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Production of Phytotoxins by *Phoma exigua* var. *exigua*, a Potential Mycoherbicide against Perennial Thistles

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The potential of the different *Phoma exigua* var. *exigua* strains for the biocontrol of the perennial weeds *Sonchus arvensis* and *Cirsium arvense*, occurring throughout temperate regions of the world, has been evaluated in previous studies. The majority of the above strains produced ascosonchine, a newly discovered enol tautomer of 4- pyridylpyruvic acid, whereas strains C-177 and S-9, though virulent to weeds, did not produce the above metabolite. In this study, it was demonstrated that the above two strains, grown in liquid and solid cultures, produced *p*-hydroxybenzaldehyde, cytochalasins B, F, Z2 and Z3, and deoxaphomin. When assayed on the leaves of both *C. arvense* and *S. arvensis*, *p*-hydroxybenzaldehyde was inactive, whereas deoxaphomin demonstrated the highest level of toxicity on leaves of *S. arvensis*. Cytochalasin Z2 appeared to be the less toxic cytochalasan on both plants according to the lack of the secondary hydroxyl group on C-7. Production of cytochalasins by *P. exigua* var. *exigua* strains isolated from *C. arvense* and *S. arvensis* is discussed in relation to chemotaxonomy and the biocontrol potential of the fungus.

KEYWORDS: *Phoma exigua* var. *exigua*; *Ascochyta sonchi*; *Sonchus arvensis*; *Cirsium arvense*; weed biocontrol; phytotoxins; *p*-hydroxybenzaldehyde; cytochalasins; fungal taxonomy

INTRODUCTION

The genus *Phoma* includes many plant pathogenic fungi responsible of severe diseases on many plant species (*1*). They cause lesions on leaves, stems, blossoms, and pods, and discoloration of the hypocotyl, cotyledons, and roots. Some of these pathogens are soil-borne and often persist in or on soil and plant debris. Despite the fact that *P. exigua* is considered an opportunistic parasite of more than 300 plant species, it is continuously being reported as a potential biocontrol agent of different weeds such as *Taraxacum officinalis* (dandelion) (*2*), *Gaultheria shallon* (salal) (*3*), and *Cirsium arvense* (Scop.) (Canada thistle) (*4–6*). Several *Phoma* species were also proposed or/and patented as mycoherbicides for the biological control of noxious weeds. This is the case with *P. herbarum*, which is effective against *T. officinalis* (*7*), *P. destructiva*, and *P. nebulosa*, and with *P. hedericola* against *C. arvense* (*8*), *P. proboscis* against *Convolvulus arvensis* (*9*), and *P. macrostoma* against different dicotyledonous weeds (*10*). A number of *Phoma* species (for instance, *P. lingam*, *P. herbarum*, *P. putaminum*, and *P. macrostoma*) were found to produce phytotoxins, and their involvement in disease symptom induction has been proposed (*11–14*). Phytotoxins were also reported for *P. exigua* var. *exigua* (cytochalasins A and B), the causal agent of potato gangrene (*15*).

In previous studies, several fungi were isolated from the diseased leaves of *C. arvense* and *Sonchus arvensis* (perennial sowthistle) and preliminarily identified as *Ascochyta sonchi* (Sacc.) Grove according to the *Ascochyta* manual (*16*). Other taxonomic studies reclassified *A. sonchi* as a component of the complex species *Phoma exigua* Desm. var. *exigua*, but thistles were not mentioned as hosts (*1, 17*). Their potential for the biological control of these composite perennial weeds widely occurring through the temperate regions of the world (*18, 19*) was evaluated (*20, 21*).

There was an attempt to select more aggressive strains using the main phytotoxin of *P. exigua* (syn. *A. sonchi*) ascosonchine (**1**, **Figure 1**) as a biomarker. However, correlation between ascosonchine production *in vitro* and virulence of the fungus were not observed (*22*). Furthermore, among the nine strains analyzed, two (C-177 and S-9) of them, with medium and high virulence to *C. arvense*, did not produce ascosonchine (*22*).

In this article, (a) the identity of *A. sonchi* strains from *C. arvense* and *S. arvensis* to *P. exigua* var. *exigua* was confirmed

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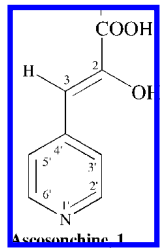

Figure 1. Chemical structure of ascosonchine (**1**).

using additional cultural markers proposed by Boerema et al. (*1*), (b) the isolation, chemical identification, and biological characterization of the toxic metabolites produced by strains C-177 and S-9 are described, and (c) the chemotaxonomy and biocontrol potential of the fungus is discussed.

MATERIALS AND METHODS

Isolation and Characterization of *Phoma exigua* var. *exigua* Strains. Fungi were isolated from necrotic lesions on leaves of both *C. arvense* and *S. arvensis* collected from different geographical places, as shown in **Table 1**, identified as *Ascochyta sonchi* (Sacc.) Grove according to Mel'nik (*16*), and then renamed as *P. exigua* Desm. var. *exigua* (*1*). Fungal strains were maintained on agar slants (PDA) at 5 °C and deposited in the collection of the All-Russian Institute of Plant Protection (St. Petersburg, Russia). For conidial production, the strains were grown on malt extract agar (Difco, Detroit, USA) or oatmeal agar (*23*) at 24 ± 2 °C, first for 4 days in the dark and then for 10 days under alternate near-UV light (14 h light/day). Under these conditions, fungal colonies sporulated abundantly. The conidia were rinsed from the agar slants by adding sterile water (containing 0.01% Tween-20). Spore suspensions were then filtered through cheesecloth and the conidial concentrations were adjusted to 1×10^7 conidia/mL. Measurements, description of fungal colonies, and NaOH spot tests were made using the *Phoma* manual (*1*).

Production of Phytotoxins in Fungal Solid and Liquid Cultures. Two liter Roux bottles containing 300 mL of M-1 D medium (*24*) were inoculated with 0.3 mL of a conidial suspension of the strain C-177 (approximately 10^7 conidia/mL). After 4 weeks of incubation under static conditions at 25 °C in the dark, cultures were filtered and then the liquid phase extracted with EtOAc (3 × 500 mL). The organic extracts were combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure to give an oily residue (101.0 mg). Strains C-177 and S-9 were also grown on autoclaved millet in ten 1000-mL Erlenmeyer flasks (millet 100 g, water 60 mL) for 14 days in the dark. Fungal metabolites were extracted from dry mycelium according to a slightly modified protocol of Evidente et al. (*25*). The dried material (800 g) was extracted with the mixture acetone−water (1:1, 2 L). After evaporation of acetone, NaCl (300 g/L) was added to the aqueous residue, and the latter was extracted with EtOAc (3 × 500 mL). The organic extracts were combined, dried (Na_2SO_4), and evaporated under reduced pressure, yielding brown oily residues of 1.43 g and 305 mg for C-177 and S-9 culture, respectively.

General Chemical Procedures. Optical rotation was measured in MeOH solution on a JASCO (Tokyo, Japan) P-1010; IR spectra were recorded as neat on a Perkin-Elmer (Norwalk, CT, USA) Spectrum One FT-IR Spectrometer, and UV spectra were taken in MeCN solution on a Perkin-Elmer Lambda 25 UV/vis spectrophotometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker (Kalsrhue, Germany) spectrometers at 600 and 400 MHz, and at 150, 100, and 75 MHz, respectively. The same solvent was used as internal standard. Carbon multiplicities were determined by DEPT (distortionless enhancement by polarization transfer) experiments (*26*). DEPT, COSY (correlation spectroscopy)-45, HSQC (heteronuclear single-quantum coherence), and HMBC (heteronuclear multiple bond correlation) experiments (*26*) were performed using Bruker microprograms. Electrospray (ESI) MS were recorded on a Micromass Q-TOF Micro (Milford, MS, USA) instrument. Analytical and preparative TLC were performed on silica gel (Kieselgel 60 F_{254}, 0.25 and 0.50 mm, respectively, Merck, Darmstadt, Germany) plates; the spots were visualized by exposure to UV light and I_2 vapors or by spraying first with 10% H_2SO_4 in methanol and then with 5% phosphomolybdic acid in ethanol, followed by heating at 110 °C for 10 min. Column chromatography (CC) was performed on Kieselgel 60, 0.063−0.200 mm (Merck, Darmstadt, Germany). Pure *p*-hydroxybenzaldehyde was purchased from Merck (Dramstadt, Germany)

Purification and Chemical Characterization of Phytotoxins from Fungal Cultures. The organic extract (1.43 g) obtained from *P. exigua* var. *exigua* strain C-177 solid culture was fractionated by CC eluted with $CHCl_3$-*i*-PrOH (92:8, v/v), yielding 10 groups of homogeneous fractions. The residues of the third (329.8 mg) and fourth (244.0 mg) fraction groups were crystallized separately, twice from EtOAc-*n*-hexane (1:5, v/v) giving white needles of cytochalasin B (220 and 200 mg, respectively, 525 mg/kg). The mother liquors (77.5 and 22.7 mg, respectively) of cytochalasin B crystallization were combined and fractionated by CC eluted with EtOAc-*n*-hexane (6:4, v/v), yielding eight groups of homogeneous fractions. The residue of the second fraction (5.2 mg) showed to be a homogeneous amorphous solid identified as *p*-hydroxybenzaldehyde (EtOAc-*n*-hexane (6:4, v/v), R_f 0.62, 6.5 mg/kg). The residue of the fifth fraction (32.5 mg) of the last column chromatography was fractionated by preparative TLC [eluent petroleum ether/acetone (65:35, v/v)] yielding three groups of fractions. The less polar of these fractions (4.6 mg) was further purified by preparative TLC [eluent EtOAc-*n*-hexane (6:4, v/v)] affording cytochalasin F as a homogeneous amorphous solid (R_f 0.43, 1.4 mg, 1.8 mg/kg). The sixth fraction (8.6 mg) of the last column chromatography was purified by preparative TLC [eluent petroleum ether/acetone (65:35)] yielding deoxaphomin as a homogeneous amorphous solid (R_f 0.31, 4.0 mg, 5.0 mg/kg).

The organic extract (100 mg) obtained from *P. exigua* var. *exigua* strain C-177 liquid culture (1 L of M1-D) was fractionated by CC eluted with $CHCl_3$-*i*-PrOH (92:8, v/v), yielding nine groups of homogeneous fractions. The residue of the second fraction (13.6 mg) was further purified by preparative TLC [eluent EtOAc-*n*-hexane (6:4, v/v)] affording *p*-hydroxybenzaldehyde (R_f 0.62, 1.0 mg) and cytochalasin B (R_f 0.32, 2.2 mg), both as homogeneous amorphous solids.

The organic extracts (305 mg) obtained from *P. exigua* var. *exigua* strain S-9 solid culture was fractionated by CC eluted with $CHCl_3$-*i*-PrOH (92:8, v/v), yielding seven groups of homogeneous fractions. The residue of the first fraction (14.4 mg) was purified by preparative TLC [eluent EtOAc-*n*-hexane (6:4, v/v)] yielding cytochalasin B (R_f 0.32, 4.2 mg). The residues of the second (51.6 mg) and third (83.1 mg) fraction groups of the first column chromatography were crystallized separately twice from EtOAc-*n*-hexane (1:5 v/v) giving white needles of cytochalasin B (32.3 and 61.1 mg, respectively, 122 mg/kg). The mother liquors (18.1 mg) of cytochalasin B crystallization of the third fraction were purified by preparative TLC [eluent EtOAc-*n*-hexane (6:4, v/v)] affording deoxaphomin (R_f 0.26, 2.5 mg, 3.1 mg/kg) as a homogeneous amorphous solid. The residue of the fourth fraction (11.0 mg) of the first column cromatography was purified by preparative TLC [eluent EtOAc-*n*-hexane (6:4, v/v)] yielding cytochalasin Z2 (R_f 0.20, 1.6 mg, 2 mg/kg) as a homogeneous amorphous solid. The fifth fraction (10.2 mg) of the first column was purified by preparative TLC with the same solvent yielding cytochalasin Z3 (R_f 0.11, 1.7 mg, 2.1 mg/kg) as a homogeneous amorphous solid.

Leaf Disk-Puncture Bioassay. The discs of 1 cm in diameter were cut out from well expanded leaves of *C. arvense* and *S. arvensis* grown in a greenhouse. The discs were placed on moistened filter paper in transparent plastic boxes and wounded with a sharp needle in the center. Samples of the toxins were dissolved in MeOH and brought up to a final concentration of 1 mg/mL with distilled H_2O. The concentration of MeOH was 2% v/v, which is nontoxic to leaves of both weeds in the control. A drop of test solution (10 μL) was placed in the leaf disk center. The treated discs were incubated under alternate artificial light and temperature: 8 h in darkness at 20 °C and 16 h under light at 24 °C. After 48 h of incubation, the leaf disk necrotic area was measured.

Table 1. Characterization of *Phoma exigua* var. *exigua* Strains by Growth Rate and Production of Antibiotic E on the Two Diagnostic Media, Malt Extract Agar (MA) and Oat-Meal Agar (OA)

strain	host plant	geographical origin	acosonchine production[a]	colony diameter, mm (7 dpi)		NaOH spot test (E + reaction)	
				MA	OA	MA	OA
S-7	*Sonchus arvensis*	Saint-Petersburg, Russia	+	80.0	55.7	+	+
S-9		Northern Osetia, Russia	−	49.7	58.3	−	+
S-10		Northern Osetia, Russia	+	26.7	36.7	+	−
C-177	*Cirsium arvense*	Oslo, Norway	−	80.0	82.7	−	+
C-180		Northern Osetia, Russia	+	85.0	80.0	+	+
C-182		Northern Osetia, Russia	+	57.0	66.7	+	−
C-208		Saint-Petersburg, Russia	+	84.7	81.7	+	+
C-216		Saint-Petersburg, Russia	+	75.7	76.7	−	+
C-240		Northern Osetia, Russia	+	58.3	80.0	+	+
LSD				8.6	7.0		
Phoma exigua var. *exigua*[b]				(25-) 40−75	(25-) 50−85	+	+

[a] Ref *23*. [b] Ref *1*.

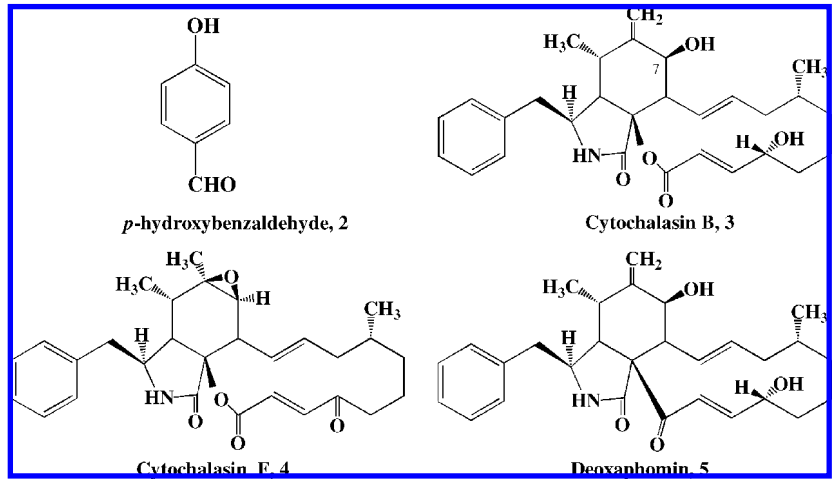

Figure 2. Chemical structure of *p*-hydroxybenzaldehyde (**2**), cytochalasins B and F, and deoxaphomin (**3**, **4**, and **5**) isolated from liquid and solid cultures of *P. exigua* var. *exigua* strain C-177.

RESULTS AND DISCUSSION

Phytotoxin Isolation and Identification. The organic extract (1.14 g/kg of a brown oil) obtained from *P. exigua* var. *exigua* strain C-177 solid culture was fractionated by silica gel column chromatography as reported in detail in the Materials and Methods section. The residues of the third and fourth fractions were combined and crystallized from EtOAc-*n*-hexane and gave the main metabolite. It was identified as the well-known cytochalasin B (**3**, **Figure 2**, 525 mg/kg) by comparing its spectroscopic (^{1}H and ^{13}C NMR and ESI-MS spectra), physical (melting point), and chromatographic behavior [R_f 0.32 by TLC, eluent EtOAc-*n*-hexane (6:4, v/v)] with those of a standard sample (*27*). The mother liquors of the cytochalasin B crystallization were combined and fractionated by a silica gel column as described in detail in the Materials and Methods section. The residue of the second fraction appeared to be a homogeneous amorphous solid. It was identified as the *p*-hydroxybenzaldehyde (**2**, **Figure 2**, 6.5 mg kg^{-1}) by comparing its spectroscopic data (^{1}H and ^{13}C NMR and ESI-MS spectra) and chromatographic behavior [R_f 0.48 by TLC, eluent $CHCl_3$-Me_2CO-AcOH (90:10:0.3, v/v/v)] with those reported in literature (*28*) and with those of a standard commercial sample. The residue of the fifth fraction of the same column was further purified by two TLC steps yielding a homogeneous amorphous solid. It was identified as cytochalasin F (**4**, **Figure 2**, 1.4 mg/kg) by comparing its spectroscopic (^{1}H and ^{13}C NMR and ESI-MS spectra) and chromatographic behavior [R_f 0.43 by TLC, eluent EtOAc-*n*-hexane (6:4, v/v)] with those of a standard sample (*29*). The residue of sixth fraction of the cited column purified by TLC gave a homogeneous amorphous solid. It was identified as deoxaphomin (**5**, **Figure 2**, 5.0 mg/kg) by comparing its spectroscopic (^{1}H and ^{13}C NMR and ESI-MS spectra) and chromatographic behavior [R_f 0.31 by TLC eluent petroleum ether/acetone (65:35, v/v)] with those of a standard sample (*30*).

The ethyl acetate organic extract (101.0 mg/L), obtained by extraction of the culture filtrates of the same strain (C-177) of *P. exigua* var. *exigua*, was fractionated by a silica gel column as reported in detail in the Materials and Methods section. The residue of the second fraction was further purified by preparative TLC yielding *p*-hydroxybenzaldehyde and cytochalisin B (1.0 and 2.2 mg/L, respectively), both as homogeneous amorphous solids.

Finally, the organic extract (377.5 mg/kg) of the solid culture of *P. exigua* var. *exigua* strain S-9, obtained as reported in detail in the Materials and Methods section and cited above for the same solid culture of strain C-177, was fractionated by silica gel column. The residue of the first fraction was purified by TLC giving cytochalasin B as a homogeneous amorphous solid (5.2 mg/kg). The residue of the second and third fractions was independently crystallized from EtOAc-*n*-hexane yielding cytochalasin B as white needles (122 mg/kg). The mother liquors were combined and purified by preparative TLC giving deoxaphomin as a homogeneous amourphous solid (3.1 mg/kg). The residue of the fourth and fifth fractions of the initial column was further purified by TLC giving two homogeneous amorphous solids, which were, in turn, identified as cytochalasins

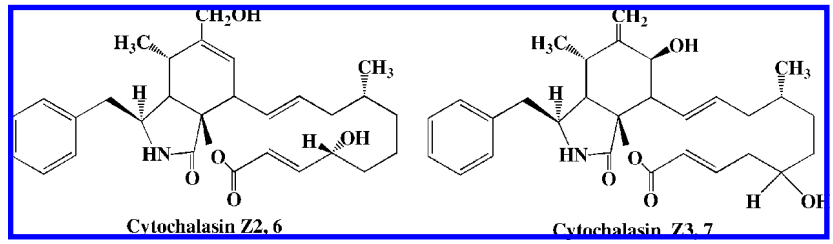

Figure 3. Chemical structure of cytochalasins Z2 and Z3 (**6** and **7**) isolated from a solid culture of *P. exigua* var. *exigua* strain S-9.

Z2 and Z3 (**6** and **7**, **Figure 3**; 2.0 and 2.1 mg/kg, respectively) by comparing their spectroscopic (^{1}H and ^{13}C NMR and ESI-MS spectra) and chromatographic behavior with those of a standard sample (*25*).

The identification of the isolated metabolites was also supported by the extended NMR investigation using bidimensional (COSY, HSQC, and HMBC spectra) techniques.

Some of the isolated cytochalasins (B, F, and deoxaphomin) are well-known metabolites isolated from different fungi (*31*), while cytochalasins Z2 and Z3 were isolated for the first time, together with other well known cytochalasins B, F, T, Z1, and deoxaphomin, from a wheat solid culture of *Pyrenophora semeniperda* (Brittlebank & Adam) Shoemaker, a pathogen proposed as a bioherbicide for the biological control of grass weeds (*25*). These two cytochalasins Z2 and Z3, which showed together with cytochalasin Z1 an original structure between the 24-oxa[14]cytochalasan subgroup, were biologically characterized by testing their capacity to inhibit the germination of wheat and tomato seedlings in comparison to the other above cited cytochalasins and the 21,22-dihydroderivative of cytochalasin B (*25*). Cytochalasins Z2 and Z3 were successively isolated from a solid culture of *Phoma exigua* var. *heteromorpha* (Schulzer et Sacc.) Noordeloos et Boerema, previously reported as *Ascochyta heteromorpha* (Schulzer et Sacc.) Curzi, grown in the same conditions (*32*). *P. exigua* var. *heteromorpha* is the causal agent of a foliar disease of oleander (*Nerium oleander* L.), observed in a nursery near Bari, Italy, during April 1985, and was extensively studied for its capacity to produce phytotoxins in liquid cultures. In fact, many already cited cytochalasins were isolated from this culture filtrate as cytochalasins A, B, 7-*O*-acetylcytochalasin B, cytochalasins F, T, and deoxaphomin as well as new cytochalasins as ascochalasin and cytochalasins U, V, and W. The first two belong to the 25,26-dioxa[16]- and the 25-oxa[15] subgroups of cytochalasans, while cytochalasin W is close to cytochalasins B (*31*). When grown on solid culture, *P. exigua* var. *heteromorpha* showed an increased capacity to synthesized cytochalasins. In fact, cytochalasin B was isolated in a very large yield (2.12 g/kg) together with cytochalasins A, F, T, and 7-*O*-acetyl cytochalasin B, while cytochalasins Z2 and Z3 were isolated in very low amounts (*32*). Three new cytochalasins, named Z4, Z5, and Z6, were successively isolated from the same organic extract and chemically identified as different and novel members of the 24-oxa[14]cytochalasans subgroup (*32*). Their activity was similarly assayed in the inhibition of tomato seedlings (*32*).

p-Hydroxybenzaldehyde was already a known phytotoxic metabolite of fungi pathogenic for important agrarian crops (e.g., apple, stone-leek and onion, and grapewine) (*33–35*) and of *Ceratocystis* spp., associated with blue stain of pine (*36*). It was also isolated as a toxin from a phytopathogenic *Monilia* sp. (*37*) and as a metabolite of *Pythium aphanidermatum*, which is the causal agent of Pythium red blight, a serious disease of bentgrass (*28*).

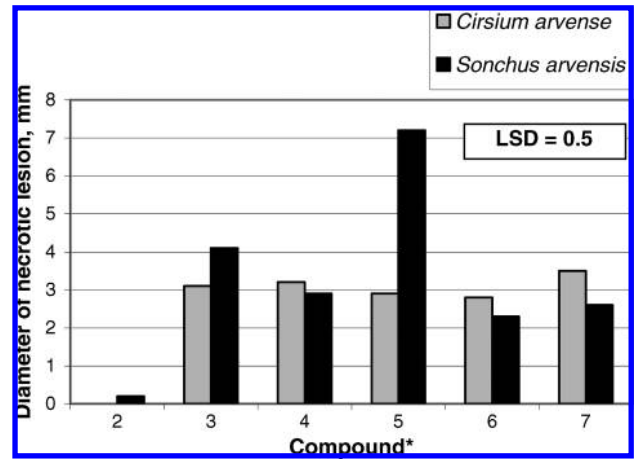

Figure 4. Effect of different toxins on *C. arvense* and *S. arvensis* using a leaf disk-puncture assay. ***2**, *p*-hydroxybenzaldehyde; **3**, cytochalasin B; **4**, cytochalasin F; **5**, deoxaphomin; **6**, cytochalasin Z2; **7**, cytochalasin Z3.

Phytotoxic Activity of Different Fungal Toxins on Leaves of *Cirsium arvense* and *Sonchus arvensis*. *p*-Hydroxybenzaldehyde (**2**, **Figure 2**) and the cytochalasins B, F, Z2, and Z3, and deoxaphomin (**3** and **4**, **Figure 2**; **6** and **7**, **Figure 3**; and **5**, **Figure 2**) were tested on *C. arvense* and *S. arvensis* by leaf disk-puncture bioassay (**Figure 4**). Among the six compounds tested, deoxaphomin (**5**) demonstrated the highest level of toxicity to leaves of *S. arvensis.* Other cytochalasins (**3**−**6**) showed lower activity. On *C. arvense*, all cytochalasins showed a medium level of activity. However, the results obtained assaying the above cited cytochalasins showed that a [13]carbocyclic or a [14]lactonic macrocyclic ring joined with an unalterated perihydroisoindolyl residue is an important feature for phytotoxicity. In this latter moiety, the presence of the secondary hydroxyl group on C-7, which is lacking in **6** and is reversibly transformed in **4**, appears to be an important feature for imparting toxicity. Furthermore, these results are in agreement with those previously described in the structure−activity relationship studies carried out by some of us while testing the phytotoxicity of several cytochalasins and their derivatives on different crop plants (*25, 29, 31, 38*) and recently on the same two weed plants (*39*).

The inactivity recorded with *p*-hydroxybenzaldehyde is in agreement with the lack of inhibitory activity observed toward bentgrass (*28*), but in contrast with the toxicity observed by leaf bioassay on 17 apple cultivars and 8 weed species. Only cultivars Supergold and Silverspur were highly sensitive, while three cultivars showed moderate resistance. Among the weed species, prickly sida (*Sida spinosa*) and morning glory (*Ipomoea*) were very sensitive (*33*). The different effects may be due to the metabolite concentration range used and to the different of plant sensibilities as previously observed for several fungal phytotoxins (*40, 41*).

Characterization of *Phoma exigua* var. *exigua in Vitro.* The strains significantly differed in growth rate ($p < 0.05$) on both diagnostic agar media. However, their 7-day old colony dimensions were in accordance with the description of *P. exigua* var. *exigua* (**Table 1**). No considerable differences in colony morphology were found in all the strains obtained from both *C. arvense* and *S. arvensis*. All the strains demonstrated E^+ reaction (green following by red staining of the agar media) to a drop of 6 N NaOH applied to colony margins (**Table 1**), which is an important species feature of *P. exigua* var. *exigua*. This reaction means the presence of antibiotic E in culture media, which is produced only by this species (*1*). Moreover, a comparison of ITS sequences from our strains of *P. exigua* var. *exigua* with those uploaded in GenBank showed their identity (Múle, G.; Vurro, M., personal communication).

A representative culture of *P. exigua* var. *exigua*, the type species of the section *Phyllostictoides*, genus *Phoma*, was reported to produce both cytochalasins A and B, and antibiotic E (*1, 17*). A strain of *P. herbarum*, which is a type species of the genus *Phoma*, was found to produce cytochalasins C, D, and E (*42*). Furthermore, the isolation of cytochalasins from cultures of *Phoma exigua* var. *heteromorpha* (*31*), *P. multipora* (*43*), and *Phoma* spp. (*44, 45*) demonstrates these metabolites to be typical for some species or their groups form the genus *Phoma*, whereas they were not found at the present time in *Ascochyta* spp. It additionally supports the reclassification of *Ascochyta sonchi*, in particular, strains C-177 and S-9 to *P. exigua* var. *exigua*, which synthesize the above-described cytochalsins (B, F, Z2, Z3, and deoxaphomin) and antibiotic E.

Several authors proposed *P. exigua* var. *exigua*, in particular strain C-177 (*20, 21*), to be a potential mycoherbicide against the Canadian thistle. However, this species was demonstrated to be capable of producing high amounts of known cytochalasins that possess both phytotoxic and cytotoxic activity. The latter activity restricts the usefulness of the fungus as a biocontrol agent.

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