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Phytotoxic Lipophilic Metabolites Produced by Grapevine Strains of *Lasiodiplodia* Species in Brazil

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ABSTRACT: Phytotoxic metabolites produced in liquid culture by six species of *Lasiodiplodia* isolated in Brazil and causing Botryosphaeria dieback of grapevine were chemically identified. As ascertained by LC/MS, *L. brasiliense*, *L. crassispora*, *L. jatrophicola*, and *L. pseudotheobromae* produced jasmonic acid, and *L. brasiliense* synthesized, besides jasmonic acid, also (3*R*,4*S*)-4-hydroxymellein. *L. euphorbicola* and *L. hormozganensis* produced some low molecular weight lipophilic toxins. Specifically, *L. euphorbicola* produced (−)-mellein, (3*R*,4*R*)-(−)- and (3*R*,4*S*)-(−)-4-hydroxymellein, and tyrosol, and *L. hormozganensis* synthesized tyrosol and *p*-hydroxybenzoic acid. This is the first report on the production of the above cited metabolites from *L. euphorbicola* and *L. hormozganensis*. The phytotoxic activity of the metabolites produced is also discussed and related to the symptoms these pathogens cause in the grapevine host plants.

KEYWORDS: *grapevine trunk diseases, Lasiodiplodia spp., Vitis vinifera, Botryosphaeria dieback, phytotoxins, melleins, tyrosol, p-hydroxybenzoic acid*

■ INTRODUCTION

Fungal species in the Botryosphaeriaceae family are cosmopolitan plant pathogens causing fruit rot, leaf spot, dieback, cankers, and root rot of various angiosperms and gymnosperms.[1] Many species of various genera in this family are important pathogens of grapevine in all the main grape-growing areas worldwide, where they cause wood cankers, stunted growth, dieback, light-brown stripes under the bark, and sectorial necrosis in the wood.[2] Some of these Botryosphaeriaceae species are also reported to cause typical foliar symptoms in grapevine,[3] and, since these species colonize only the wood, there is an increasing interest in the toxic compounds that they produce during the pathogenic process, and the role that these compounds may have in that process. In the genus *Lasiodiplodia*, 11 species, including *L. brasiliense*, *L. crassispora*, *L. egyptiacae*, *L. euphorbicola*, *L. jatrophicola*, *L. hormozganensis*, *L. missouriana*, *L. parva*, *L. pseudotheobromae*, *L. theobromae*, and *L. viticola*, have so far been reported as pathogenic on grapevine.[2,4−7] Of these, *L. theobromae* is one of the most widespread and aggressive, occurring mostly in the tropical and subtropical regions of the Americas and Australia,[2,8] but also in the warmer areas of Europe and in the Mediterranean.[9−11]

Lasiodiplodia species were also investigated for their production of phytotoxic metabolites. A number of substances (jasmonic acid, mellein, lasiodiplodin, theobroxide, butyrolactones, botryospaeran, botryorodines, and lasiodiplodan among others) are produced in vitro by various isolates of *L. theobromae* and other *Lasiodiplodia* spp. in hosts other than grapevine, and these substances have been tested for their toxic activity.[12−22] *L. mediterranea*, recently isolated from grapevine and closely related to *L. pseudotheobromae*,[23] produces in vitro three jasmonic acid esters, named lasiojasmonates A−C, and 16-*O*-acetylbotryosphaerilactones A and C, (1*R*,2*R*)-jasmonic acid, its methyl ester, botryosphaerilactone A, (3*S*,4*R*,5*R*)-4-hydroxymethyl-3,5-dimethyldihydro-2-furanone, and (3*R*,4*S*)-botryodiplodin.[24] Two different strains of *L. pseudotheobromae*, isolated from hosts other than grapevine, i.e., decaying fruit and root crops during storage, synthesize four palmarumycins, of which two are reported to have a cytotoxic effect on human promyelocytic leukemia cells,[25] and six sulfureous diketopiperazines, designated as lasiodiplines A−F, with lasiodipline E having a strong antibacterial effect.[26] None of the secondary metabolites produced by *L. pseudotheobromae* strains have been detected in the strains isolated from declining grapevines.

In a recent study on grapevines growing in Brazil and showing Botryosphaeria dieback symptoms, *L. brasiliense*, *L. euphorbicola*, *L. hormozganensis*, and *L. jatrophicola* were isolated, together with other *Lasiodiplodia* species previously reported on grapevine.[7]

The present study reports on the identification of low molecular weight (LMW) phytotoxins produced by six strains and in particular by *L. euphorbicola* and *L. hormozganensis*.

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■ MATERIALS AND METHODS

General Experimental Procedure. Optical rotations were measured in $CHCl_3$ on a Jasco P-1010 digital polarimeter (Tokyo, Japan). IR spectra were recorded as a deposit glass film on a Thermo Electron Corporation Nicolet 5700 FT-IR spectrometer (Madison, WI, USA), and UV spectra were measured in MeCN on a Jasco V-530 spectrophotometer; ^{1}H NMR spectra were recorded at 400 or 500 MHz in $CDCl_3$ on Bruker (Karlsruhe, Germany) and Varian (Palo Alto, CA, USA) instruments. The same solvent was also used as an internal standard. ESI MS and LC/MS analyses were performed using the LC/MS TOF system (AGILENT 6230B, HPLC 1260 Infinity, Milan, Italy). The HPLC separations were performed with a Phenomenex LUNA (C18(2) 5 μm 150 × 4.6 mm). Analytical and preparative TLCs were carried out on silica gel (Kieselgel 60, F_{254}, 0.25 and 0.5 mm respectively) and on reverse phase (Kieselgel 60 RP-18, F_{254}, 0.20 mm) plates (Merck, Darmstadt, Germany). The spots were visualized by exposure to UV radiation, or by spraying first with 10% H_2SO_4 in MeOH, and then with 5% phosphomolybdic acid in EtOH, followed by heating at 110 °C for 10 min. Column chromatography was performed using silica gel (Kieselgel 60, 0.063−0.200 mm) (Merck). Standard samples of racemic jasmonic acid and 4-hydroxybenzoic acid and tyrosol were procured from Sigma-Aldrich (Saint Louis, MO, USA). Standard samples of scytalone and isosclerone and *cis*-(3*R*,4*R*)- and *trans*-(3*R*,4*S*)-(−)-4-hydroxymelleins were isolated, repectively, from *Phaeoacremonium minimum* (syn. *P. aleophilum*) and *Diplodia africana* as reported in earlier works.[27−29]

Fungal Strains and Culture Conditions. The *Lasiodiplodia* strains used in this study were obtained from the collection of Universidade Federal Rural de Pernambuco, Recife, Brazil, and were *L. crassispora* CMM0390, *L. euphorbicola* CMM0181, *L. hormozganensis* CMM0126, *L. jatrophicola* CMM0840, *L. pseudotheobromae* CMM0204, and *L. brasiliense* CMM0418. All the strains were grown in stationary culture in 0.5 L Roux flasks, each containing 100 mL of modified Difco Czapek Dox medium (Benton, MD, USA) with 0.5% yeast and 0.5% malt extract (both from Difco), for 21 days at 25 °C in the dark. To obtain a greater amount of culture filtrate, *L. euphorbicola* CMM0181 and *L. hormozganensis* CMM0126 were also grown in six 2 L Roux flasks, each containing 600 mL of culture medium to reach a total volume of 3.6 L of liquid culture. Mycelium plugs from one-week-old colonies grown on potato dextrose agar were used to seed liquid cultures of *Lasiodiplodia* spp. At harvest, the mycelial mats were removed and the liquid cultures filtered through filter paper folded double and lyophilized prior to the extraction procedure.

Phytotoxic Activity Assay. The phytotoxic activity of the crude extract and chromatographic fractions were assayed on nonhost lemon fruits, as reported in a previous work.[30] Briefly, the samples were dissolved in DMSO and diluted in distilled water to a final concentration of 1 mg/mL and 4% of DMSO. The peel of the lemon fruits was disinfected with sodium hypochlorite (50 μg/mL) and subsequently rinsed three times with sterile distilled water. A 10 μL drop of the test solution was placed on lemon peel, and the peel underneath the solution was punctured three times with the needle of a sterile syringe. The infiltrations were carried out in spring, at room temperature (16−22 °C), and were examined after 72 h. Ophiobolin and 5% DMSO in Milli-Q water were used as positive and negative controls, respectively.

LC/MS Analysis of the Culture Filtrates of *Lasiodioplodia* spp. Strains. The lyophilized residue of 100 mL of the culture filtrates of the *Lasiodiplodia* strains was dissolved in 10 mL of Milli-Q water; and five volumes of cold ethanol were added under stirring at 10 °C. The suspensions were centrifuged at 7000 rpm at the same temperature. The ethanolic phases were recovered and the precipitates resuspended and again precipitated as above. The polysaccharide content of the precipitates was measured following a recently described procedure.[31] The standards of jasmonic acid (**6**), *cis*-(3*R*,4*R*)- and *trans*-(3*R*,4*S*)-(−)-4-hydroxymelleins (**2** and **3**), scytalone, and isosclerone were compared by TLC analysis with the residues obtained from both ethanolic phases. The samples containing jasmonic acid were also analyzed by LC/MS. The LC/MS analyses were performed using the Agilent LC/MS TOF system. Samples were injected using a 20 μL loop, eluted in isocratic conditions with CH_3CN/H_2O 0.1% TFA (50:50) at a flow rate of 1 mL/min, and UV monitored for 10 min. The metabolites were identified according to their retention time (t_R) and by ESI MS.

Extraction of LMW Phytotoxic Metabolites from *L. euphorbicola* CMM0181 and *L. hormozganensis* CMM0126. The lyophilized residues of the culture filtrates of *L. euphorbicola* CMM0181 (3.5 L) and *L. hormozganensis* CMM0126 (3.7 L) were dissolved in 400 and 300 mL of water, respectively. Three aliquots of 20 mL were taken from each culture filtrate: in the first aliquot the pH was acidified to pH 2 with 1 M formic acid, in the second the pH was alkalized to pH 9 with 1 M NH_4OH, and in the third the pH of the culture filtrates (pH 6) was kept unchanged. The organic phase was extracted with EtOAc (3 × 60 mL). The organic extracts were then combined, dried (Na_2SO_4), filtered, and evaporated under low pressure. The organic extracts were tested for toxicity on the lemon fruits by a preliminary procedure as described above.

Purification of LMW Phytotoxins from the Organic Extracts of *L. euphorbicola* CMM0181 and *L. hormozganensis* CMM0126. The residual aliquot of 340 mL of culture filtrate of *L. euphorbicola* was extracted at pH 6 with EtOAc (3 × 300 mL). The organic extracts were combined, dried (Na_2SO_4), and evaporated under low pressure. The residue (305 mg) was purified by silica gel column chromatography using $CHCl_3$−*i*-PrOH (95:5, v/v). After TLC, the fractions were collected in seven separate groups, each group being tested for its phytotoxicity on lemon fruits. The residue of fraction one was purified by preparative TLC on silica gel using $CHCl_3$ as an eluent; this yielded a white solid, which was identified as (−)-mellein (**1**, 9.4 mg). The residue of fraction two was purified by TLC on reverse phase, eluent EtOH−H_2O (1:1, v/v), yielding two amorphous solids, identified as (3*R*,4*R*)- and (3*R*,4*S*)-(−)-4-hydroxymelleins (**2** and **3**, 1.2 and 1.9 mg). The residue of fraction four was purified on preparative TLC on silica gel, using $CHCl_3$−*i*-PrOH (9:1, v/v) as an eluent; this yielded a white solid substance identified as tyrosol (**4**, 2.4 mg). The organic extract obtained from the residual culture filtrates (240 mL) of *L. hormozganensis* was extracted at pH 6 with EtOAc (3 × 250 mL). The organic extracts were combined, dried (Na_2SO_4), and evaporated under low pressure. The residue (325 mg) was purified by silica gel column chromatography, using $CHCl_3$−*i*-PrOH (97:3, v/v) as an eluent, and yielded ten different fraction groups. Residue fraction six (14 mg) was purified by preparative TLC, eluent *n*-hexane−acetone (1:1, v/v), and yielded a white solid identified as tyrosol (**4**, 2.5 mg). Lastly, the residue of fraction eight (18 mg) was purified by preparative TLC eluted with *n*-hexane−Me_2CO (1:1, v/v), yielding an amorphous solid identified as *p*-hydroxybenzoic acid (**5**, 1.9 mg).

Identification of Compounds 1−6. Compounds **1**−**6** were identified by comparing their spectroscopic data (^{1}H NMR and ESI/MS) with those already reported in the literature.[14,24,32−40]

*(−)-Mellein (**1**).* ^{1}H NMR (500 MHz, in $CDCl_3$), δ: 11.03 (s, HO-8), 7.41 (t, *J* = 8.4 Hz, H-6), 6.89 (d, *J* = 8.4 Hz, H-7), 6.69 (d, *J* = 8.4 Hz, H-5), 4.74 (tq, *J* = 6.9 and 6.3 Hz, H-3), 2.93 (d, *J* = 6.9 Hz, H_2-4), 1.53 (d, *J* = 6.3 Hz, Me-3). ESI/MS (+), *m*/*z*: 179 $[M + H]^+$. These data are in agreement with the data previously reported.[32,34−37]

*(3R,4R)-(−)-4-Hydroxymellein (**2**).* ^{1}H NMR (400 MHz, in $CDCl_3$), δ: 10.99 (s, HO-8), 7.55 (t, *J* = 7.6 Hz, H-6), 7.03 (d, *J* = 7.6 Hz, H-7), 7.00 (d, *J* = 7.6 Hz, H-5), 4.60 (m, H-3 and H-4), 1.53 (d, *J* = 6.1 Hz, Me-C3). ESI/MS (+), *m*/*z*: 195 $[M + H]^+$. These data are in agreement with the data previously reported.[32,34−37]

*(3R,4S)-(−)-4-Hydroxymellein (**3**).* ^{1}H NMR (400 MHz, in $CDCl_3$) and ESI/MS (+) data are very similar to those of **2**. These data are also in agreement with the data previously reported.[32−37]

*Tyrosol (**4**).* ^{1}H NMR (500 MHz, in $CDCl_3$), δ: 7.20 (d, *J* = 8.0 Hz, (H-2 and H-6), 6.80 (d, *J* = 8.0 Hz, H-3 and H-5), 4.90 (s, OH), 3.80 (t, *J* = 6.4 Hz, H_2-8), 2.80 (t, *J* = 6.4 Hz, H_2-7). ESI/MS (+), *m*/*z*: 295 $[2 M + Na]^+$, 159 $[M + Na]^+$. These data are in agreement with those previously reported.[37,38]

*p-Hydroxybenzoic Acid (**5**).* ^{1}H NMR (500 MHz, in $CDCl_3$), δ: 7.91 (d, *J* = 8.6 Hz, H-2 and H-6), 7.04 (d, *J* = 8.6 Hz, H-3 and H-5).

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Table 1. LMW Lipophilic Compounds Identified in the Organic Extract of Culture Filtrates of *Lasiodiplodia* spp. Isolated from Grapevine in Brazil[a]

		metabolites isolated from culture filtrates					
strain	species	(*R*)-(−)-mellein (**1**)	(3*R*,4*R*-)-(−)-4-hydroxymellein (**2**)	(3*R*,4*S*)-(−)-4-hydroxymellein (**3**)	tyrosol (**4**)	*p*-hydroxybenzoic acid (**5**)	jasmonic acid (**6**)
CMM0418	*L. brasiliense*			×			×
CMM0390	*L. crassispora*						×
CMM0181	*L. euphorbicola*	×	×	×	×		
CMM0126	*L.hormozganensis*				×	×	
CMM0840	*L. jatrophicola*						×
CMM0204	*L. pseudotheo-bromae*						×

[a]The presence of the metabolite is indicated with "×".

ESI/MS, m/z: 139 [M + H]$^+$. These data are in agreement with the data previously reported.[40]

Jasmonic Acid (6). ^{1}H NMR (400 MHz, in $CDCl_3$), δ: 5.46 (m, H-10), 5.29 (m, H-9), 2.70−1.40 (12 H, m), 0.98 (t, J = 7.7 Hz, Me-12). ESI/MS, m/z: 211 [M + H]$^+$. These data are in agreement with the data previously reported.[14,24]

RESULTS

Analysis of the ethanolic supernatants obtained by EPS precipitation indicated that only *L. brasiliense*, *L. crassispora*, *L. jatrophicola*, and *L. pseudotheobromae* produced jasmonic acid (JA, **6**), as revealed by TLC and LC/MS (Table 1). JA present in all organic phases showed by TLC and HPLC the same R_f of 0.29 and t_R of 4.30 min also when coinjected with a standard commercial sample of racemic jasmonic acid. Moreover, with ESI/MS analysis the corresponding peak showed the expected pseudomolecular ion [M + H]$^+$ at m/z 211. Lastly, JA purified from the ethanolic phase of *L. brasiliense* had spectroscopic properties (^{1}H NMR and ESI/MS) identical to those reported for jasmonic acid (**6**).[14,24] In *L. brasiliense* one more compound was identified that had the same R_f of 0.37 and t_R of 2.76 min of (3*R*,4*S*)-(−)-4-hydroxymellein (**3**) (Table 1) when it was coinjected with the corresponding standard, while with ESI/MS analysis the corresponding peak showed the expected pseudomolecular ion [M + H]$^+$ at m/z 195. The compound had the same physical and spectroscopic (^{1}H NMR and ESI/MS) properties as those previously reported for (−)-(3*R*,4*S*)-(−)-4-hydroxymellein (**3**).[32−34] Thus, the metabolites **1**, **2**, **4**, and **5** were not produced from these *Lasiodiplodia* strains as reported in Table 1.

An aliquot (20 mL) of the culture filtrates of *L. euphorbicola* CMM0181 and *L. hormozganensis* CMM0126 was extracted at unmodified pH (pH 6), and in acidic (pH 2) and basic (pH 9) conditions, and the corresponding organic extracts were assayed on lemon fruits. Both of these were most toxic on lemon when the extraction was carried out at unchanged culture filtrate pH. This also produced the highest yield of the metabolites extracted. The organic extracts of the residual culture filtrates of *L. euphorbicola* (340 mL, 305 mg) and *L. hormozganensis* (240 mL, 325 mg) were purified by combined column and TLC on normal and reverse phases. Only fractions 1, 2, and 4 and fractions 6 and 8, obtained by purification of the above from *L. euphorbicola* and *L. hormozganensis* extracts, respectively, were toxic on lemon. Purification of the extracts allowed four pure metabolites to be isolated from *L. euphorbicola*, and two from *L. hormozganensis*.

The four metabolites isolated from *L. euphorbicola* were identified as (*R*)-(−)-mellein, (3*R*,4*R*)-(−)- and (3*R*,4*S*)-(−)-4-hydroxymellein, and tyrosol (**1−4**, Figure 1) by

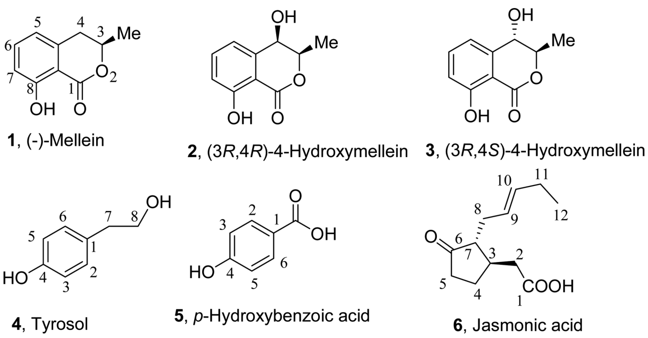

Figure 1. Structure of (−)-mellein, (3*R*,4*R*)- and (3*R*,4*S*)-4-hydroxymellein, and tyrosol (**1−4**) isolated from *Lasiodiplodia euphorbicola* and *p*-hydroxybenzoic acid (**5**) isolated from *L. hormozganensis* together with tyrosol. Structure of jasmonic acid (**6**) produced by the other *Lasioplodia* strains.

comparing their physical ($[\alpha]^{25}_{D}$) and spectroscopic (^{1}H NMR and ESI/MS) properties with those reported in earlier studies.[12,32−39] The two metabolites isolated from *L. hormozganensis* were identified as tyrosol and 4-hydroxybenzoic acid (**5**, Figure 1) by comparing their spectroscopic properties (^{1}H NMR and ESI-MS) with those reported in the literature for **4** (as above) and for **5**,[40] and also by comparison with a commercial standard.

None of the *Lasiodiplodia* strains tested produced scytalone or isosclerone.

DISCUSSION

In this study we reported on the production of secondary metabolites by various species of *Lasiodiplodia* associated with grapevine trunk diseases in Brazil. Four (*L. brasiliense*, *L. crassispora*, *L. jatrophicola*, and *L. pseudotheobromae*) out of six species examined synthesized the well-known plant hormone jasmonic acid (**6**) usually produced in response to wounding caused by insects and necrothrophic microbes.[41,42] Jasmonic acid (**6**) was also isolated from *L. theobromae*[43] and recently also as the main phytotoxin produced by *L. mediterranea*, together with its methyl ester its three new furanonenyl esters named lasiojasmonates A−C.[24] In the same work these authors reported that jasmonic acid (**6**) also caused vein necrosis on detached grapevine leaves. Only *L. brasiliense* and *L. euphorbicola* produced (3*R*,4*S*)-(−)-4-hydroxymellein (**3**). *L. euphorbicola* also synthesized (*R*)-(−)-mellein (**1**) and (3*R*,4*R*-)-(−)-4-hydroxymellein (**2**), which had previously been isolated as toxic metabolites produced by Botryosphaeriaceae species causing grapevine decline, namely, *Neofusiccoccum parvum*,[36,37] *Diplodia seriata*,[42,43] and *Sphaeropsis sapinea*, the

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Figure 2. Decline and cluster wilting in a vine (cultivar Isabel) heavily affected with wood cankers caused by *Lasiodiplodia* species in the Valley of Siriji in Pernambuco, Brazil.

causal agent of pine decline.[34] The phytotoxic effect of (3*R*,4*R*)-(−) and (3*R*,4*S*)-(−)-4-hydroxymellein (**2** and **3**) has previously been assayed on tomato cuttings,[37] on which it produced only a slight wilting, and on grapevine leaves,[35,36] where it caused necrosis.

Melleins are metabolites produced by many fungi in various genera such as *Aspergillus*, *Cryptosporiopsis*, *Hypoxylon*, *Microsphaeropsis*, *Pezicula*, *Phoma*, *Plectophomella*, *Septoria*, and *Xylaria*, which showed different phytotoxic, zootoxic, and moderate antifungal effects.[37] (−)-Mellein (**1**) was toxic on grapevine leaves and grapevine calli.[37,36,44,45] Moreover, on grapevines affected with Botryosphaeria dieback and grapevine leaf stripe disease, (−)-mellein (**1**) was detected in symptomatic and asymptomatic wood samples and in green shoots.[38] Since (−)-mellein (**1**) is produced by many Botryosphaeriaceae species, its role in pathogenesis was investigated by examining the extent to which it caused the expression of defense-related genes in grapevine calli. A significant expression of defense-related genes was recorded only after 6 days, and at a dose of 500 μg mL^{-1}, which is 100 times greater than the amount of (−)-mellein (**1**) produced in vitro by *N. parvum*, calling into question the involvement of (−)-mellein (**1**) in the Botryosphaeria dieback and leaf stripe disease.[46]

Tyrosol (**4**), produced by *L. euphorbicola* and *L. hormozganensis*, was also isolated from *D. seriata*,[44,45] *Neofusicoccum australe*, associated with grapevine cordon and branch dieback,[47] and *N. parvum*.[37] Tyrosol (**4**) not only is toxic to tomato cuttings[37] but also is a quorum sensing molecule in *C. albicans*, controlling growth, morphogenesis, and biofilm formation.[48]

4-Hydroxybenzoic acid (**5**) has been found in the root exudate of grapevine and other plants[49−51] and has more recently been reported as one of the metabolites produced by *Diaporthe gulyae*, a fungal species that has been proposed as a mycoherbicide to control the annual weed *Carthamus lanatus*.[52] 4-Hydroxybenzoic acid (**5**) is toxic to lettuce seedlings[51] and inhibits radish and grain sorghum germination and growth,[50] the growth of *Brassica kaber* roots and hypocotyls,[53] germination of lettuce seeds, and the growth of various seaweeds.[54] It also has a significant autotoxicity effect on tissue culture plantlets, and potted cuttings of grapevine.[49] It is also produced by oil palm plants, in which at high concentrations it inhibits in vitro growth of the fungal pathogen *Ganoderma boninense*.[55]

It is still difficult to link the occurrence of these metabolites in the culture filtrate of *Lasiodiplodia* strains from grapevine to the disease symptoms seen in those vines in the field. The disease reported in general terms as Botryosphaeria dieback[2] also includes *Lasiodiplodia* species as its causal agents. V-shaped cankers are the symptoms most definitely associated with these *Lasiodiplodia* species (mainly *L. theobromae*, but also *L. missouriana*, *L. crassispora*, and *L. viticola*).[24] There are occasional reports of other symptoms: dark streaks, root necrosis, and fruit rot.[2] *L. theobromae* was reported as possibly causing foliar chlorosis, in a vine where colonization was particularly heavy.[10] But all the *Lasiodiplodia* species isolated from grapevine in Brazil reported here were associated with V-shape necrosis that caused a general decline in the vines, and death of the cordon or main branches, but with no specific or characteristic leaf symptoms (Figures 2 and 3). Nevertheless it can be hypothesized that when phytotoxic metabolites are produced, they are translocated to the vine leaves by the plant sap, and cause damage to different components of the cell, affecting the physiology and normal growth of the vine, even if no symptoms become visible other than a decline. This is what happens with *Eutypa lata*.[56] Further study is therefore required to ascertain whether the phytotoxins here isolated from the Brazilian *Lasiodiplodia* strains have a role in the Botryosphaeria dieback of grapevine, relating the production of these phytotoxins in vivo to their activity in planta.

However, the secondary bioactive metabolites produced by phytopathogenic fungi can contribute, together with the traditional taxonomic investigations, to better characterize the biodiversity and systematic of species belonging to the same fungal genus. Our findings extend the knowledge on the

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Figure 3. Trunk portions of infected vines and V-shaped necroses caused by *Lasiodiplodia*.

secondary metabolite pattern of *Lasiosiplodia* spp., which could represent a fingerprint of the studied species. For example, jasmonic acid (**6**) was biosythesized by four (*L. brasiliense*, *L. crassispora*, *L. jatrophicola*, and *L. pseudotheobromae*) of the *Lasiodiplodia* species studied in the present work, as well as by *L. mediterannea*.[24] Also (−)-mellein (**1**) and 4-hydroxymelleins (**2** and **3**) were produced from other species such as *L. brasiliense* and *L. euphorbicola*. On the other hand, specific metabolites occur in a single species only; for example, lasiodiplodins synthesized from *L. theobromae*;[57] jasmonic acid (**6**), its methyl and 4-hydroxymethyl-3,5-dimethyldihydro-2-furanone and 4-(4-hydroxy-3,5-dimethyltetrahydro-furan-2-yloxymethyl)-3,5-dimethyl dihydro-2-furanone esters and 16-*O*-acetylbotryosphaerilactones A and C were produced only from *L. mediterranea*;[24] and *p*-hydroxybenzoic acid (**5**) from *L. hormozganensis* as reported in this work.

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Notes

The authors declare no competing financial interest.

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