (Churchill et al., 2001) can be isolated from pathogenic *Periconia circinata* strains. The *A. alternata* ACR-toxins are also typical polyketides (Gardner et al., 1985b), products of a PKS type I enzyme (Izumi et al., 2012). The major form with the highest toxicity, ACR-toxin I, is a polyalcohol with 19 C atoms and an α-dihydropyrone ring. The other ACR-toxins, showing less potent biological activity, have a pyrone ring and a polyalcohol chain of various lengths (Kono et al., 1985b). Lastly, the AAL-toxins of *A. alternata* possess an aminopentol backbone to which is attached, through esterification, the tricarboxylic acid group propane-1,2,3-tricarboxylic acid (PTCA) (Bottini and Gilchrist, 1981) (Figure 1). The most significant feature of the backbone, whose precise formula is 1-amino-11,15-dimethylheptadeca-2,4,5,13,14-pentol, is its resemblance to sphingosine and

sphinganine. The AAL-toxins resemble structurally a group of non-HST mycotoxins called fumonisins and together these related metabolites are termed sphinganine-analogue mycotoxins (SAM or SAMT) (Du et al., 2008). The difference between the AAL-toxins and the fumonisins is that while AAL-toxins have only one PTCA side chain, the fumonisins have two of them.

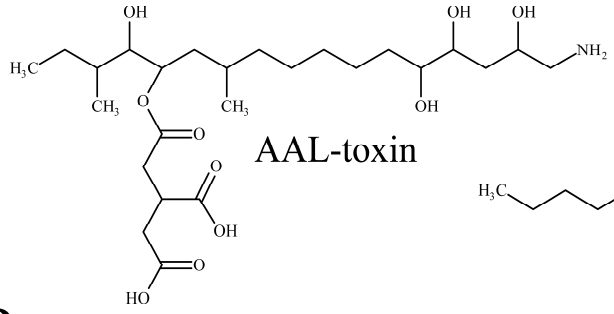
Another category of secondary metabolites, involving representatives of the HSTs, comprises peptides and depsipeptides. Two HSTs, victorin and HC-toxin are examples of cyclic peptides. In reality *C. victoriae* secretes in culture several related chemical compounds of which victorin C is the most abundant one (Wolpert et al., 2002). Victorin C is a very intriguing molecule, consisting of glyoxylic acid and a cyclic pentapeptide containing unusual amino acids: 5,5-dichloroleucine, threo-β-hydroxylysine, erythro-β-hydroxyleucine, α-amino-β-chloro-acrylic acid and 2-alanyl-3,5-dihydroxycyclopentenone (victalanine) (Wolpert et al., 1985). The rest of the structural variants of this HST are designated victorin B, D, E, victoricine and HV-toxin M and have only minor differences from victorin C, for instance – a different degree of chlorination of the leucine residue in victorins B and E (Wolpert et al., 1986). In turn, HC-toxin is a group of cyclic tetrapeptides, of which the main form produced by race 1 isolates is cyclo-(D-proline-L-alanine-D-alanine-L-2-amino-8-oxo-9,10-epoxydecanoic acid) (Walton et al., 1982) (Figure 1). It is synthetized non-ribosomally by a large enzyme termed HC-toxin synthetase (HTS). Fungal cyclic peptides often contain D-amino acids and HC-toxin is a typical case with 2 of them: the first one, D-proline, is made by an epimerase domain within the HTS enzyme, while for the second, D-alanine, is required a separate enzyme (alanine racemase, EC 5.1.1.1) (Cheng and Walton, 2000). While the non-ribosomal origin of HC-toxin has been confirmed, details on the biosynthesis of victorin, including the site and mechanism of its production, still remain to be elucidated.

Depsipeptides are peptides which have some of their amide groups replaced by ester linkages and therefore contain both peptide and ester bonds (Stawikowski and Cudic, 2007). HSTs with such structures are the AM-toxin, phomalide and depsilairdin. The AM-toxin I molecule is a four-membered depsipeptide with cyclical arrangement: cyclo(-L-α-amino-*p*-methoxy-phenylvaleryl-dehydroalanyl-L-alanyl-L-α-hydroxyisovaleryl-lactone) (Okuno et al., 1974). In turn, phomalide is a cyclic pentadepsipeptide containing three α-amino acids and two α-hydroxy acids: cyclo(Val-(E)-Aba-Hpp-Hmp-(R)-Leu), with Aba designating 2-amino-2-butenoic acid, Hpp - (2S)-2-hydroxy-3-phenylpropanoic acid and Hmp - (2S)-2-hydroxy-4-methylpentanoic acid (Ward et al., 1999). Interestingly, the presence and configuration of the (E)-double bond in the structure is important for the biological activity, since the analogue isophomalide with a (Z)-double bond does not damage Brassica species, while the saturated equivalent dihydrophomalide shifts its phytotoxicity from canola to the resistant brown mustard (Ward et al., 1996). Finally, depsilairdin is a unique acyclic combination of a tripeptide and a sesquiterpene elements, the first of which contains the previously unknown amino acid (2S,3S,4S)-3,4-dihydroxy-3-methylproline (Dhmp) (Figure 1). This unusual amino acid is crucial for the effects and host-selectivity of depsilairdin (Pedras et al., 2004).

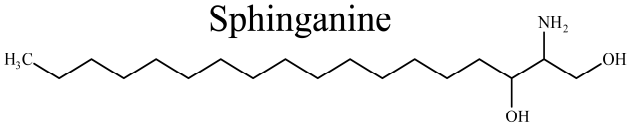
A



T-toxin

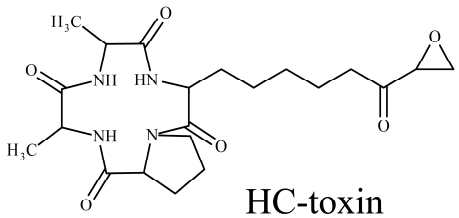


AAL-toxin

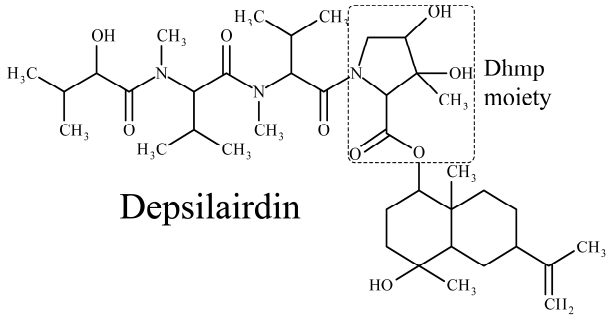


Sphinganine

B

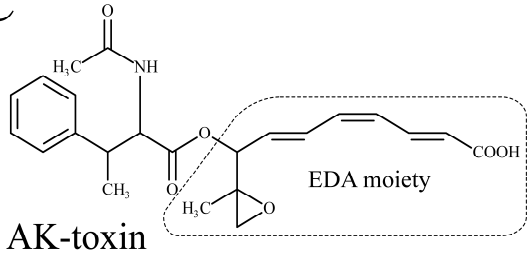


HC-toxin



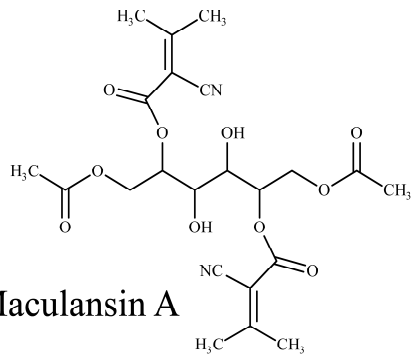
Depsilairdin

C



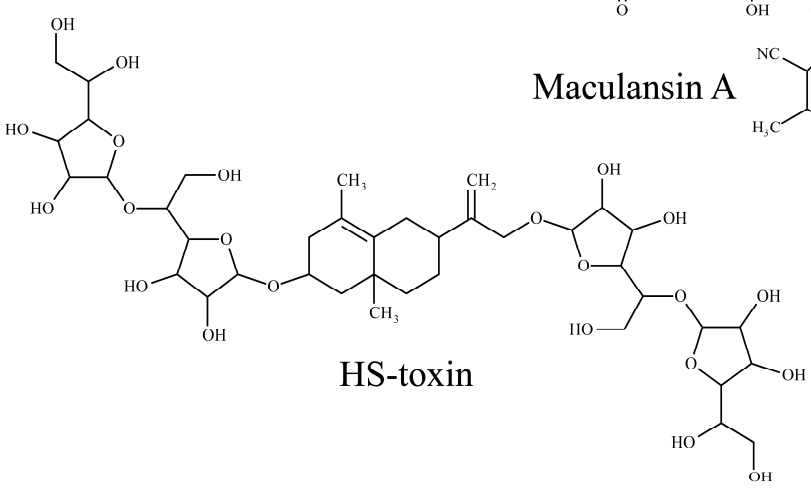
AK-toxin

D



Maculansin A

E



HS-toxin

Figure 1. Chemical composition of different types of secondary metabolite HSTs. **A**. T-toxin and AAL-toxin T_a as examples of polyketide HSTs. The AAL-toxin is an analogue of the complex lipid constituent sphinganine. **B**. The cyclic peptide HC-toxin and the depsipeptide depsilairdin. In the formula of the latter is marked the unusual amino acid 3,4-dihydroxy-3-methylproline (Dhmp). **C**. The AK-toxin, which is a derivative of 9,10-epoxy-8-hydroxy-9-methyl-decatrienoic acid (EDA). **D**. Maculansin A as an example of a mannitol-derived HST. **E**. Structure of the HS-toxin, which contains a sesquiterpene aglycone and four galactofuranose fragments.

The AK-, AF- and ACT-toxins, characteristic for three of the pathotypes of *A.alternata*, form a specific family of 9,10-epoxy-8-hydroxy-9-methyl-decatrienoic acid (EDA) derivatives. All of them are EDA esters (Tsuge et al., 2013) (Figure 1). The more prevalent and toxic form of AK-toxin (AK-toxin I) has the EDA moiety esterified at the 8–hydroxy position with the phenylalanine derivative N-acetyl-β-methyl-phenylalanine (Nakashima et al., 1985). The strawberry *A.alternata* pathotype produces 3 AF-toxins, in which EDA is esterified in a similar manner, but with 2-hydroxyvaleric acid. Additionally, AF-toxin I has a 2,3-dihydroxy isovaleric acid attached at the 2'-position, while in the case of AF-toxin III it is a 2-hydroxy isovaleric acid (Nakatsuka et al., 1986; Tsuge et al., 2013). The third members of the family, the ACT-toxins, consist of 3 chemical parts, the EDA, valine and a polyketide residue (Akimitsu et al., 2016).

Two other HSTs, the maculansins and brassicicolin A, are a type of pretty rare fungal metabolites that have their origin from the polyol D-mannitol. The maculansins contain also an unusual 2-isocyano-3-methyl-2-butenoyl chromophore (Pedras and Yu, 2008). The final structure of maculansin A has been identified as 1,6-diacetyl-2,5-bis(2-isocyano-3-methyl-2-

butenoyl)-D-mannitol (Figure 1), while the closely related maculansin B is proposed to be 1,6-diacetyl-2-(2-isocyano-3-methyl-2-butenoyl)-5-(2-iso-cyano-3-methylbutanoyl)-D-mannitol. The main difference in their chemical composition is that one of the 2-isocyano-3-methyl-2-butenoyl fragments in maculansin A is replaced with a 2-isocyano-3-methylbutanoyl moiety in maculansin B, which as a result has one less double bond. Unlike the maculansins, brassicicolin A is a completely acylated derivative of D-mannitol that possesses two acetyl units at positions 3 and 4, two 2-hydroxy-3-methylbutanoyl residues at positions 1 and 6 and two epimerized 2-isocyano-3-methylbutanoyl moieties attached to carbon atoms 2 and 5 of the polyol (Gloer et al., 1988).

The last known HST, which falls into the category of fungal secondary metabolites, is the HS-toxin. This molecule can be considered of mixed origin and consists of a sesquiterpene aglycone core with the formula $C_{15}H_{24}O_2$, to which are connected four galactofuranose fragments arranged in pairs. The linkage is by β-1,5-bonds to carbon atoms 2 and 13 of the aglycone, respectively (Macko et al., 1983) (Figure 1). Three isomeric forms of the toxin are possible and they are designated HS-toxin $A_{2,2}$, $B_{2,2}$ or $C_{2,2}$ (Wolpert et al., 2002). The secreted by *B. sacchari* analogous toxoid compounds of lower molecular weight, that were mentioned above, lack one or more of the galactose moieties (Livingston and Scheffer, 1984).

3.2. Proteinaceous HSTs – Some phytopathogenic fungi provoke diseases by secreting small toxic ribosomally synthesized peptides. Typical examples are the Ptr toxins of *Pyrenophora tritici-repentis*. Ptr ToxA was the first identified of these HSTs (Ballance et al., 1989). It is encoded by a single gene in the genome of the fungus and after cleavage of the N-terminal 60 amino acids, which constitute a signal peptide and a pro-domain, is released the mature 13.2 kDa product (Tuori et al., 2000). Structurally, this one-domain protein is characterized with the

presence of a β-sandwich fold and a solvent-exposed loop containing the so called RGD-motif (arginylglycylaspartic acid) (Sarma et al., 2005), which is important for the phytotoxicity effect. In contrast to Ptr ToxA, Ptr ToxB is encoded by two to ten genes in the different races of the pathogen and the virulence of the corresponding race correlates with the Ptr ToxB gene copy number (Amaike et al., 2008; Martinez et al., 2004). The protein is small, with 64 amino acid residues, rich in cysteine, and a molecular weight of only 6.5 kDa. One of its intriguing features is the resistance to heat, organic solvents and proteases, presumably due to a tightly folded conformation (Ciuffetti et al., 2010). Based on mass spectrometry analysis, four of the cysteines in Ptr ToxB are predicted to contribute to the compact structure by forming two disulfide bridges. These properties are often characteristic for fungal apoplastic effectors and thus the site of action of Ptr ToxB was suggested to be the apoplast.

All eight HSTs isolated from *S. nodorum* are proteins (Stergiopoulos et al., 2013). The mature peptide of the first of them, SnTox1, consists of 100 amino acids of which 16 residues are cysteines. Therefore, SnTox1 was also proposed to act in the apoplast (Liu et al., 2012). Another interesting structural peculiarity of SnTox1 is the presence of a chitin-binding domain (ChtBD) reminiscent of the plant chitin-binding proteins. It has been recently confirmed that SnTox1 is able to bind chitin and protects against wheat chitinases (EC 3.2.1.14) (Liu et al., 2016). The second described *S. nodorum* HST is SnToxA (Friesen et al., 2006). This protein shares a high sequence and structural similarity with Ptr ToxA and it has been suggested that the *ToxA* gene has been very recently horizontally transferred from *S. nodorum* to *P. tritici-repentis*. This interspecific virulence gene transfer probably occurred shortly before 1941 and lead to the emergence of the *P. tritici*-repentis-caused tan spot disease on wheat (Friesen et al., 2006). SnTox2 has been only partially purified and was determined to be a protease sensitive small

protein between 7-10 kDa in size (Friesen et al., 2007). On the other hand, SnTox3 is probably synthesized as a relatively large pre-pro protein with 230 amino acids, of which 20 constitute a signaling peptide and amino acids 21 to 72 are additionally cleaved to form the final mature product of around 18 kDa molecular weight. Six of the SnTox3 cysteines are predicted to build three disulfide bridges (Liu et al., 2009). The four other *S. nodorum* HSTs, which have been identified in the last decade: SnTox 4 (Abeysekara et al., 2009), SnTox5 (Friesen et al., 2012), SnTox6 (Gao et al., 2015) and SnTox7 (Shi et al., 2015) are 10-30 kDa polypeptides, but still not completely characterized structurally.

The second *C. heterostrophus* HST, ChToxA, is also a polypeptide, demonstrating the ability of this fungal pathogen to produce HSTs of both secondary metabolite (T-toxin, a polyketide) and proteinaceous nature (ChToxA) (Lu et al., 2015). Remarkably, ChToxA is another Ptr ToxA and SnToxA orthologue with moderate similarity in sequence (64%) and significant overlap of the three-dimensional structure with Ptr ToxA. However, ChToxA does not possess the RGD-motif of Ptr ToxA. Lastly, The *C. cassiicola* HST cassiicolin is a very small peptide of just 27 amino acid residues, which bears a resemblance to a family of inhibitors of trypsin-like proteases. Despite the tiny size, this molecule contains three disulfide bridges between its six cysteines, a common feature with other described HSTs. Moreover, cassiicolin is O-glycosylated on the Thr2 moiety (Barthe et al., 2007).

3.3. HSTs with unresolved chemical identity - Unlike the other Ptr toxins, Ptr ToxC is not ribosomally synthesized, but is rather a nonionic, polar molecule with a low weight (Effertz et al., 2002). However, other details on its composition are currently unknown. The other HSTs whose chemical structures have not been elucidated yet include the AT-toxin of *A. longipes* and the SV-toxins of *Stemphylium vesicarium*.

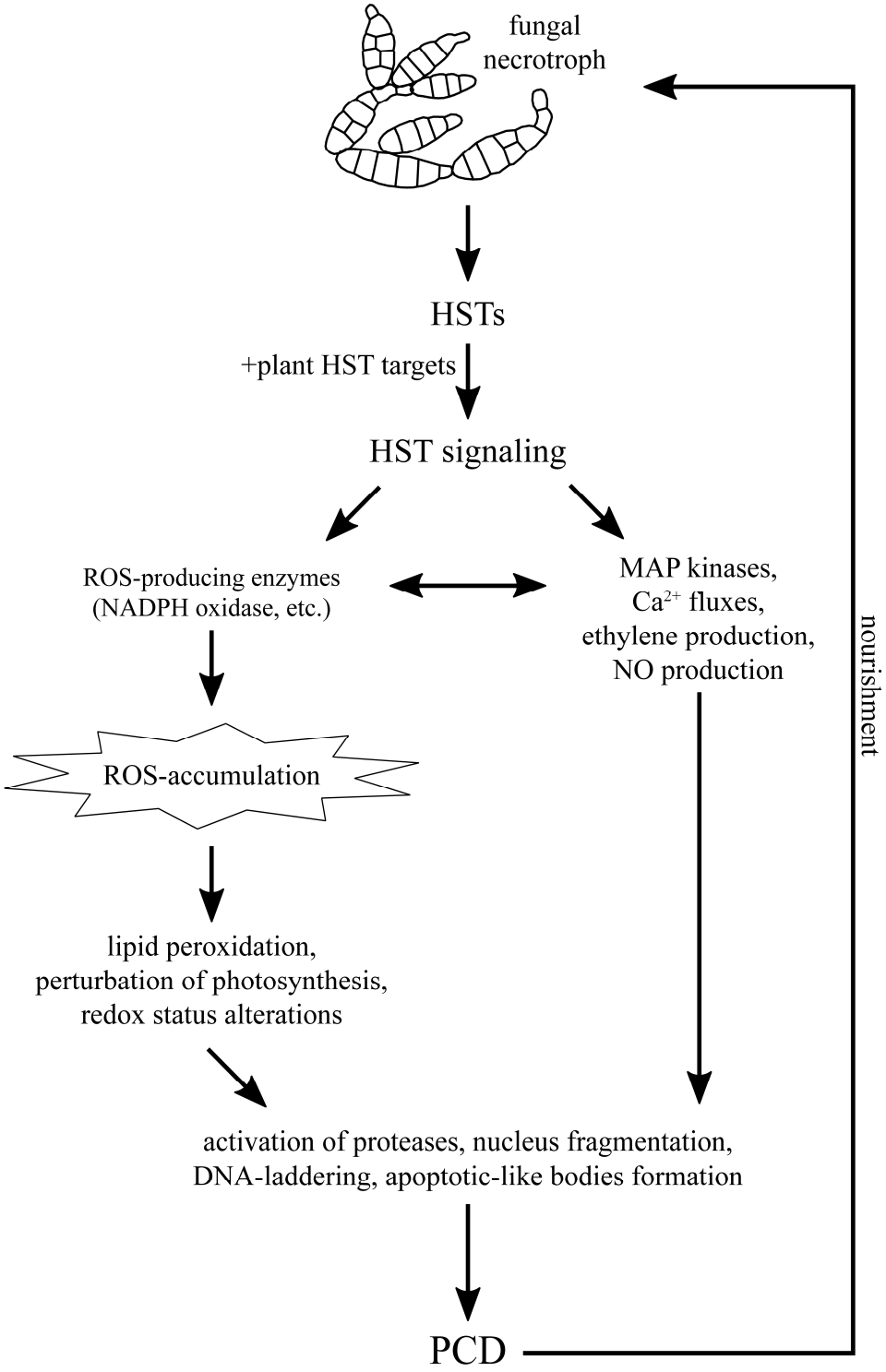

fungal
necrotroph
HSTs
+plant HST targets
HST signaling
ROS-producing enzymes
(NADPH oxidase, etc.)
MAP kinases,
Ca2+ fluxes,
ethylene production,
NO production
ROS-accumulation
lipid peroxidation,
perturbation of photosynthesis,
redox status alterations
activation of proteases, nucleus fragmentation,
DNA-laddering, apoptotic-like bodies formation
PCD
nourishment

Figure 2. Accumulation of ROS is a key phenomenon in the mode of action of many HSTs. Activation of ROS-producing enzymes like NADPH oxidases is either directly induced by recognition of HSTs or is a downstream event dependent on other signal transduction processes, involving Ca^{2+} influxes, MAP kinases, etc. The outcome is an oxidative burst, affecting membranes, the photosynthetic apparatus and the cellular redox status, which contributes to the onset of PCD. This cascade is normally utilized by plants to resist biotrophic pathogens, but necrotrophic species exploit it in order to derive nourishment.

4. Mechanisms of toxicity

Most of the HSTs, with the exception of the HC-toxin, exert their effects by inducing host-specific and rapid plant cell death by various mechanisms. As a result of their action *in vivo*, the infected tissue ultimately necrotizes and provides nourishment for the fungus. The same phenomenon is observed after manual application of purified toxins on susceptible plants. Accumulation of reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and superoxide ($O_2^{\cdot -}$), is a common event following the recognition of an HST by sensitive cells (Figure 2). This results in dramatic changes in the cellular biochemistry which are crucial for the onset of genetically regulated programmed cell death (PCD) (Petrov et al., 2015).

4.1. Alternaria EDA derivative HSTs – The primary site of action of the AK-, AF- and ACT-toxins in sensitive cells is the plasma membrane. Ultrastructural studies revealed that treatment with these compounds provokes plasmalemma invagination, caused by accumulation of membrane fragments between the membrane and the cell wall, enhanced traffic of exocytotic Golgi vesicles exuding polysaccharides (mostly β-1,3-glucans) in the apoplast and desmotubule elongation (Park and Ikeda, 2008). Thus, inclusion of membrane fragments, polysaccharides and

desmotubules were proposed to be ultrastructural markers for HST-toxin effects. These modifications of the plasma membrane are associated with permeability changes, for example a considerable increase of the K^+-efflux. Most extensively has been studied the mode of action of AK-toxin I. Since the alterations it triggers were observed mainly at sites near the plasmodesmata, it is hypothesized that a specific toxin receptor is situated on plasmodesmatal membranes (Park et al., 1976). In the membrane fragments of AK-treated cells was detected a significant boost in H_2O_2 generation (Shinogi et al., 2001) while in membrane-enriched fractions of host fruits was demonstrated an enhanced activity of the ROS generating enzyme NADPH oxidase (EC 1.6.3.1) (Shimizu et al., 2006). The production of these ROS leads to lipid peroxidation of the affected membrane areas, aggravating the damage (Figure 2). In turn, electrophysiological experiments provided evidence that another symptom of AK- as well as AF-application is depolarization of the plasmalemma (Namiki et al., 1986; Otani et al., 1989). It is important to point out that unlike some non-host toxins, these HSTs never cause the complete destruction of the membrane before cell death. They trigger only partial dysfunction that facilitates the colonization of infected tissues by the fungus. Moreover, the continuous supply of materials by Golgi vesicles for the regeneration of the plasmalemma demonstrates that an active membrane repair mechanism in the host is simultaneously operational with the HST action (Shimizu et al., 2006). Interestingly, it was shown that the ACT-toxin actually leads to significant upregulation of the expression of defense-related genes in resistant species like rough lemon. Therefore, it was proposed that the toxin acts as an effector of the defense systems with dual nature: in resistant plants it seems to be an elicitor, while in susceptible ones – a suppressor (Tsuge et al., 2013).

4.2. AM-toxin – Unlike the previously described Alternaria EDA-derived HSTs, the depsipeptide AM-toxin has two target sites: the plasma membrane and the chloroplasts. The effects on the plasma membrane are reminiscent to those induced by the EDA HSTs. However, while the latter are classified as a severely damaging subtype, the AM-toxin falls in the category of a slightly-damaging subtype (Park and Ikeda, 2008). The difference is that in the second case the polysaccharide exudates and membrane fragments are in a lower content. The reduced deposition of polysaccharide inclusion materials triggered by the AM-toxin suggests a decreased activity of the Golgi complex, despite the similar mechanism of modifying the membrane. The AM-toxin provokes also ultrastructural changes and disorganization of the chloroplast. This is caused by disruption of the grana lamellae, which leads to emergence of membrane fragments and vesicles in the stroma. The structural damage to the chloroplasts is coupled to disturbance of their physiological functions since the photosynthetic CO_2 assimilation activity and the chlorophyll concentrations drop (Kohmoto and Otani, 1991).

4.3. ACR-toxin - The primary site of action for this toxin are the mitochondria. After treatment of the susceptible host are observed swelled mitochondria characterized with partial destruction of the cristae, disappearance of dense granules and bulge formation on the mitochondrial membranes (Kohmoto et al., 1984). In addition, biochemical studies showed that ACR-toxin leads to uncoupling of the oxidative phosphorylation from the transfer of electrons in the mitochondrial electron transport chains, loss of membrane potential, and most remarkably – the leakage of NAD^+ from the Krebs cycle (Akimitsu et al., 1989). These processes are proposed to cause the emergence of pores in the mitochondrial membranes (Akimitsu et al., 2004). Not surprisingly, the gene in rough lemon (*ACRS*) which confers susceptibility to the ACR-toxin was found to be in the mitochondrial genome. Transformation of *E.coli* with *ACRS* renders them

sensitive to ACR-toxin as well. However, the effects of the ACR-toxin in plants are not determined by the presence or absence of *ACRS* transcripts, but rather by a specific posttranscriptional modification (Ohtani et al., 2002).

4.4. AAL-toxin –Since AAL-toxin is an analogue of sphinganine, it is able to inhibit the enzyme sphingosine-N-acyltranspherase (ceramide synthase, EC 2.3.1.24) in the endoplasmatic reticulum and thus perturb the metabolism of ceramide-containing lipids (Gilchrist et al., 1994). These lipids are bioactive compounds which participate in numerous signaling processes as second messengers and thus regulate cell fate both in animals and plants (Hannun and Luberto, 2000; Liang et al., 2003). When challenged with AAL-toxin, susceptible tomato tissues accumulate sphinganine and phytosphinganine (Abbas et al., 1994) while at the same time complex sphingolipids are depleted (Gechev et al., 2008). As a result the affected cells undergo PCD, characterized by fragmentation of the nucleus, DNA-laddering and formation of apoptotic-like bodies (Wang et al., 1996a). This process is similar to a hypersensitive response (HR) and involves also calcium, ethylene and MAP kinases (EC 2.7.11.24) (Mase et al., 2012; Moore et al., 1999; Wang et al., 1996a). Other signaling molecules taking part in the PCD cascade of reactions are NO and ROS (especially H_2O_2) (Gechev et al., 2004) (Figure 2). Interestingly, AAL-toxin and the structurally related fumonisins provoke an increase in the concentration of sphinganine and phytosphinganine in resistant tomato genotypes as well, but in this case the effect is much less pronounced, suggesting a greater *in vivo* inhibition of ceramide synthase in the sensitive plants (Abbas et al., 1994). An important discovery, throwing light on the mechanism of AAL-toxin action, was that supplementing susceptible tomato leaves with ceramide prevents the AAL-toxin triggered PCD, which demonstrates that the ceramide balance is crucial for the onset of PCD (Brandwagt et al., 2000). On the other hand, resistance to AAL-

toxin in tomato is conferred by the gene *Asc1* (Alternaria stem canker resistance gene 1), which is a homologue of the yeast *Lag1* (longevity assurance gene 1), encoding a ceramide synthase (Brandwagt et al., 2000). Lag1 homologues determine the response to AAL-toxin and other SAMs also in other plant species, for example the gene *loh2* (*lag1* homologue 2) in *Arabidopsis thaliana* (Gechev et al., 2004).

4.5. AT-toxin – The HST of *Alternaria longipes* leads to ultrastructural modifications of the mitochondria of affected cells similar to those described for the ACR-toxin (Kodama et al., 1990; Park and Ikeda, 2008). At the molecular level, the cellular death caused by AT-toxin application is characterized with a massive H_2O_2 accumulation as well as an increase in the quantities of the stress markers malondyaldehyde and free proline, and the total protease activity (Yakimova et al., 2009). Moreover, the manifestation of lesions and stress markers production can be suppressed by pre-infiltration of the susceptible tobacco tissues with caspase-specific peptide inhibitors. These results suggest that caspase-like proteases and ROS-homeostasis play an important role in the PCD process induced by the AT-toxin (Yakimova et al., 2009).

4.6. HC-toxin – The *Cochliobolus carbonum* HC-toxin is unique among the other HSTs with that it is actually not able to kill plants cells by itself. Many of its effects are the opposite of those provoked by the rest of the HSTs. For example, unlike the AK-, AM- and other Alternaria HSTs, which depolarize the plasma membrane, HC-toxin leads to hyperpolarization (Gardner, 1974). Moreover, instead of inducing electrolyte leakage, HC-toxin stimulates the uptake of organic acids by susceptible maize roots (Yoder and Scheffer, 1973a) and the assimilation of nitrate (Yoder and Scheffer, 1973b). HC-toxin is not involved in the elicitation of host defense systems since it does not induce ROS-accumulation or production of the protective compounds phytoalexins (Wolpert et al., 2002). These observations demonstrated that the HC-toxin is not

cytotoxic, but rather participates in pathogenicity by preventing the adequate and timely host defense responses (Cantone and Dunkle, 1991; Markham and Hille, 2001). This is achieved by inhibition of the enzymes histone deacetylases (HDACs, EC 3.5.1.98) which are involved in the modification of histones, especially H3 and H4 (Brosch et al., 1995). HDACs catalyze the removal of acetyl groups from the N-terminal lysine residues of these histones, which correlates with alteration of the chromatin architecture. As a result is adopted a more closed structure, which is inaccessible to the transcription apparatus, thus normally leading to transcriptional repression (Liu et al., 2014). Therefore, it is presumed that HC-toxin compromises the host's ability to protect itself against the pathogen by modulating the expression of crucial regulatory genes of the defense pathways (Stergiopoulos et al., 2013).

4.7. T-toxin and PM-toxin – The target of the action of the related T- and PM-toxins in *cms-T* maize is the 13 kDa oligomeric protein URF13, which is the product of the mitochondrial gene *T-urf13* (Dewey et al., 1986). Expression of *T-urf13* in *E. coli* and yeast renders these organisms sensitive to the cytotoxic effects of the toxins (Dewey et al., 1988; Huang et al., 1990), confirming the key role of URF13 for susceptibility. T- and PM-toxins bind to URF13, which is found on the inner mitochondrial membrane, and trigger conformational changes in the protein. As a result are formed pores on the inner membrane resulting in swelling of the organelle, uncoupling of the oxidative phosphorylation, leakage of small molecules like NAD^+ and ions like Ca^{2+}, disruption of the electrochemical gradient, and ultimately – cell death (Levings et al., 1995). The functions of URF13 in the host are currently unknown.

4.8. Victorin - The PCD that victorin induces is apoptosis-like and includes features like DNA-laddering, heterochromatin condensation and cell shrinkage. The process is mediated by Ca^{2+} ions, an oxidative burst that follows the production of ROS, as well as activation of serine- and

cysteine proteases, and nucleases (Navarre and Wolpert, 1999; Tada et al., 2001). In addition, it has been demonstrated that victorin is able to provoke a decrease of the mitochondrial membrane potential which precedes the cell death markers (Curtis and Wolpert, 2004). Initially, it was presumed that the toxin triggers PCD by its binding to the P-subunit (EC 1.4.4.2) of the glycine decarboxylase complex (GDC) in oats mitochondria, which plays a key role in photorespiration (Navarre and Wolpert, 1995). However, later it was shown that victorin-induced PCD symptoms occur before the toxin even enters the target cells, supporting the hypothesis that the recognition takes place outside the plant cells, not by GDC in mitochondria (Tada et al., 2005). Moreover, the dominant *Vb* gene, which confers susceptibility to victorin, is indistinguishable from the crown rust resistance gene *Pc2* that determines resistance against the biotrophic fungus *Puccinia coronate*, despite the numerous attempts to separate the two loci (Gilbert and Wolpert, 2013). This suggests that the mechanism of victorin action may actually reside in manipulation of the host's defense responses. In agreement with this idea is the fact that some of the processes observed in victorin-affected cells, like callose deposition, extracellular alkalization, K^+ efflux and phytoalexin synthesis, are typical of plant hypersensitive response (HR) reactions against biotrophic pathogens. The power of *Arabidopsis thaliana* genetics threw additional light on this topic when the gene *Locus Orchestrating Victorin Effects1* (*LOV1*), rendering Arabidopsis sensitive to the toxin, was discovered (Lorang et al., 2007). LOV1 turned out to be a nucleotide-binding-site leucine-rich repeat (NB-LRR) protein which is a member of the RPP8 family of disease resistance proteins. Another study aiming to identify how LOV1 mediates its effects revealed the direct target of victorin in Arabidopsis, which is the thioredoxin TRX-h5, another participant in plant defense systems. Victorin binds to TRX-h5 *in vivo*, which activates LOV1 to induce an HR-like response, ultimately resulting in cell death (Lorang et al., 2012). Therefore, it

seems that at least in Arabidopsis victorin exploits the innate protective mechanisms of the host cells to trigger PCD. Such sacrifice of the affected tissues in order to halt the spread of an infection is a legit instrument against biotrofic fungi, but in a case when the same process is initiated by a necrotrophic species like *C.victoriae*, only the pathogen benefits. The strategy of *C. victoriae* against the natural host oats could be the same, but it is still not definitively confirmed.

4.9. ToxA HSTs – The main site of action of Ptr ToxA are the chloroplasts since the toxin induces light-dependent loss of chlorophyll, disorganization of the thylakoids and damage to the photosystems (Manning et al., 2007). The RGD-motif containing loop of Ptr ToxA is crucial for its functions and is required for the internalization of the toxin in the target mesophyll cells and for the development of typical symptoms (Manning et al., 2008; Meinhardt et al., 2002). This suggests that the RGD-motif binds to a putative membrane receptor, which facilitates the transition of the toxin in the plant cells. Once inside the cells, Ptr ToxA migrates in the chloroplasts, where it interacts with ToxA binding protein 1 (ToxABP1) (Manning et al., 2007). As a result are triggered changes in the photosystems that in the presence of light lead to ROS-accumulation and disruption of the photosynthetic apparatus (Manning et al., 2009) (Figure 2). The observed overproduction of ROS is necessary for the Ptr ToxA-mediated PCD since treatment with antioxidants like N-acetyl cysteine inhibits the cell death. Interestingly, silencing of the *ToxABP1* gene does not have the same PCD-limiting effect as ROS scavenging, which shows that additional proteins also participate in the mechanism of Ptr ToxA action (Ciuffetti et al., 2010). The genetic factor in wheat that confers sensitivity to Ptr ToxA is the dominant allele of *Tsn1*, encoding a member of the NB-LRR family of disease resistance proteins (Faris et al., 2010). *Tsn1* transcription is regulated by the circadian clock and light, which implies that Tsn1 is related to photosynthesis as well. Remarkably, Tsn1 is crucial for the toxicity not only of Ptr

ToxA, but also for the orthologous SnToxA (Liu et al., 2006). Therefore, the wheat Tsn1 seems to serve as a major determinant of susceptibility to both *P. tritici-repentis* tan spot and *S. nodorum* glume blotch. Moreover, the appurtenance of *Tsn1* to a family of disease resistance genes suggests that the necrotrophic *P. tritici-repentis* and *S. nodorum* subvert the host's own defense strategy to derive benefits in a way similar to the approach of *C. victoriae* described above. This hypothesis is further supported by the recent report that the dimeric PR-1 type pathogenesis related protein PR-1-5 interacts with ToxA and potentially participates in the ToxA-mediated cell death (Lu et al., 2014). The third representative of the ToxA HSTs, ChToxA, is not extensively studied yet, but the leaf-necrosis it induces in maize is also light-dependent. It must be noted that due to the lack of an RGD-motif, ChToxA needs to exploit a different mechanism for internalization in the cells than Ptr ToxA and SnToxA (Lu et al., 2015).

4.10. Ptr ToxB – The mode of action of Ptr ToxB is less studied than the one of Ptr ToxA. While Ptr ToxA is generally associated with necrosis, Ptr ToxB causes chlorosis. Remarkably, the Ptr ToxB-triggered chlorosis is also light-dependent and involves disturbance of the photosynthetic processes that results in the accumulation of ROS and subsequent chlorophyll photo-oxidation (Kim et al., 2010). Additionally, transcriptome adjustments after treatment with Ptr ToxA and Ptr ToxB seem fairly similar, which shows that despite their structural differences the two HSTs modulate analogous host defense responses (Ciuffetti et al., 2010). However, although Ptr ToxB provokes changes inside the cell, its three-dimensional structure is characteristic of fungal apoplastic effectors. In addition, Ptr ToxB can be reisolated from the apoplastic fluid after infiltration of host tissues and fluorescently labelled toxin appears in the apoplast. Thus, several lines of evidence demonstrate that the Ptr ToxB does not cross the plasma membrane and orchestrates cellular events from the outside (Figueroa et al., 2015).

4.11. SnTox HSTs – SnTox1 induces necrosis in wheat genotypes carrying the dominant allele of *Snn1*. The SnTox1-Snn1 interaction leads to DNA laddering, oxidative burst due to ROS overproduction and increase in pathogenesis-related gene expression. As in the case of the SnToxA-Tsn1 couple, the SnTox1 effects are also light-dependent (Liu et al., 2012). The cloning of *Snn1* showed that it is a member of the cell wall-associated kinase receptors, which are implicated in resistance to biotrophic pathogens (Shi et al., 2016). The mechanisms of action of three of the other SnTox proteins: SnTox2, SnTox4 and SnTox5 are not studied in detail yet, but like SnToxA and SnTox1 they need light in order to exert their toxicity. Conversely, the SnTox3 functions in a light-independent manner, pointing at a different strategy to induce cellular damage. Recently, yeast-two hybrid assays and *in planta* co-immunoprecipitation were used to demonstrate that SnTox3 is able to bind to the wheat pathogenicity related protein PR-1-1 and a PR-1-1 derived defense signaling peptide, called CAPE1, enhances the SnTox3-mediated infection (Breen et al., 2016). Since SnToxA interacts with the closely related PR-1-5, it appears that *P. nodorum* has evolved complementary approaches to exploit similar host protective proteins. Finally, the newly-identified SnTox7 is able to provoke some necrosis development even in the absence of light, thus it resembles more SnTox3 than the other SnTox HST proteins (Shi et al., 2015).

4.12. Peritoxins (PC-toxins) – Exposition of sensitive sorghum plants to PC-toxins induces typical disease symptoms manifested at the cytological level with a variety of alterations like inhibition of mitosis, electrolyte leakage, highly condensed heterochromatin, enlargement of vacuoles and autolytic processes (Dunkle and Macko, 1995). Notably, the effects of the toxins are prevented by pretreatment with inhibitors of RNA and protein synthesis, which suggests that high protein turn-over is necessary for the toxins' mode of action. Not surprisingly, the sorghum

Pc-B gene, controlling the susceptibility to peritoxins, has been shown to encode another NB-LRR disease resistance protein (Nagy and Bennetzen, 2008).

4.13. SV-toxins – The HSTs produced by *S. vesicarium* provoke changes in the plasmalemma similar to those of the AK-, AF- and ACT-toxins. Most probably the SV-toxins have their target sites located on the plasma membrane of the host cells near plasmodesmata since membrane invaginations following SV-toxin treatment are observed mainly at both plasmodesmatal ends (Singh et al., 2000).

Details on the mechanism of toxicity of other HSTs like phomalide, depsilairdin, brassicicolin A, cassiicolin and maculansins still remain to be revealed.

5. Effects on animals and humans

By definition the HSTs are compounds conferring noteworthy specificity to the pathogens that synthesize them and normally do not trigger biological reactions even in resistant genotypes of the same species. For decades the pathosystems HST producing fungi-susceptible crops have been studied as a model for elucidating the intricate mechanisms of plant-microbe interactions. Given the economic importance of HST-induced diseases, a lot of efforts have been devoted to the characterization of their chemical structures, the genetic and biochemical factors leading to their synthesis and their mode of action in sensitive plants. However, little is known about the effects, if any, of most of the HSTs on animal organisms. The HST secreting necrotrophs attack widespread crop species utilized for food and feed and therefore both humans and livestock can be exposed to HSTs. For example maize and maize-based feeds may be contaminated with the AAL-toxin. The investigation of the effect of agronomic practices and weather conditions on the occurrence and concentrations of the two forms of the AAL-toxin in maize silage revealed that

temperature was negatively while moisture levels were positively correlated with AAL-toxin Ta presence. In addition, ensiling and other agronomic practices did not seem to affect the quantities of the toxin (Mansfield at al., 2007).

Up to date there are no reports in the literature of HSTs taking part in fungal pathogenesis in animals (Sexton and Howlett, 2006). In this regard, the fact that a lot of the identified host HST targets are typical plant disease resistance proteins and the specificity of protein-protein interactions render unlikely that HSTs of proteinaceous nature may affect animal cells. On the other hand, many other HSTs are secondary metabolites, molecules possessing remarkable functional plasticity which may provoke various responses in distant species. Indeed, there are two well-described cases of fungal secondary metabolite HSTs influencing animal cells: the AAL-toxin and the HC-toxin. A brief summary of the mechanism of action of both toxins is presented in Figure 3.

5.1. AAL-toxin – The sphinganine analogue AAL-toxin appears to trigger PCD by a universal mechanism efficient in both the plant and animal kingdoms, involving disturbance of the sphingolipid metabolism. This is achieved by inhibiting similar biochemical targets - the enzymes ceramide synthases (Merrill et al., 1993; Riley et al., 1996). Among the first animal *in vitro* experimental systems shown to react to AAL-toxin were African green monkey kidney cells (CV-1). The treatment with AAL-toxin induced typical apoptosis hallmarks like DNA laddering, chromatin condensation, cell shrinkage and emergence of apoptotic bodies (Wang et al., 1996b). Interestingly, while the structurally and functionally related fumonisin B_1 arrested the CV-1 cells in the G1 cell cycle phase, AAL-toxin was not able to do this, demonstrating that both SAM compounds cause apoptosis, but only the fumonisins affect also the cell cycle. Another example of the AAL-toxin exerting cytotoxic effects on mammalian cells are rat

primary hepatocytes (van der Westhuizen et al., 1998). The bioactivity of the tested SAMs in hepatocytes did not seem to be dependent solely on their ability to inhibit ceramide synthase since not in all cases there was a direct correlation between the cytotoxicity and the elevation of the sphinganine levels. SAMs were shown to be toxic also to dog kidney MDCK cells and rat hepatoma lines, but not to mouse fibroblast and pig kidney cells (Mirocha et al., 1992). In all these experimental systems the AAL-toxin had sufficient, but weaker toxicity than its fumonisin counterparts. Nevertheless, its apoptosis-driving properties combined with the widespread presence in food and animal feed justifies additional research on the potential risk for humans and livestock. Moreover, both AAL-toxin and fumonisins are most often accompanied by other mycotoxins, which increases the chances for additive or synergistic effects (Mansfield et al., 2007). Fumonisins have been much more extensively studied than the AAL-toxin and they were reported to be hepatotoxic, neurotoxic, immunotoxic and cancerogenic, especially for the liver and esophagus in humans (Mary et al., 2017). In addition, they can induce ROS accumulation, further contributing to toxicity, by a not completely clarified mechanism (Theumer et al., 2010). All these detrimental features, combined with their similar structure and mode of action, indicate that the fumonisins' analougue AAL-toxin should not be underestimated as a putative threat. Fortunately, new screening and decontamination methods targeting all SAMs in foodstuffs have been developed. For instance, a high resolution LC-MS approach, based on product ion filtering with rapid polarity switching, was reported recently to successfully identify and discriminate all known fumonisins, AAL-toxins and related compounds (Renaud et al., 2015). Another group demonstrated that cold atmospheric pressure plasma (CAPP) using ambient air as working gas is able to efficiently and completely degrade a variety of pure mycotoxins, including SAMs, when they are confined to or enriched on surfaces like cereal grains (Ten Bosch et al., 2017).

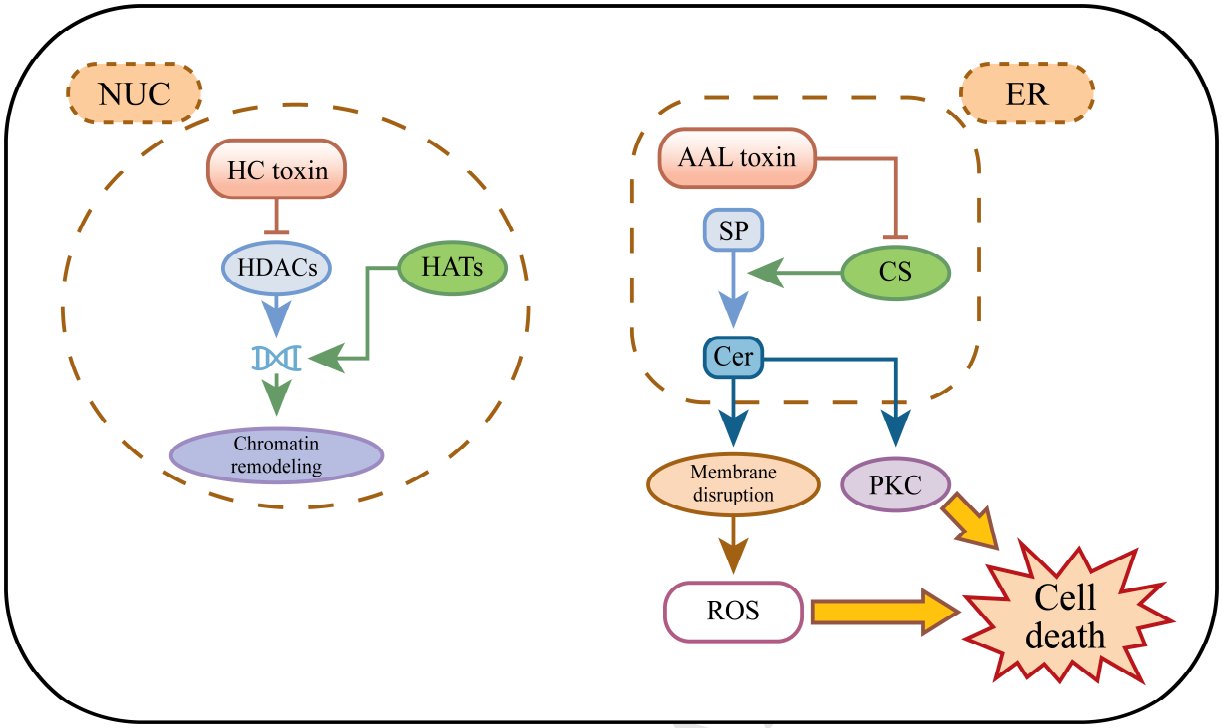


Figure 3. Known mechanisms of action of HC- and AAL-toxins. The HC-toxin inhibits histone deacetylases (HDACs) in the nucleus (NUC), indirectly leading to hyperacetylation of histones via histone acyltransferases (HATs). The altered profile of histone acetylation causes chromatin remodeling with various effects. On the other hand, the AAL-toxin acts in the endoplasmic reticulum (ER) where it inhibits ceramide synthase (CS), which is involved in the synthesis of ceramide (Cer) from sphingosine (or sphinganine) (SP) and acyl-CoA. In turn, the perturbed sphingolipid metabolism results in disruption of the integrity of cellular membranes. In plants, increased ROS production and oxidative stress further contribute to induce programmed cell death (PCD). Both ceramide and sphingosine are compounds that can influence protein kinase C (PKC) activity in animals, which also leads to cell death (apoptosis).

5.2. HC-toxin – The ability of HC-toxin to inhibit HDACs is not restricted only to the host of *C. carbonum*. In the first study on the topic, the HC-toxin was shown to affect the histone deacetylases of maize embryos and the same enzymes of taxonomically distant species like the myxomycete *Physarum polycephalum* and chicken (Brosch et al., 1995). With time, many authors confirmed the universal HDAC-modulating properties of HC-toxin in various other organisms – from parasitic protozoa species (Darkin-Rattray et al., 1996) to humans (Witt et al., 2003; Yang et al., 2001). One of the most important characteristics of HC-toxin is that it is cytostatic in mammalian cells, not cytotoxic (Markham and Hille, 2001). Together with other compounds with a similar mode of action (HDAC inhibitors or HDACIs), it is regarded as a promising chemotherapeutic agent. The antitumor activity of HC-toxin and HDACIs has been demonstrated on numerous occasions. A good example is the suppression of the growth and stimulation of the differentiation of neuroblastoma cells (Deubzer et al., 2008). Among all the HDACIs tested in this experimental system, HC-toxin was the most efficient and even in nanomolar concentrations caused reduction of tumor marker proteins. When applied to estrogen receptor positive human breast cancer T47D cells, HC-toxin manifested potent antiproliferative, cell cycle-arresting and apoptosis-inducing effects in a dose-dependent manner (Joung et al., 2004). HDACs are highly expressed in the rare but dangerous intrahepatic cholangiocarcinoma (ICC) and are markers of poor prognosis for the patients. In a screening for appropriate HDACIs, the HC-toxin was found to exert the strongest antitumor properties against ICC cells *in vitro* and proposed as a potential chemotherapeutic for this condition (Zhou et al., 2016). HDACIs may be a suitable therapeutics choice for another much more common malignant liver disease – hepatocellular carcinoma (HCC). In this case, however, the outcome is dependent on the downregulation of the *inhibitor of DNA binding 2* (ID2) (Tsunedomi et al., 2013). Treatment of

HD-MB03 cells, which are a model for Group 3 medulloblastomas, with HC-toxin and other HDACIs, yielded promising results as well. The HD-MB03 cells were not only susceptible to HDACIs, but were additionally sensitized to radiation with a significantly increased cell death upon concomitant use (Milde et al., 2012). All these examples demonstrate the significant interest towards the HC-toxin and HDACIs as a novel class of antitumor agents. But this is not their only possible application. They have been studied extensively also for their antiparasitic properties as chemotherapeutic compounds for diseases like malaria, toxoplasmosis, trypanosomiasis, coccidiosis and others (Andrews et al., 2012; Darkin-Rattray et al., 1996). For instance, the T-toxin was shown to possess comparable activities *in vitro* and *in vivo* to the potent broad spectrum antiprotozoal effector apicidin (Darkin-Rattray et al., 1996). Moreover, according to an intriguing recent report, the HC-toxin may serve even as an exercise mimetic. In mouse myoblasts it was able to stimulate two exercise-triggered pathways (Tan et al., 2015). Therefore, by unlocking the metabolic benefits of exercise the HC-toxin may promote hypertrophy and suppress atrophy.

The AAL- and HC-toxins provide a good example that fungal-derived metabolites whose normal purpose is destined to interactions with plants may as well provoke a broad spectrum of responses in other organisms. Thus, these compounds may either pose a health risk due to potential toxicity, like the AAL-toxin, or be of prospective use in different fields, not only pharmacology and medicine, like in the case of the HC-toxin, but also agriculture, cosmetics, etc. Additional research on the rest of the HSTs described in this review will give clues whether they can find similar applications outside the domain of plant pathology.

6. Concluding remarks

The present work summarizes some of the key aspects of our current knowledge on the variety, taxonomical distribution, structural characteristics and mode of action of the fungal host-selective toxins in different organisms. Other important issues like the genetic and biochemical determinants of HST production were not included in the scope of this review, but have been described in detail elsewhere (Meena et al., 2017; Stergiopoulos et al., 2013; Tsuge et al., 2013). Over the years, the HST-releasing fungi and their susceptible hosts have proven to be an indispensable experimental system for elucidating the fundamental problems of plant-pathogen interactions. From the practical point of view, understanding the mechanisms underlying HST recognition and effects will provide instruments to obtain new crop cultivars which are resistant to biotic stress. Moreover, one of the HST representatives, the HC-toxin, has been shown to evoke various responses in non-plant cells and at present incites the interest with a variety of possible applications in pharmacology and medicine. Whether in this regard the HC-toxin is an exception among the HSTs will be clear when the modes of action and biological activities of other less studied HST compounds like AT-toxin, brassicicolin A, phomalide, cassiicolin, etc., are elucidated. The rapid development and wide accessibility of –omics and systems biology approaches are expected to significantly contribute to speed up this process by providing information on the genomic structure of HST-producing fungi and transcriptional and proteomic footprints after HST treatment of host and non-host organisms alike.

Acknowledgements

The authors would like to thank Mihail Angelov for designing two of the figures. VP and TG acknowledge the financial support of the European Union's Horizon 2020 research and innovation programme, project PlantaSYST (SGA-CSA No. 739582 under FPA No. 664620), and FP7 project PlantAgeing (Grant No. 612630).

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Table 1. A summary of the species variety of fungal pathogens synthetizing HSTs, their HST products and provoked diseases in specific hosts.

Table 2. A summary of the chemical identities of all of the HSTs described in the text and studies in which these HSTs were isolated and structurally characterized.

Figure 1. Chemical composition of different types of secondary metabolite HSTs. **A.** T-toxin and AAL-toxin as examples of polyketide HSTs. The AAL-toxin is an analogue of the complex lipid constituent sphinganine. **B.** The cyclic peptide HC-toxin and the depsipeptide depsilairdin. In the formula of the latter is marked the unusual amino acid 3,4-dihydroxy-3-methylproline (Dhmp). **C.** The AK-toxin, which is a derivative of 9,10-epoxy-8-hydroxy-9-methyl-decatrienoic acid (EDA). **D.** Maculansin A as an example of a mannitol-derived HST. **E.** Structure of the HS-toxin, which contains a sesquiterpene aglycone and four galactofuranose fragments.

Figure 2. Accumulation of ROS is a key phenomenon in the mode of action of many HSTs. Activation of ROS-producing enzymes like NADPH-oxidases is either directly induced by recognition of an HSTs or is a downstream event dependent on other signal transduction processes involving Ca^{2+} influxes, MAP-kinases, etc. The outcome is an oxidative burst, affecting membranes, the photosynthetic apparatus and the cellular redox status, which contributes to the onset of PCD. This cascade is normally utilized by plants to resist biotrophic pathogens, but necrotrophic species exploit it in order to derive nourishment.

Figure 3. Known mechanisms of action of HC- and AAL-toxins. The HC-toxin inhibits histone deacetylases (HDACs) in the nucleus (NUC), indirectly leading to hyperacetylation of histones via histone acyltransferases (HATs). The altered profile of histone acetylation causes chromatin remodeling with various effects. On the other hand, the AAL-toxin acts in the endoplasmic reticulum (ER) where it inhibits ceramide synthase (CS), which is involved in the synthesis of ceramide (Cer) from sphingosine (or sphinganine) (SP) and acyl-CoA. In turn, the perturbed sphingolipid metabolism results in disruption of the integrity of cellular membranes. In plants, increased ROS production and oxidative stress further contribute to induce programmed cell death (PCD). Both ceramide and sphingosine are compounds that can influence protein kinase C (PKC) activity in animals, which also leads to cell death (apoptosis).

Highlights

- Some fungal necrotrophs induce plant diseases by secreting specific host-selective toxins (HSTs).
- HSTs are either secondary metabolites or proteinaceous compounds.
- HSTs provoke cell death in sensitive plant tissues by different mechanisms, often involving reactive oxygen species (ROS).
- There are examples of HSTs having cytotoxic or cytostatic biological activities in animal and human cells.