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ORIGINAL PAPER

# The secondary metabolites profile of *Stemphylium lycopersici*, the causal agent of tomato grey leaf spot, is complex and includes host and non-host specific toxins

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## Abstract

The aim of this work was to characterize the secondary metabolite (SMs) profile of *Stemphylium lycopersici*, a necrotrophic fungus that causes the disease known as grey leaf spot. We detected twenty-four SMs in cultures of *S. lycopercisi* within the extracts of 19 isolates. Each of them presented a characteristic unique profile. While highly virulent isolates synthesized a high number of metabolites (15), the low virulent ones synthesized a low number of SMs (2). However, a PCA analysis could not establish the relationship between SMs and virulence. Infectopyrone was the most frequent metabolite produced by the isolates (89%).

**Keywords** Grey leaf spot · *Stemphylium lycopersici* · Fungal tomato disease · Secondary metabolism

## Introduction

Fungi synthesize an ample array of low-molecular-mass compounds e.g., alkaloids, non-ribosomal peptides, polyketides, terpenes and their hybrids. Based on the moment of the life cycle of the organism when these compounds are produced as well as their role, they are considered secondary metabolites (SMs, Keller et al. 2005; Andersson 2012; Collemare et al. 2014). Their roles are mainly related with organisms' survival under specific environmental conditions, and in the case of plant pathogens many of these SMs are virulence factors with toxic activity on plant cells and, others are considered potential mycotoxins (Boruta 2018). Also, SMs might act as metal chelating agents, sexual hormones that trigger fungal development, or as chemical signals between the fungus and the host in mutualistic or pathogenic interactions (Demain and Fang 2000). So evidently SMs play a key role either as pathogenicity or virulence factors (Wolpert et al. 2002; Berestetskiy 2008).

Phytopathogenic fungi synthesize many different compounds, including SMs that protect them against molecules released by the plant defense mechanisms, particularly at the early stage of infection (Jashni et al. 2015). Specifically, necrotrophic fungi secrete ribosomal and non-ribosomal peptides along with other toxins that induce necrosis in host tissues during plant-pathogen interactions (De Wit 2016). Furthermore, some toxins synthesized by necrotrophic fungi can be recognized by host plants following a gene-for-gene relationship model (Oliver and Solomon 2010). An example of such type of fungi might be *Stemphylium lycopersici*, a

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necrotrophic fungus that is the causative agent of grey leaf spot disease in tomato (Lo Presti et al. 2015; Franco et al. 2017a).

Advances in molecular biology suggest that a re-examination of several phylogenetically related type-isolates is needed to clarify their neotypification, particularly into the genus *Stemphylium* (Woudenberg et al. 2017). These taxa form a well-defined monophyletic clade in Pleosporaceae (Inderbitzin et al. 2009), a family that also includes other genera such as *Alternaria*, *Bipolaris* and *Curvularia*, that are known for their ability to synthesize an array of SMs (Woudenberg et al. 2017; Khiralla et al. 2019; Patriarca et al. 2019). Interestingly, representatives of the genus *Stemphylium* are known for their ability to provoke disease in a wide array of crops (Inderbitzin et al. 2009; Franco 2019). Although the ability of several representatives of *Stemphylium* to synthesize SMs has already been studied leading to the characterization of metabolites such as stemphylin, stemphyloxin II, stemphyperylenol and stemphol (Solfrizzo et al. 1994; Andersen et al. 1995), there is a lack of studies aimed at analyzing the profile and the synthesis of SMs within representatives of *S. lycopersici* (Andersen and Frisvad 2004; Li et al. 2017; Olsen et al. 2018; Li et al. 2019).

Many gene sequences and/or gene clusters coding for the synthesis of small molecules have been identified by bioinformatic analysis in sequenced genomes. These molecules are putative SMs and may play key biological roles in nature (Khaldi et al. 2010; Keller 2019). Within the draft genome of *S. lycopersici* CIDEFI 216, 32 gene clusters encoding enzymes involve in the synthesis of SMs were predicted (Franco et al. 2016). SMs might act as pathogenicity and/or virulence factors during fungal infection, either as host-specific or non-host-specific toxins (HST and non-HST), respectively (Stergiopoulos et al. 2013). Still, their expression like that of most genes is highly dependent on the physiological stage of the fungus as well as the growth conditions (Nielsen and Nielsen 2017; Medina et al. 2018a).

Here, we investigated and compared the SM profile of 19 *S. lycopersici* isolates that differed in virulence (Franco et al. 2017a) and tried to identify the main compounds synthesized by these pathogens as well as their role and/or relationship with their virulence. Such knowledge might be helpful tools to develop a strategy to manage the disease. These data can provide the basis to identify target compounds for further evaluation of their actual role within the pathosystem.

## Materials and methods

### Biological material

*Stemphylium lycopersici* isolates used in this work were obtained from the Culture Collection of the Centro de Investigaciones de Fitopatología (CIDEFI), Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, La Plata, Argentina (Franco et al. 2017a). They were isolated from tomato (*Solanum lycopersicum* L.) plants showing typical symptoms of grey leaf spot that were collected from the main tomato-growing areas in Argentina in 2010, 2011 and 2013 (Table 1). They were identified as *S. lycopersici* through morphological, molecular tools as well as virulence (Franco et al. 2017a). For the sake of simplicity all along with the text, we will refer to the isolates only by their number.

### Secondary metabolite production

The metabolites synthesized by 19 *S. lycopersici* isolates on 14-day-old DRYES (Dichloran Rose Bengal Yeast Extract Sucrose medium) cultures that were grown at 25 °C in darkness, were extracted using a modification of the micro-scale extraction method used to study *Alternaria* metabolites (Andersen et al. 2015). Briefly, three agar plugs (6 mm diameter) were cut from the centre of three colonies and were placed in a 4 mL vial. Then, 1 mL ethyl acetate containing 1% formic acid (vol/vol) was added to each vial and the plugs were extracted by sonication for 30 min. The extract was transferred to a clean 2 mL vial, evaporated to dryness under a gentle stream of $N_2$ and re-dissolved in 400 μL methanol HPLC grade. The methanol extract was filtered through a 0.45 μm PTFE filter into a clean 2 mL vial and kept at −18 °C until analysis.

### UHPLC–HRMS analyses

Analyses were performed using ultra-high-performance liquid chromatography (UPHLC) with diode array detector (DAD) and high-resolution (HR) maXis 3G QTOF mass spectrometer (MS) (Bruker Daltonics, Bremen, Germany) equipped with an ESI source and connected to an Ultimate 3000 UHPLC system (Dionex, Sunnyvale, USA) equipped with a Kinetex 2.6-μm $C_{18}$, 100 mm × 2.1 mm column (Phenomenex, Torrance, CA). According to Klitgaard et al. (2014), a linear water-acetonitrile gradient was used (buffered with 20 mM formic acid) starting from 10% (vol/vol) acetonitrile and increased to 100% in 10 min, maintained for 3 min before returning to the starting conditions. MS was performed in ESI+ and ESI- in the scan range m/z 100–1000, with a mass accuracy <1.5 ppm. UV/VIS spectra were collected at wavelengths from 200 to 700 nm. Data processing was performed using DataAnalysis 4.0 and TargetAnalysis 1.2 (Bruker Daltonics) by the aggressive dereplication approach (Klitgaard et al. 2014). This method is based on accurate mass and isotopic patterns from a list of putative compounds and can handle many thousands of data inputs in a very short time. In this study, a database of 251 compounds reported in the

**Table 1** Isolates of *Stemphylium lycopersici*: site, tomato cultivar, year of isolation, virulence and accession number of the ITS and *gdp* sequences used for their identification

| Isolate | Sampling site | Tomato cultivar | Sampling year | Virulence group[a] | GenBank accession numbers | |
|---|---|---|---|---|---|---|
| | | | | | ITS | *gpd* |
| CIDEFI 200 | Lavalle[b] | Elpida | 2011 | L | KF709429 | KJ624421 |
| CIDEFI 201 | Lavalle | Elpida | 2011 | L | KF624431 | KJ624422 |
| CIDEFI 203 | Lavalle | Elpida | 2011 | L | KP026206 | KP026202 |
| CIDEFI 206 | Lavalle | Torry | 2011 | L | KJ624432 | KJ624423 |
| CIDEFI 207 | Bella Vista[b] | Elpida | 2011 | H | KJ624433 | KJ624424 |
| CIDEFI 208 | Lavalle | Elpida | 2011 | M | KJ624434 | KJ624425 |
| CIDEFI 210 | Bella Vista | Elpida | 2010 | L | KJ624435 | KJ624426 |
| CIDEFI 211 | Lavalle | Elpida | 2011 | H | KJ624436 | KJ624428 |
| CIDEFI 213 | Bella Vista | Elpida | 2011 | L | KJ624438 | KJ624427 |
| CIDEFI 214 | Lavalle | Elpida | 2011 | H | KP026208 | KP026198 |
| CIDEFI 215 | Bella Vista | Elpida | 2011 | H | KP026209 | KP026197 |
| CIDEFI 216 | Bella Vista | Elpida | 2010 | H | KJ624439 | KJ624429 |
| CIDEFI 225 | La Plata[c] | Platense | 2013 | M | KJ624449 | KP026189 |
| CIDEFI 226 | La Plata | Platense | 2013 | H | KJ624450 | KP026188 |
| CIDEFI 227 | Lavalle | Elpida | 2013 | M | KJ624446 | KP026183 |
| CIDEFI 228 | Lavalle | Elpida | 2013 | H | KJ624447 | KP026186 |
| CIDEFI 229 | Lavalle | Elpida | 2013 | H | KJ624448 | KP026187 |
| CIDEFI 230 | La Plata | Elpida | 2013 | H | KJ624441 | KP026185 |
| CIDEFI 231 | La Plata | Elpida | 2013 | L | KJ664442 | KP026184 |

[a] Virulence assessment by means of a detached leaf assay and quantification of affected area: L, between 0.42 to 1.00 $cm^2$; M, 1 .00 to 3.00 $cm^2$; H, higher than 3.00 $cm^2$. [b] Province of Corrientes, Argentina. [c] Province of Buenos Aires, Argentina. indicates Low virulence; indicates Medium virulence; indicates High virulence

literature for *Stemphylium*, *Alternaria* and other related genera was used to identify compounds based on accurate mass (deviation<1.5 ppm) and isotopic pattern (isotope fit<50).

## Data analyses

Relations between the isolates studied and the presence of each SM synthesized by them were analyzed by principal components analysis (PCA) using NTSYS-pc software (Rohlf 1992).

## Results

*Stemphylium lycopersici* is a necrotrophic pathogen that synthesizes, like other plant pathogens, SMs whose roles in plant-microbe interactions are unknown. This study was performed with a subset of isolates belonging to the CIDEFI culture collection that includes fungi isolated from different tomato growing areas, on different cultivars, that were all identified as *S. lycopersici* based on *gdp* and ITS gene sequences and that differ in virulence (Table 1). In addition to this, we obtained the draft genome sequence of two of these isolates (Franco et al. 2015; Medina et al. 2018b) and the first mitogenome sequence of a representative of Pleosporales (Franco et al. 2017b), fact that not only confirmed their identity but also showed that their genomes contained many gene clusters coding for the synthesis of SMs. The results of the analysis performed in this work showed that the SM profile of *S. lycopersici* consisted of an array of 15–20 major peaks that included both known and unknown compounds. In Fig. 1, we present a representative HPLC-MS chromatogram of an extract of an *S. lycopersici* isolate (214) that includes the major peaks identified. Although the analysis of the extracts of all

**Table 2** Secondary metabolites (SMs) profile synthesized by each of the 19 isolates from *Stemphylium lycopersici*. The last column of the table on the right shows the number of isolates that synthesized each compound, while the last row at the bottom of the table shows the number of compounds produced by each isolate

| | ***Stemphylium lycopersici* isolates CIDEFI collection** | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Compound** | 200 | 201 | 203 | 206 | 207 | 208 | 210 | 211 | 213 | 214 | 215 | 216 | 225 | 226 | 227 | 228 | 229 | 230 | 231 | **Number of producers** |
| **Stemphylin** | | | | | X | | X | | X | X | | X | X | X | X | X | | | X | **10** |
| **Altersolanol B** | | X | X | | | | | | X | | | X | X | X | X | | X | X | | **9** |
| **Alterporriol D** | | | | | | | | | | X | | | | | X | X | | | | **3** |
| **Altersolanol C** | | | | | | | | | X | | | | | | X | | | | | **2** |
| **Pyrenochaetic acid A** | | | | | | | | | | X | | | | | X | | | | | **2** |
| **Stemphypyrone** | X | X | | | X | X | X | X | | X | X | X | X | | | | | | X | **11** |
| **Deoxyuvidin** | X | | | | | | | | | X | | X | | | | | | | | **3** |
| **Phomapyrone D** | | | | | X | | | | | X | | X | | | | | | | | **3** |
| **Stemphyperylenol** | | | | | | | | X | | | | | | | | | | | | **1** |
| **Stemphyloxin I** | | | | | | | | | | | | | | | | X | | | | **1** |
| **Altertoxin** | X | | | | | | | | | | | | | | | | | | | **1** |
| **Deoxyuvidin B** | X | | | | X | X | | X | | | X | X | | | | | | X | | **7** |
| **Infectopyrone** | X | X | X | | X | X | X | | X | X | X | X | X | X | X | X | X | X | X | **17** |
| **Phomapyrone C** | | | | | | | | | | | | X | | | | | | | | **1** |
| **Alterporriol G** | | | | | | | | | | X | | | | | X | X | | | | **3** |
| **Phomapyrone F** | | | | | | | | | | | | | | | X | | | | | **1** |
| **Albrassitriol** | X | | | | | X | | X | | X | | X | | | | | | X | X | **7** |
| **Phomapyrone G** | | | | | | | | X | | | | X | | | | | | | | **2** |
| **Macrosporin** | | | | | | X | | | | X | | | | | X | | | | | **3** |
| **Phomapyrone A** | X | X | X | X | | | X | | | X | X | X | | X | X | X | X | X | X | **14** |
| **Stemphol** | X | | | X | X | X | X | | | X | X | X | | | | X | | | | **9** |
| **Brefeldin A** | X | | | | X | X | | | | X | | X | | | | | X | | X | **7** |
| **7-Oxo-brefeldin** | X | X | | | | X | | | | X | | X | | | | | | | | **5** |
| **Brassicadiol** | X | | | | X | X | | | | X | | X | | | | | | X | | **6** |
| **Number of SMs** | **11** | **5** | **3** | **2** | **8** | **9** | **5** | **5** | **4** | **15** | **5** | **15** | **4** | **4** | **10** | **7** | **4** | **6** | **6** | |

indicates 10 or more; indicates between 10 and 5; indicates 4 or less

the isolates studied showed that each presented a unique profile, a joint analysis on all isolates showed that *S. lycopersici* synthesizes 24 major peaks that were identified based on available databases. While a list of the SMs produced by *S. lycopersici* isolates, including the number that each isolate synthesized and number of isolates that produce each metabolite, is presented in Table 2, in Fig. 2 we presented the chemical structure of some of the major metabolites, and in Supplementary Material 1 the mass spectra obtained from the 6 most abundant compounds. Different altersolanols, alterporriols and phomapyrones were synthesized by the isolates of *S. lycopersici*. Isolates 200, 214, 216 and 227 presented the more complex profiles that contained the highest number of SMs (between 15 and 10) of known structure, though only two (214 and 216) were stated by Franco et al. (2017a) as highly virulent (Table 1). Other isolates, like 203, 206 and

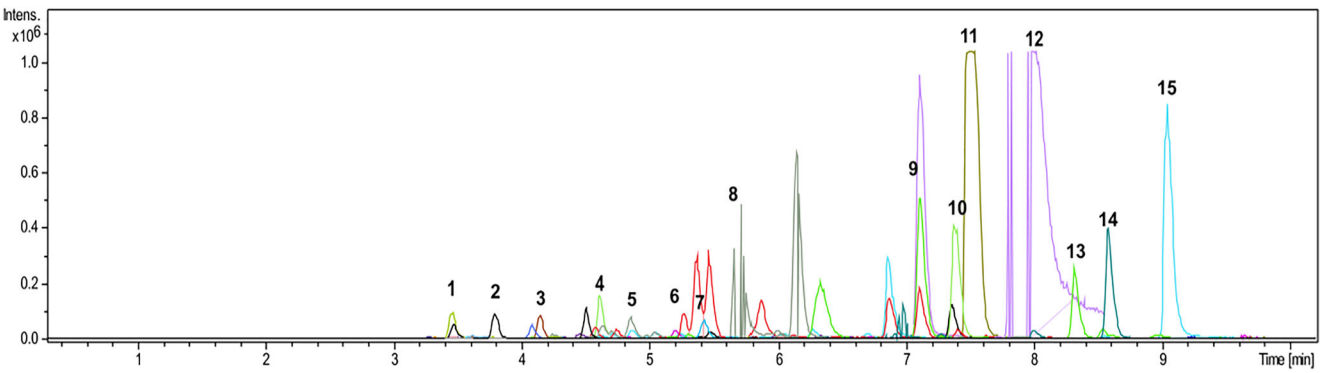


**Fig. 1** Metabolite profile (HPLC-MS chromatogram) of *Stemphylium lycopersici* isolate CIDEFI 214. X-axis shows retention time [min] while Y- axis shows signal intensity (intens.). (1) Stemphylin, (2) Alterporriol D, (3) Pyrenochaetic acid A, (4) Stemphypyrone, (5) Deoxyuvidin, (6) Phomapyrone D, (7) Infectopyrone, (8) Alterporriol G, (9) Albrassitriol, (10) Macrosporin, (11) Phomapyrone A, (12) Stemphol, (13) 7-Oxo-brefeldin, (14) Brefeldin A, (15) Brassicadiol

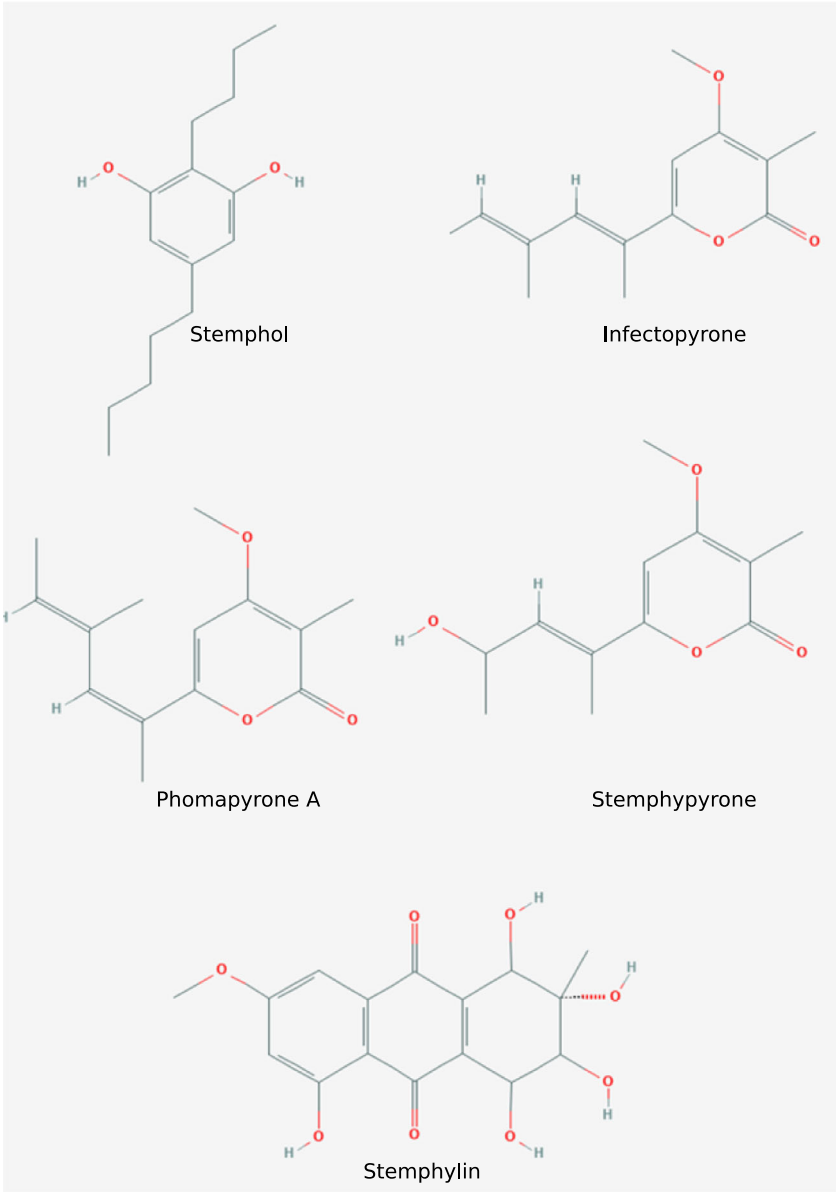


**Fig. 2** Structures of the SMs more commonly synthesized by *Stemphylium lycopersici* isolates analysed. The figure shows the structure of stemphol, infectopyrone, phomapyrone A, stemphypyrone and stemphylin. Chemical structure of compounds were obtained from https://pubchem.ncbi.nlm.nih.gov/

213, that are low virulent ones, as well as the medium and highly virulent isolates 226, 229 and 225, secreted only a few secondary metabolites (2 to 4).

Among all the SMs synthesized by representatives of *S. lycopersici*, infectopyrone, an α-pyrone resembling known toxins, was the most frequent, which was synthesized by 89% of the isolates. Phomapyrone A and stemphypyrone, two compounds formed through cyclisation of a growing polyketide chain that has antimicrobial as well as other biological activities were produced by 73% and 58% of the isolates, respectively. Stemphylin (*syn.* altersolanol A), and altersolanol B are two cytotoxic anthraquinone derivatives that were produced by 53 and 47% of the isolates, respectively. Finally, stemphol, a resorcinolic lipid is a cytotoxic compound that was synthesized by a 47% of the isolates. *S. lycopersici* isolates 225, 200 and 210 synthesized the highest level of infectopyrone; isolate 200 that of phomapyrone A and stemphypyrone and isolate 231 that of stemphylin (See Supplementary Material 2). *S. lycopersici* isolates 200, 211, 216, 227 and 228 synthesize altertoxin I, stemphyperylenol, phomapyrone C, phomapyrone F and stemphyloxin I, respectively. Furthermore, isolates like 214, 227 and 228 synthesize alterporriol D and alterporriol G. Isolates 216 and 227 were those whose SM profiles diverged the most. Interestingly, most low and medium virulence isolates were unable to synthesize deoxyuvidin, phomapyrone D, deoxyuvidin B, albrassitriol, stemphol, brefeldin A (*syn.* cyanein), 7-oxo-brefeldin and / or brassicadiol (See Supplementary Material 2).

A PCA was performed to establish if there is any relationship between the isolates' ability to synthesize specific SMs

and their virulence (See Supplementary Material 3). Two of them, 200 and 208, were clustered together apart from the other virulent isolates, which seemed to be due to their ability to synthesize deoxyuvidin, deoxyuvidin B and brassicadiol (See Supplementary Material 2). Interestingly, highly virulent isolates were not clustered together along the PC1 axis, showing that there was no relationship with their ability to synthesize SMs, which is not surprising considering that clustering along PC1 and PC2 explains only 44.3% of the variance that changed to an 80% when 7 axes of ordination were considered (See Supplementary Material 3).

By comparing the phenotype as well as the metabolic profile, it appears that high virulence was associated with the number of SMs synthesized as well as by the synthesis of phomapyrone D and brefeldin A (See Supplementary Material 2). Specifically, isolate 228 synthesized 7 different SMs, and among them stemphyloxin I, while the others cannot. Isolates 214 and 228 as well as 227 the formers one high virulent and the later medium virulent synthesized alterporriol D and alterporriol G. Finally, isolates 216 and 227 were the most segregated along the PC1 axis, while the former one synthesized 15 different metabolites, the latter one synthesized only 10 (Table 2). The comparison of the SM profiles between these two isolates showed that they differed in the synthesis of 17 different compounds. Within the PC2 axis, the isolates 211 and 227 were the most divergent. Interestingly these two isolates hardly had compounds in common within their SM profiles.

## Discussion

Andersen et al. (1995) characterized the SM profile of *Stemphylium* species such as *S. alfalfae, S. botryosum, S. herbarum*, *S. majusculum, S. sarciniforme* and *S. vesicarum.* They found stemphylin, stemphyloxin II, stemphyperylenol, stemphol, macrosporin (Barash et al. 1975; Arnone et al. 1986; Assante and Nasini 1987; Solfrizzo et al. 1994; Andersen et al. 1995; Andersen and Frisvad 2004; Debbab et al. 2009), as well as compounds related with stemphol, physcione, and others including a red pigment, polyphenols and compounds with anthraquinones structures (Debbab et al. 2012). Olsen et al. (2018) reported that an isolate of *S. xanthosomatis*, which is a homotypic synonym of *S. lycopersici*, synthesized only two SMs, macrosporin and stemphylin. We characterized the SM profile of 19 *S. lycopersici* isolates, which were previously identified through the ITS as well as the *gpd* sequences (Franco et al. 2017a) as well as by the draft genome of three isolates (Franco et al. 2015; Medina et al. 2018b), recovering 24 major compounds that have already been reported in other *Stemphylium* species and/or related genera belonging to Pleosporaceae.

More than 50% of SMs synthesized by isolates of *S. lycopersici* are compounds with cytotoxic activity against plants like stemphylin, altersolanol B, altersolanol C, pyrenochaetic acid A, phomapyrone D, stemphyloxin I, altertoxin I, deoxyuvidin B, phomapyrone C, phomapyrone F, albrassitriol, phomapyrone G, macrosporin, phomapyrone A, stemphol and 7-oxo-brefeldin A (Table 3). Thus, our results confirmed that *S. lycopersici* synthesizes a quite similar SMs profile to other species of the genus because of three main reasons, we used an HPLC-MS analysis to detect the compounds, which is a reliable method in line with the suggestion of Perez et al. (2016); we performed the studies using accurately identified isolates (Franco et al. 2015, 2017a; Medina et al. 2018b); and we used standard culture conditions like ones on the DRYES medium, which was also previously used by Andersen and Thrane (1996), Andersen et al. (2008, 2015) and Olsen et al. (2018). In summary, we not only characterized the SMs profile of *S. lycopersici*, but also proved that, even though the isolates shared compounds represented by the major peaks, their profiles were different.

Several SMs produced by fungi have cytotoxic activity against plants, which has been related to their phytopathogenic lifestyle. It has been suggested that necrotrophic fungi synthesize and release a larger number of cytotoxic SMs than biotrophic fungal pathogens (Collemare et al. 2014; Medina et al. 2019).

As mentioned above, *S. lycopersici* synthesized SMs (Table 3). Among them, several compounds are cytotoxic for plant cells that have already been reported in cultures of other *Stemphylium* species as well as representatives of other fungi (Table 3). Many of the low-molecular-mass compounds are part of the mechanisms of diseases in plants, and when they are applied in vitro they triggered symptom development on leaves and/or fruits (Agrios 2005; Medina et al. 2019).

Franco et al. (2017a) described the diversity within a *S. lycopersici* population of approximately 70 isolates based on sporulation, conidia morphometry and pigmentation, genetic diversity, and their ability to provoke disease. They grouped isolates in low, moderately and highly virulent ones, which might be related to the number of SMs produced by each isolate, as well as to the ability to co-synthesize phomapyrone D and brefeldin A. However, the SMs-based PCA of isolates did not group them according to their virulence. Therefore, it appears that soluble SMs per se do not explain the virulence of *S. lycopersici*, which might be a function of other compounds whose activity is under the influence of the environment (Medina et al. 2019). The analysis of the *S. lycopersici* genome allowed to predict 1005 secreted-proteins that might be involved in pathogenicity and virulence (Franco et al. 2017c). Intriguingly, the studied isolates synthesized SMs anywhere between 15 and 2 compounds, raising a question regarding the putative secreted-proteins within the *Stemphylium* genome.

**Table 3** Summary of secondary metabolites synthesized by *Stemphylium lycopersici*: biological activity, if available the toxin type to which they belong, and other reported fungal sources

| Compound | Biological activity | Toxin type | Other producing-fungi | Reference |
|---|---|---|---|---|
| Stemphylin | Phytotoxic[a] and antibiotic activities. | Not reported. | *Stemphylium botryosum, S. globuliforme, Alternaria* spp. | Barash et al. 1975; Assante and Nasini, 1987; Andersen et al. 1995; Debbab et al., 2009, 2012. |
| Altersolanol B | Phytotoxic activity. | Not reported. | *Alternaria* spp. | Debbab et al. 2009. |
| Alterporriol D | Toxic for mouse cells and antibacterial activities. | Not reported. | *Alternaria* spp. | Debbab et al. 2009. |
| Altersolanol C | Phytotoxic activity. | | *Alternaria* spp. | Debbab et al. 2009. |
| Pyrenochaetic acid A | Phytotoxic activity. | Non-HST[c]. | *Pyrenochaeta terrestris.* | Ichihara et al. 1987. |
| Stemphypyrone | Not reported[b]. | Not reported. | *S. botryosum, S. globuliforme.* | Barash et al. 1975; Assante and Nasini, 1987; Andersen et al. 1995, Debbab et al. 2009. |
| Deoxyuvidin | Not reported. | Not reported. | *Alternaria brassicae.* | Saharan et al. 2016 |
| Phomapyrone D | Phytotoxic activity. | Not reported. | *Phoma* and *Alternaria* spp. | Pedras and Yu 2009; Andersen et al. 2015; Evidente et al. 2019. |
| Stemphyperylenol | Antifungal activity. | Not reported. | *Stemphylium* sp. | Arnone et al. 1986; Olsen et al. 2018; Stringlis et al. 2018. |
| Stemphyloxin I | Phytotoxic activity. | Non-HST. | *S. lycopersici..* | Barash et al. 1982. |
| Altertoxin I | Phytotoxic, antibacterial, toxic for HeLa cells and mutagenic by Ames test. | Not reported. | *Alternaria* spp., *Stemphylium* sp. | Stinson et al. 1982; Olsen et al. 2018. |
| Deoxyuvidin B | Phytotoxic activity. | HST[d]. | *A. brassicae.* | Ayer and Pena-Rodriguez 1987; Evidente et al. 2019. |
| Infectopyrone | Mycotoxin. | Not reported. | *Alternaria* spp., *Ulocladium consortiale*, *S. eturmiunum*, *S. sarciniforme* and *S. vesicarium.* | Larsen et al., 2003; Andersen and Frisvad 2004; Pedras and Yu 2009. |
| Phomapyrone C | Phytotoxic activity. | Not reported. | *Phoma* and *Alternaria* spp. | Pedras and Yu 2009; Andersen et al. 2015; Evidente et al. 2019. |
| Alterporriol G | Toxic for mouse cells. | Not reported. | *Alternaria* spp. | Debbab et al. 2009. |
| Phomapyrone F | Phytotoxic activity. | HST. | *Phoma* and *Alternaria* spp. | Pedras and Yu 2009; Andersen et al. 2015; Evidente et al. 2019. |
| Albrassitriol | Phytotoxic activity. | HST. | *A. brassicae.* | Ayer and Pena-Rodriguez 1987; Evidente et al. 2019. |
| Phomapyrone G | Phytotoxic activity. | Not reported. | *Phoma* and *Alternaria* spp. | Pedras and Yu 2009; Andersen et al. 2015; Evidente et al. 2019. |
| Macrosporin | Phytotoxic activity. | Non-HST. | *S. lycopersici.* | Trigos et al. 2011. |
| Phomapyrone A | Phytotoxic activity. | HST. | *Phoma* and *Alternaria* spp. | Pedras and Yu 2009; Andersen et al. 2015; Evidente et al. 2019. |
| Stemphol | Phytotoxic and antibiotic activities. | Not reported. | *S. botryosum, S. majusculum, S.* cf, *lycopersici; Stemphylium* sp. | Solfrizzo et al., 1994; Andersen et al. 1995; 1994; Andersen and Frisvad 2004; Li et al. 2017. |
| Brefeldin A | Toxic for HeLa cells, antifungal, antiviral, and antimitotic activities. | Not reported. | *Penicillium*, *Ascochyta*, *Alternaria* spp. and *Curvularia.* | Betina 1989; Cimmino et al. 2013; Saharan et al. 2016. |
| 7-Oxo-brefeldin | Phytotoxic activity. | Not reported. | *Penicillium.* | Brase et al. 2009. |
| Brassicadiol | Non-phytotoxic. | Non-phytotoxic. | *A. brassicae.* | Ayer and Pena-Rodriguez 1987; Evidente et al. 2019. |

These findings along with the results of this work and the proposal of Franco et al. (2017c) suggest that the biological role of SMs in pathogenicity should be studied in further detail.

*Stemphylium lycopersici* synthesizes SMs such as deoxyuvidin, deoxyuvidin B, albrassitriol, brefeldin A, brassicadiol, 7-oxo-brefeldin and pyrenochaetic acid A. These SMs were also produced by other fungi including pathogens like *Alternaria*, *Aspergillus, Curvularia, Ascochyta* and *Penicillium* species (Brase et al. 2009; Ebel 2010; Khallaf 2013; Cimmino et al. 2013; Saharan et al. 2016, Table 3). Probably, these SMs, which are quite frequent within plant pathogens, are key factors involved in triggering symptom development in plant-pathogen interactions.

Other SMs synthesized by *S. lycopersici* and other plant pathogens, such as alterporriol D, alterporriol G, stemphyperilenol, infectopyrone, brefeldin A, and brassicadiol, were cytotoxic, mutagenic or might inhibit growth in in-vitro studies. However, their biological role as well as that of stemphypyrone and deoxyuvidin, in nature alone or together, remain unknown.

In this work, we show for the first time that *S. lycopersici* synthesizes compounds already defined as both HST or non-HST, which might explain the broad host range of crops affected by this pathogen. Fourteen out of 19 isolates synthesized phomapyrone A and other phomapyrones, such as C, D, F and G, also known as phomenins, though in low quantities. These SMs are considered HST, particularly phomapyrones A and G (Evidente et al. 2019), which were also detected in *Alternaria* (Andersen et al. 2015) and *Phoma* (Pedras and Chumala 2005). Furthermore, here we reported that *S. lycopersici* synthesizes in culture two other HST like deoxyuvidin B and albassitriol, such toxins have also been isolated from cultures of *Alternaria brassicae* (Ayer and Pena-Rodriguez 1987; Evidente et al. 2019).

Isolates of *Stemphylium lycopersici* analyzed in this work also synthesized stemphol and stemphylin, two compounds that have shown to be toxic against a wide array of microorganisms, including several pathogens (Solfrizzo et al. 1994; Debbab et al. 2012). Andersen et al. (1995), Barash et al. (1975), and Debbab et al. (2009) reported previously that *S. lycopersici, S. botryosum* and *S. globuliferum,* synthesizes stemphylin. Purified stemphylin provoked on lettuce leaves sunken brown lesions that look like those produced by *S. botryosum* when the pathogen infects plants under natural conditions (Barash et al. 1975; Assante and Nasini 1987). In addition to this, stemphylin had a strong biocide activity against several pathogenic microorganisms (Debbab et al. 2012). Interestingly, stemphol, a compound previously reported for *S. botryosum, S. majusculum* and *S.* cf. *lycopersici* (Solfrizzo et al. 1994; Andersen et al. 1995; Li et al. 2017; Andersen and Frisvad 2004), also was found in mouldy tomatoes infected with *S. lycopersici,* as well as in oilseed rape infected with *S. majusculum* (Solfrizzo et al. 1994), thus suggesting that stemphol might be one of the SMs responsible of tomato grey leaf spot development.

Isolates of *S. lycopersici* analyzed also synthesized several non-HST, like altersolanols and alterporriols, that also are synthesized by representatives of *Alternaria*, as well as *S. globuliferum* (Debbab et al. 2009) and a *Stemphylium* sp. (Debbab et al. 2012; Olsen et al. 2018). Other non-HST synthesized were macrosporin and stemphyloxin I and pyrenochaetic acid A (Li et al. 2017). Interestingly, while alterporriols E, D, and N, are growth inhibitors of pathogenic bacteria, alterporriol G is cytotoxic for mouse lymphoma cells E (Debbab et al. 2009). The other molecules like macrosporin provoke leaf necrosis by producing singlet oxygen (Trigos et al. 2011), and stemphyloxin I specifically causes necrosis and turgor loss on tomato and eggplant (Barash et al. 1982). Furthermore, macrosporin and altertoxin I might be toxigenic for bacteria, animals and plant cells or they might have mutagenic activity based on the by Ames test (Stinson et al. 1982; Debbab et al. 2012). *S. lycopersici* also synthesized pyrenochaetic acid A, which has already been defined as a non-HST that inhibits root growth of onion and lettuce seedlings (Ichihara et al. 1987).

Only one isolate of *S. lycopersici* synthesized stemphyperylenol, an inhibitory compound of fungal growth (Stringlis et al. 2018). Its synthesis has already been reported in other *Stemphylium* species (Arnone et al. 1986; Olsen et al. 2018).

We found that each *S. lycopersici* isolates presented a specific profile of SMs, being infectopyrone, an alpha-pyrone, the most frequent one, which also is synthesized by *Alternaria* spp., *Ulocladium consortiale*, *S. eturmiunum*, *S. sarciniforme* and *S. vesicarium* (Larsen et al. 2003; Andersen and Frisvad 2004). Probably this SM might be a widely used tool for pathogenesis within necrotrophic pathogens.

Other SMs synthesized by *S. lycopersici* were brefeldin A and 7-Oxo-brefeldin, a brefeldin A analogue, that had cytotoxic, antifungal, antiviral, and antimitotic activity (Betina 1989), and inhibited secretion in a eukaryotic cell system (Driouich et al. 1997). Studies should be aimed at studying the role of these SMs in plant-microbe interactions like tomato leaf gray spot.

## Conclusions

The SM profile of St*emphylium lycopersici* contains 24 major compounds that have already been reported in other *Stemphylium* species and/or related genera belonging to Pleosporaceae.

The SM profile within isolates of *Stemphylium lycopersici* is variable and unrelated with their virulence, although like in other plant pathogens they might play key roles in triggering symptoms development on the host plants.

*Stemphylium lycopersici* synthesizes host specific and non-host specific toxins that might explain the ability of the fungus to provoke disease on several plant species.

Stemphol is the putative SM that triggering grey leaf spot symptom development on tomato.

*Stemphylium lycopersici* SMs profile includes compounds that also are synthesized by other necrotrophic plant pathogens.

Since infectopyrone was the most frequent metabolite produced by the isolates and its synthesis also reported for other necrotrophic fungi such as *Alternaria* spp., *Ulocladium consortia*, and other *Stemphylium* species, it might be a target compound for studying pathogenesis within necrotrophic pathogens.

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## Compliance with ethical standards

**Declaration of interest** The authors have declared that they have not declaration of interest.

## References

Agrios GN (2005) Plant pathology, 5th ed. Academic Press, 922 p. ISBN: 0120445654

Andersen B, Frisvad JC (2004) Natural occurrence of fungi and fungal metabolites in moldy tomatoes. J Agric Food Chem 52:7507–7513. https://doi.org/10.1021/jf048727k

Andersen B, Thrane U (1996) Differentiation of *Alternaria infectoria* and *Alternaria alternata* based on morphology, metabolite profiles, and cultural characteristics. Can J Microbiol 42(7):685–689. https://doi.org/10.1139/m96-093

Andersen B, Solfrizzo M, Visconti A (1995) Metabolite profiles of common *Stemphylium* species. Mycol Res 99(6):672–676. https://doi.org/10.1016/S0953-7562(09)80526-1

Andersen B, Dongo A, Pryor BM (2008) Secondary metabolite profiling of *Alternaria dauci*, *A. porri*, *A. solani*, and *A. tomatophila*. Mycol Res 112:241–250. https://doi.org/10.1016/j.mycres.2007.09.004

Andersen B, Nielsen KF, Fernández Pinto V, Patriarca A (2015) Characterization of *Alternaria* strains from Argentinean blueberry, tomato, walnut and wheat. Int J Food Microbiol 196:1–10. https://doi.org/10.1016/j.ijfoodmicro.2014.11.029

Andersson PF (2012) Secondary metabolites associated with plant disease, plant defense and biocontrol. Doctoral thesis, Swedish University of Agricultural Sciences, Uppsala. ISSN 1652-6880, ISBN 978-91-576-7699-3

Arnone A, Nasini G, Merlini L, Assante G (1986) Secondary mould metabolites. Part 16. Stemphyltoxins, new reduced perylenequinone metabolites from *Stemphylium botryosum var. Lactucum*. J Chem Soc Perkin Trans 1:525–530. https://doi.org/10.1039/P19860000525

Assante G, Nasini G (1987) Identity of the phytotoxin stemphylin from *Stemphylium botryosum* with altersolanol a. Phytochemistry 26(3): 703–705. https://doi.org/10.1016/S0031-9422(00)84768-8

Ayer WA, Pena-Rodriguez LM (1987) Metabolites produced by *Alternaria brassicae*, the black spot pathogen of canola. J Nat Prod 50:400–407. https://doi.org/10.1021/np50051a010

Barash I, Karr AL, Strobel GA (1975) Isolation and characterization of Stemphylin, a chromone glucoside from *Stemphylium botryosum*. Plant Physiol 55:646–651. https://doi.org/10.1104/pp.55.4.646

Barash I, Pupkin G, Netzer D, Kashman Y (1982) A novel enolic β-ketoaldehyde phytotoxin produced by *Stemphylium botryosum* f. sp. *lycopersici*: partial chemical and biological characterization. Plant Physiol 69:23–27. https://doi.org/10.1104/pp.69.1.23

Berestetskiy AO (2008) A review of fungal phytotoxins: from basic studies to practical use. Appl Biochem Microbiol 44(5):453–465. https://doi.org/10.1134/S0003683808050013

Betina V (1989) Structure-activity relationships among mycotoxins. Chem Biol Interact 71(2–3):105–146. https://doi.org/10.1016/0009-2797(89)90030-6

Boruta T (2018) Uncovering the repertoire of fungal secondary metabolites: from Fleming's laboratory to the international Space Station. Bioengineered. 9(1):12–16. https://doi.org/10.1080/21655979.2017.1341022

Brase S, Encinas A, Keck J, Nising CF (2009) Chemistry and biology of mycotoxins and related fungal metabolites. Chem Rev 109(9): 3903–3990. https://doi.org/10.1021/cr050001f

Cimmino A, Andolfi A, Abouzeid M, Evidente A (2013) Polyphenols as fungal phytotoxins, seed germination stimulants and phytoalexins. Phytochem Rev 12(4):653–672. https://doi.org/10.1007/s11101-013-9277-5

Collemare J, Griffiths S, Iida Y, Karimi Jashni M, Battaglia E, Cox RJ, de Wit PJGM (2014) Secondary metabolism and biotrophic lifestyle in the tomato pathogen *Cladosporium fulvum*. PLoS One 9(1):e85877. https://doi.org/10.1371/journal.pone.0085877

De Wit PJGM (2016) Apoplastic fungal effectors in historic perspective; a personal view. New Phytol 212(4):805–813. https://doi.org/10.1111/nph.14144

Debbab A, Aly AH, Edrada-Ebel R, Wray V, Müller WEG, Totzke F, Zirrgiebel U, Schächtele C, Kubbutat MHG, Lin WH, Mosaddak M, Hakiki A, Proksch P, Ebel R (2009) Bioactive metabolites from the endophytic fungus *Stemphylium globuliferum* isolated from *Mentha pulegium*. J Nat Prod 72:626–631. https://doi.org/10.1021/np8004997

Debbab A, Aly AH, Edrada-Ebel R, Wray V, Pretsch A, Pescitelli G, Kurtan T, Proksch P (2012) New anthracene derivatives–structure elucidation and antimicrobial activity. Eur J Org Chem 7:1351–1359. https://doi.org/10.1002/ejoc.201101442

Demain AL, Fang A (2000) The natural functions of secondary metabolites. In: Fiechter A (ed) Adv. Biochem. Eng./Biotechnol, History of modern biotechnology I. Springer, Germany, pp 1–39. https://doi.org/10.1007/3-540-44964-7_1

Driouich A, Jauneau A, Staehelin LA (1997) 7-Dehydrobrefeldin a, a naturally occurring brefeldin a derivative, inhibits secretion and causes a cis-to-trans breakdown of Golgi stacks in plant cells.

Plant Physiol 113(2):487–492. https://doi.org/10.1104/pp.113.2.487

Ebel R (2010) Terpenes from marine-derived fungi. Mar Drugs 8(8): 2340–2368. https://doi.org/10.3390/md8082340

Evidente A, Cimmino A, Masi M (2019) Phytotoxins produced by pathogenic fungi of agrarian plants. Phytochem Rev 18(3):843–870. https://doi.org/10.1007/s11101-019-09624-0

Franco MEE (2019) *Mancha gris de la hoja del tomate: identificación, biología y genómica del agente etiológico*. (Doctoral dissertation, Facultad de Ciencias Naturales y Museo)

Franco MEE, López SMY, Medina R, Saparrat MCN, Balatti P (2015) Draft genome sequence and gene annotation of *Stemphylium lycopersici* strain CIDEFI-216. Genome Announc 3(5):1–2. https://doi.org/10.1128/genomeA.01069-15

Franco MEE, López SMY, Saparrat MCN, Balatti PA (2016) *In silico* screening of genes coding for secondary metabolites in the phytopathogenic fungus *Stemphylium lycopersici*. In: *American phytopathological society annual meeting,* Tampa, USA

Franco MEE, Troncozo MI, López SMY, Lucentini G, Medina R, Saparrat MCN, Ronco LB, Balatti PA (2017a) A survey on tomato leaf gray spot in the two main production areas of Argentina led to the isolation of *Stemphylium lycopersici* representatives which were genetically diverse and differed in their virulence. Eur J Plant Pathol 149(4):983–1000. https://doi.org/10.1007/s10658-017-1248-z

Franco MEE, López SMY, Medina R, Lucentini CG, Troncozo MI, Pastorino GN, Saparrat MCN, Balatti PA (2017b) The mitochondrial genome of the plant-pathogenic fungus *Stemphylium lycopersici* uncovers a dynamic structure due to repetitive and mobile elements. PLoS One 12(10):e0185545. https://doi.org/10.1371/journal.pone.0185545

Franco MEE, López SMY, Medina R et al (2017c) El Secretoma Estimado del Hongo Fitopatógeno *Stemphylium lycopersici* CIDEFI-216. In XXXVI Jornadas Argentinas de Botánica - XXVIII Reunión Anual de la Sociedad Botánica de Chile - II Reunión Científica de la Asociación Micológica Carlos Spegazzini

Ichihara A, Murakami K, Sakamura S (1987) Synthesis of pyrenocines A, B and pyrenochaetic acid A. Tetrahedron. 43(22):5245–5250. https://doi.org/10.1016/S0040-4020(01)87700-0

Inderbitzin P, Mehta YR, Berbee ML (2009) *Pleospora* species with *Stemphylium* anamorphs: a four locus phylogeny resolves new lineages yet does not distinguish among species in the *Pleospora herbarum* clade. Mycologia. 101(3):329–339. https://doi.org/10.3852/08-071

Jashni MK, Mehrabi R, Collemare J, Mesarich CH, de Wit PJGM (2015) The battle in the apoplast: further insights into the roles of proteases and their inhibitors in plant–pathogen interactions. Front Plant Sci 6: 584. https://doi.org/10.3389/fpls.2015.00584

Keller NP (2019) Fungal secondary metabolism: regulation, function and drug discovery. Nat Rev Microbiol 17:167–180. https://doi.org/10.1038/s41579-018-0121-1

Keller NP, Turner G, Bennett JW (2005) Fungal secondary metabolism—from biochemistry to genomics. Nat Rev Microbiol 3(12):937–947. https://doi.org/10.1038/nrmicro1286

Khaldi N, Seifuddin FT, Turner G, Haft D, Nierman WC, Wolfe KH, Fedorova ND (2010) SMURF: genomic mapping of fungal secondary metabolite clusters. Fungal Genet Biol 47(9):736–741. https://doi.org/10.1016/j.fgb.2010.06.003

Khallaf I (2013) Biotransformations of fungal phytotoxins in plants and Indolyl-3-acetaldoxime in fungi. Doctoral thesis, University of Saskatchewan, Saskatoon

Khiralla A, Spina R, Saliba S, Laurain-Mattar D (2019) Diversity of natural products of the genera Curvularia and Bipolaris. Fungal Biol Rev 33(2):101–122. https://doi.org/10.1016/j.fbr.2018.09.002.

Klitgaard A, Iversen A, Andersen MR, Larsen TO, Frisvad JC, Nielsen KF (2014) Aggressive dereplication using UHPLC–DAD–QTOF: screening extracts for up to 3000 fungal secondary metabolites. Anal Bioanal Chem 406:1933–1943. https://doi.org/10.1007/s00216-013-7582-x

Larsen TO, Perry NB, Andersen B (2003) Infectopyrone, a potential mycotoxin from *Alternaria infectoria*. Tetrahedron Lett 44(24): 4511–4513. https://doi.org/10.1016/S0040-4039(03)01018-9

Li F, Xue F, Yu X (2017) GC–MS, FTIR and Raman analysis of antioxidant components of red pigments from *Stemphylium lycopersici*. Curr Microbiol 74(4):532–539. https://doi.org/10.1007/s00284-017-1220-3

Li J, Zheng YB, Kurtán T, Liu MX, Tang H, Zhuang CL, Zhang W (2019) Anthraquinone derivatives from a coral associated fungus *Stemphylium lycopersici*. Nat Prod Res 1-8:2116–2123. https://doi.org/10.1080/14786419.2019.1576041

Lo Presti L, Lanver D, Schweizer G, Tanaka S, Liang L, Tollot M, Zuccaro A, Reissmann S, Kahmann R (2015) Fungal effectors and plant susceptibility. Annu Rev Plant Biol 66:513–545. https://doi.org/10.1146/annurev-arplant-043014-114623

Medina R, Franco MEE, Lucentini CG et al (2018a) *Stemphylium lycopersici* isolates virulence depends on the synthesis of phytotoxic metabolites, which is modified by the environment. In: International Congress of Plant Pathology (ICPP), Boston, USA

Medina R, Franco MEE, Lucentini CG et al (2018b) Draft genome sequences of sporulating (CIDEFI-213) and nonsporulating (CIDEFI-212) strains of *Stemphylium lycopersici*. Microbiol Resour Announc 7(6):e00960–e00918. https://doi.org/10.1128/MRA.00960-18

Medina R, Franco MEE, Lucentini CG, Rosso JA, Saparrat MCN, Bartel LC, Balatti PA (2019) Secondary metabolites synthesized by *Stemphylium lycopersici* and *Fulvia fulva*, necrotrophic and biotrophic fungi pathogen of tomato plants. Curr Plant Biol 20: 100122. https://doi.org/10.1016/j.cpb.2019.100122

Nielsen JC, Nielsen J (2017) Development of fungal cell factories for the production of secondary metabolites: linking genomics and metabolism. Synth Syst Biotechnol 2(1):5–12. https://doi.org/10.1016/j.synbio.2017.02.002

Oliver RP, Solomon PS (2010) New developments in pathogenicity and virulence of necrotrophs. Curr Opin Plant Biol 13(4):415–419. https://doi.org/10.1016/j.pbi.2010.05.003

Olsen KJK, Rossman A, Andersen B (2018) Metabolite production by species of *Stemphylium*. Fungal Biol 122(2–3):172–181. https://doi.org/10.1016/j.funbio.2017.12.012

Patriarca A, da Cruz CL, Pavicich MA et al (2019) Secondary metabolite profiles of small-spored *Alternaria* support the new phylogenetic organization of the genus. Int J Food Microbiol 291:135–143. https://doi.org/10.1016/j.ijfoodmicro.2018.11.022

Pedras MSC, Chumala PB (2005) Phomapyrones from blackleg causing phytopathogenic fungi: isolation, structure determination, biosyntheses and biological activity. Phytochemistry. 66(1):81–87. https://doi.org/10.1016/j.phytochem.2004.10.011

Pedras MSC, Yu Y (2009) Phytotoxins, elicitors and other secondary metabolites from phytopathogenic "blackleg" fungi: structure, phytotoxicity and biosynthesis. Nat Prod Commun 4(9): 1934578X0900400. https://doi.org/10.1177/1934578X0900400927

Perez ER, Knapp JA, Horn CK, Stillman SL, Evans JE, Arfsten DP (2016) Comparison of LC–MS-MS and GC–MS analysis of benzodiazepine compounds included in the drug demand reduction urinalysis program. J Anal Toxicol 40(3):201–207. https://doi.org/10.1093/jat/bkv140

Rohlf FJ (1992) NTSYS-pc: numerical taxonomy and multivariate analysis system. Applied Biostatistics

Saharan GS, Mehta N, Meena PD, Dayal P (2016) *Alternaria* diseases of crucifers: biology, ecology and disease management. Springer Singapore https://doi.org/10.1007/978-981-10-0021-8

Solfrizzo M, Strange RN, Sabia C, Visconti A (1994) Production of a toxin stemphol by *Stemphylium* species. Nat Toxins 2(1):14–18. https://doi.org/10.1002/nt.2620020104

Stergiopoulos I, Collemare J, Mehrabi R, De Wit PJGM (2013) Phytotoxic secondary metabolites and peptides produced by plant pathogenic *Dothideomycete* fungi. FEMS Microbiol Rev 37(1):67–93. https://doi.org/10.1111/j.1574-6976.2012.00349.x

Stinson EE, Osman SF, Pfeffer PE (1982) Structure of altertoxin I, a mycotoxin from *Alternaria*. J Org Chem 47(21):4110–4113. https://doi.org/10.1021/jo00142a020

Stringlis IA, Zhang H, Pieterse CM et al (2018) Microbial small molecules–weapons of plant subversion. Nat Prod Rep 35(5):410–433. https://doi.org/10.1039/C7NP00062F

Trigos Á, Mendoza G, Espinoza C, Salinas A, Fernández JJ, Norte M (2011) The role of macrosporin in necrotic spots. Phytochem Lett 4: 122–125. https://doi.org/10.1016/j.phytol.2010.12.007

Wolpert TJ, Dunkle LD, Ciuffetti LM (2002) Host-selective toxins and avirulence determinants: what's in a name? Annu Rev Phytopathol 40(1):251–285. https://doi.org/10.1146/annurev.phyto.40.011402.114210

Woudenberg JHC, Hanse B, Van Leeuwen GCM et al (2017) *Stemphylium* revisited. Stud Mycol 87:77–103. https://doi.org/10.1016/j.simyco.2017.06.001

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