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Fungal phytotoxins with potential herbicidal activity: chemical and biological characterization

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Fungal phytotoxins are secondary metabolites playing an important role in the induction of disease symptoms interfering with host plant physiological processes. Although fungal pathogens represent a heavy constraint for the agrarian production, forest and environmental heritage, they can also represent an ecofriendly alternative to manage weeds. Indeed, the phytotoxins produced by weed pathogenic fungi are an efficient tool to design natural safe bioherbicides. Their use could avoid that of synthetic pesticides causing resistance in the host plants and long term impact of residues in agricultural products with risk for human and animal health. The isolation and structural and biological characterization of phytotoxins produced by fungi pathogenic for weeds including parasitic plants are described. Structure activity relationship and mode of action studies for some phytotoxins are also reported to elucidate the herbicide potential of these promising fungal metabolites.

1 Introduction

Weeds infest economical important crops causing marked losses in the agrarian production, in forest, and ornamental heritage. Weed pests, including parasitic plants, have always being recognised as one of the most serious agricultural and environmental problems due to competition with the growth of agrarian crops and forest plants by subtraction of water, nutrients, light and by the serious obstacles they represents for the agronomic activities.[1-3]

A number of weed management strategies have been followed in agriculture production, including mechanical, cultural, chemical, and biological. The use of soil solarisation (physical method), hand pulling (mechanical method), and crop rotation, delay in the sowing date, the use of catch and trap crops, soil amendments, crop genotypes with better competitive and allelopathic ability (cultural methods), do not provide a satisfactory solution to the weed problems. On the other hands, the use of chemical pesticides differing widely in respect to spectrum, unit activity, crop safety, toxicology, and environmental effects, have increased herbicidal resistance and environmental and toxicological concerns arise a question mark on their large scale use.[1,4] Consequently, many efforts were devoted to develop alternative strategies based on the use of natural products and in particular on the use of fungal phytotoxins alone as natural herbicides and/or in combination with the fungal producers in a more efficient integrated management.[5-7]

Fungal phytotoxins, are secondary metabolites that play an important role in the induction of disease symptoms on agrarian and forest plants and weeds.[8-15] They belong to different classes of naturally occurring compounds as: aromatic, aminoacids, coumarins and isocoumarins, cytochalasans, ethanones, furopyrans, nonenolides, oxazatricycloalkalenones, pyrones,

spirophytotoxins, terpenes, trichothecenes, and some others with a complex and original carbon skeleton.[2,3,10,11]

Phytotoxins produced by microbial plant pathogens known up to the end of 2005 were well reviewed by Strange in 2007,[12] while the use of fungal natural products in research and development were reviewed by Schueffler and Anke in 2014.[15] Also strategic approaches for discovering fungal phytotoxins,[14,16,17] their modes of action,[13,18,19] and the relationships between the stereochemistry and biological activity[20,21] were recently reviewed.

In this review the chemical and biological characterization of fungal phytotoxins is reported focusing on their potential herbicidal activity. It is the first review of this kind and in our opinion it will serve as an excellent source for researchers who are involved in alternative and innovative biological control of weeds, including parasitic plants, in agriculture. It could also attract interest for the potential practical application of promising fungal metabolites as ecofriendly herbicides.

2 Phytotoxins produced by fungi phytopathogenic for weeds

2.1 Non Proteinogenic amino acids

Ascaulitoxin (**1**, Fig. 1, Table 1 ESI) is a new *N*-glucoside of a very unusual *bis*-amino acid, characterized as the N^2-β-D-glucopyranoside of the 2,4,7-triamino-5-hydroxy octandioic acid.[22] Its relative configuration was assigned by NMR analysis, applying for the first time the Murata's method[23] to a compound containing an amino group.[24] **1** was isolated together with its aglycone (letteratura) and another non proteic toxic amino acid characterized as *trans*-4-aminoproline (**2**, Fig. 1, Table 1 ESI)[25] from the culture filtrates of the perthotrophic fungal species *Ascochyta caulina,* which was proposed as mycoherbicides against *Chenopodium album* L.. The absolute

configuration of **2** was determined by a chemical method adopting a well known synthetic procedure[26,27]and the toxin finally identified as (+)-*trans*-4-D-aminoproline. Although the *trans*-4-aminoprolines are well known as synthetic compounds, this represents the first isolation of the (+)-*trans*-4-D-aminoproline as a naturally occurring compound and as a phytotoxin produced by *A. caulina*.

C. album L. (common lambsquarters or fat-hen) is one of the most successful colonizing species[22 28] that is able to grow in all inhabited areas except in extreme desert climates and on all soil types over a wide range of pH values.[29] *C. album* L. has been reported to be a trouble in plantations of sugar beets, potatoes, maize, cereals, and vegetables all over the world.[30] In most crops it is currently controlled by herbicides, but in maize and some vegetable crops the weed is relatively insensitive or has become resistant to them.[31] Biological control using pathogens was considered a suitable option and thus, the post-emergence application of fungal pycnidiospores to young plants causes the appearance of large necrosis on leaves and stems and, depending on the amount of necrosis developed, a retarded growth or death of plants.[32,33]

Assayed at 30 μg droplet^{-1} on punctured leaves of host and non host weeds and cultivated plants, ascaulitoxin (**1**) showed a different degree of phytotoxicity on the tested species. Clear necrosis appeared on some tested weeds (common sowthistle, annual fleabane, noogoora burr and tree of heaven) and on cultivated plants (pea and cucumber) and still clear, but in reduced size, on tomato and redroot pigweed. Compound **1** showed no antimicrobial activity when assayed up to 100 μg per disc on fungi (*Geothricum candidum*) and on bacteria (*Pseudomonas syringae* and *Escherichia coli*).[22] Assayed at 1 mg mL^{-1} on punctured leaves **2** had a severe effect on the host plant, causing the rapid appearance of large necrosis surrounding the puncture point. An interesting aspect is the lack of toxicity when it was assayed on several monocots, both cultivated

(*Triticum durum*, *Avena sativa* and *Hordeum vulgare* L.) and wild (*Phalaris canariensis* L., *Alopecurus myosuroides* and *Avena fatua* L.). The toxin lacked antifungal and antibiotic activities when assayed up to 50 μg $disk^{-1}$ on *G. candidum* and on *P. syringae* and *E. coli*, as already described above for ascaulitoxin (**1**) and had no zootoxicity when tested up to 40 μg mL^{-1} of sea solution on brine shrimp larvae (*Artemia salina*).[25] Finally, the aglycone of **1**, the 2,4,7-triamino-5-hydroxyoctandioic acid (**3**, Fig. 1, Table 1 ESI), was isolated from the mixture of toxins obtained by basic eluate of cation exchange chromatography carried out on the fungal culture filtrates.[34] The three toxins produced by *A. caulina* were used in mixture to enhance the efficacy of this mycoherbicide agent for the biological control of the noxious weed *C. album* L.. Greenhouse experiments showed that the use of toxin solutions (at 1 mg mL^{-1}) in conjunction with spores of *A. caulina* (at 10^6 mL^{-1}) strongly improved the biocontrol efficacy of this fungus by more than 30%. Furthermore, the simultaneous application of toxins or fungal spores, together with low doses of synthetic herbicides (metribuzin and rimsulfuron at 1/5 of the labeled rate), gave better results than single-agent treatments.[35] To optimize and maximize the toxins production by fermentation, a new, simple and inexpensive HPLC method was developed. This method, using a not expensive gel-filtration high density column and a water based solvent,[36] appeared to be more convenient in respect to the other two previously reported to analyze qualitatively and quantitatively the same toxins mixture.[34,37] Such new HPLC method allows rapid evaluation of the toxin content in the fungal culture filtrates and thus easy selection of the best fungal strain to use for toxin large scale production. The simplification and scaling up of the entire process was optimized allowing the fast and easy purification of large volumes of fungal filtrates produced also by a bioreactor and the harvest of large amounts of toxins mixture. The method was proposed as potential for scale up to a pre-industrial level.[36] The mode of action of

the aglycone of **1** was studied on *Lemna paucicostata* by biochemical methods. The results obtained can be explained by three hypotheses: (1) the toxin inhibits one or more aminotransferases not examined, (2) ascaulitoxin aglycone affects amino acid transporters, (3) ascaulitoxin aglycone is a protoxin that is converted *in vivo* to an aminotransferase inhibitor.[38]

The ecotoxicological profile of the toxins produced by *A. caulina* was investigated both on aquatic (algae, *Daphnia*, fish) and terrestrial organisms (earthworms). With the exception of *Daphia magna*, the aquatic organisms were not particularly sensitive. In fact, for algae (*Pseudokirchneriella subcapita*) and fish (*Brachydanio rerio*), according to the GHS (Globally Harmonised System of Classification and Labelling of Chemicals), the mixture of toxins exceeded both class III and class II, respectively, for the acute and chronic aquatic toxicity categories (not harmful). On the other hand, for *D. magna*, the mixture of toxins can be categorized in acute class III and chronic class II, respectively (hazardous to the aquatic environment). Finally, the results obtained in both acute and chronic toxicity tests with *Eisenia foetida* indicated a very low toxicity for terrestrial organisms. In general, the comparison of the ecotoxicological profile of the toxins with other herbicides has shown a lower ecotoxicity for the tested mixture revealing their potential as ecofriendly herbicide.[39]

A new phytotoxic enol tautomer of 4-pyridylpyruvic acid, named ascosonchine (**4**, Fig. 4, Table 1 ESI), was isolated from the culture filtrate of *Ascochyta sonchi*, proposed as a potential biocontrol agent of *Sonchus arvensis* L., a very dangerous perennial weed of important agrarian crops. Ascosonchine (**4**), characterised as (*Z*)-2-hydroxy-3-(4-pyridyl)-2-propenoic acid, showed selective herbicidal properties, not associated with antibacterial, antifungal or zootoxic activities.[40]

Assayed on several weedy and cultivated plants, both monocots and dicots, at 15 mg/droplet, **4** showed interesting selective herbicidal properties, that are not associated with antibacterial, antifungal or zootoxic activities. In fact, **4** was completely ineffective on all the solanaceous species assayed (*Lycopersicon esculentum* L., *Solanum melongena* L., *Caspicum annum* L., *Solanum tuberosum* L.), was slightly active or almost inactive on leguminous (*Phaseolus vulgaris* L. and *Cicer arietinum* L.) and cucurbitaceous (*Cucumis melo* L. and *Cucurbita pepo* L.) plants, but caused severe necrosis on many other species, such as *Euphorbia helioscopia* L., *Salvia officinalis* L., *Valerianella locusta* L., or *T. durum* L.. This semi-selective toxin could have practical applications as a herbicidal compound. It is interesting to note that the toxin is still very active when used at a quite low concentration.

A. sonchi was proposed as mycoherbicide for biocontrol of the perennial weeds *Cirsium arvense* L. and *Sonchus arvensis* L. (Asteraceae), commonly called Canada thistle and perennial sowthistle, respectively. They give rise to widespread problems in crop production and are especially harmful in agricultural systems with reduced herbicide usage due to the ineffectiveness of mechanical weed control. The few herbicides recommended for chemical control of these perennials non-organic cropping systems have low selectivity.[41-43]

2.2 Cytochalasans

Different strains of *A. sonchi*, collected in Russia and Norway, were investigated for their ability to produce **4** by applying an optimized HPLC method. The majority of them produced ascosonchine, whereas two strains, C-177 and S-9, though virulent to weeds, did not produce such toxin. The fungus was re-classified on the basis of epidemiological, morphological and biochemical studies as *Phoma exigua* var. *exigua*.[44] When grown in liquid and solid cultures, they were able to produce *p*-hydroxybenzaldehyde, cytochalasins B, F, Z2 and Z3, and

deoxaphomin (**6**, **7**, **10**, **11** and **12**, Fig. 2, Table 1 ESI). Cytochalasins Z2 and Z3 (**10** and **11**) were isolated for the first time, together with other well known cytochalasins B, F, T, Z1, and deoxaphomin (**6-9** and **12**, Fig. 2, Table 1 ESI) from a wheat solid culture of *Pyrenophora semeniperda*, a pathogen proposed for the biological control of grass weed *Bromus tectorum*, as reported below.[45,46] Cytochalasins are a large group of fungal metabolites produced by several genera of fungi. The first two fungal cytochalasins isolated were cytochalasins A and B (**5** and **6**, Fig. 2) but then sixty different cytochalasins have been purified, identified and grouped in different sub-groups based on the size of the macrocyclic ring and the substituent at C-3 of the perhydroisoindolyl-1-one residue. The structure of cytochalasins, their biosynthesis, and relationships between structure and activity were recently reviewed.[47] The two cytochalasins Z2 (**10)** and Z3 (**11)**, which showed together with cytochalasin Z1 (**9**) an original structure between the 24-oxa[14]cytochalasans subgroup, were biologically characterized by testing their capacity to inhibit the germination of wheat and tomato seedlings in comparison with the other above cited cytochalasins and the 21,22-dihydroderivative of cytochalasin B.[46] Cytochalasins **10** and **11** were later isolated from a solid culture of *P. exigua* var. *heteromorpha*, previously reported as *Ascochyta heteromorpha*, grown in the same conditions.[48] Assayed on the leaves of both *C. arvense* L. and *S. arvensis* L., *p*-hydroxybenzaldehyde was inactive, whereas deoxaphomin demonstrated the highest level of toxicity on leaves of *S. arvensis* L.. Cytochalasin **10** appeared to be the less toxic cytochalasan on both plants due to the lack of the secondary hydroxy group on C-7,[47 44] which in the previous SAR study appeared to be one of the most important feature to impart activity.[45,46]

Four new cytochalasins, named phomachalasins A-D (**13-16**, Fig. 2, Table 1 ESI) were successively isolated from the two above cited strains C-177 and S-9 of *P. exigua* var. *exigua*.

They were characterized as three new closely related 26-oxa[16] and one new [15]cytochalasans belonging to a new subgroup of cytochalasans bearing a 1,2,3,4,6,7-hexasubstituted bicycle[3.2.0]heptene joined to the macrocyclic ring between the C-20 and C-23. Phomachalasin B (**14**) displays one oxygen atom less than phomachalasin A (**13**) because its macrocyclic ring has a carbocyclic nature as in deoxaphomin and lacks of the lactone functionality. Phomachalasins C (**15**) and D (**16**), which are two novel 26-oxa[16]cytochalasans, are diastereomers of **13** for the different stereochemistry of the substituent of the 1,2,3,4,6,7-hexasubstituted bicycloheptene.[49] None of the four new metabolites showed either phytotoxic or antimicrobial activity probably due to the heavy modification of both functionalities and the lower conformational freedom of the macrocyclic ring due to its junction with the bulky and quite rigid new bicycle, namely bicycle[3.2.0]heptene moiety.[49]

As cited above also *P. semeniperda*, proposed as mycoherbicide for biological control of *B. tectorum*, produced cytochalasins and in particular an Australian strain of it produced for the first time the above cited Z1, Z2 and Z3 (**9-11**, Fig. 2, Table 1 ESI) together with the other well known cytochalasins B, F, T, and deoxaphomin (**6-8** and **12**, Fig. 2, Table 1 ESI).[46] *B. tectorum* (cheatgrass, downy brome) is an exotic winter annual grass weed that causes serious losses in intensive agriculture, particularly in winter cereal crops[50,51] and is also a major problem on rangelands in the western U.S.[52] The fungus *P. semeniperda*, a naturally occurring necrotrophic seed pathogen, proposed as a potential biocontrol agent for this weed,[53] is a generalist pathogen with a cosmopolitan distribution.[54,55] The production of all the above cited cytochalasins, except for cytochalasins Z1 (**9**) and Z2 (**10**), was confirmed working with solid wheat seed cultures of ten strains collected from Utah (USA) populations, and a rapid and sensitive HPLC method for quantification of cytochalasin B (**6**) in the organic extracts was also developed.[56]

2.3 Nonenolides

A new nonenolide named stagonolide (**17**, Fig. 3, Table 1 ESI) was characterized using chiroptical, spectroscopic and computational methods.[5] It was the main phytotoxin isolated from pycnidial fungus *Stagonospora cirsii,* a foliar pathogen proposed as mycoherbicide for the biocontrol of *C. arvense* L..[3] Assayed on the host plant, **17** caused first symptoms (necrotic) about 10 h post-application. At 5 x 10^{-3}M, **17** showed no selectivity among host and non host Asteraceae members. Besides *C. arvense* L., *Alcea rugosa* , *Heliantus annuus* L., *Lactuca sativa* L., *S. arvensis* L., and *Mentha piperita* were also highly sensitive to the toxin. However, two Solanaceae species were insensitive. Compound **17** was low toxic to *Colpoda steinii* (Protozoa) while weakly suppressive against the fungus *Candida tropicalis* and bacteria.[57] The same fungus, grown in solid culture, exhibited an increased capacity to produce nonenolides. Eight new nonenolides, named stagonolides B-I (**18-25**, Fig. 3, Table 1 ESI), were isolated together with the well known modiolide A (**26**, Fig. 3, Table 1 ESI), a nonenolide previously isolated from *Paraphaeosphaeria* sp., a fungus separated from the horse mussel.[58,59] They differed from **17** and between them for both the functionalities and the conformational freedom of nonenolide ring. Leaf disk-puncture assays at 1 mg mL^{-1} showed that only stagonolides H-I (**24** and **25**) and modiolide A (**26**) were phytotoxic to *C. arvense* L.. Only **24** inhibited chicory seedling root growth. The most potent toxin, stagonolide H, showed selectivity when tested on leaves of eight different plants. *C. arvense* L. being the most sensitive to the compound.[59]

A novel disubstituted nonenolide named putaminoxin (**28**, Fig. 3), was isolated from *Phoma putaminum*, a mycoherbicide proposed for *Erigeon annuus* L. biocontrol.[60] This is an indigenous weed from North America widely found in fields and pastures all over Europe. Compound **28**

was isolated together with the minor close nonenolides named putaminoxin B-E.[61,62] When it was assayed on leaves of host and non-host plants, only putaminoxin (**28**) and putaminoxin C showed a wide range of toxicity, with leaves of *E. annuus* L. being the most sensitive.[60-62]

A novel phytotoxic trisubstituted nonenolide (**34**, Fig. 3, Table 1 ESI) was isolated from solid cultures of the endophytic fungus *Phomopsis sp*. HCCB03520, together with three known compounds, cytochalasin H, cytochalasin N and epoxycytochalasin H. Compound **34** showed phytotoxic activity on germination and radicle growth of *Medicago sativa* L., *Trifolium hybridum* L., and *Buchloe dactyloides*.[63]

Bioguided fractionation of culture filtrate extracts of *Phoma herbarum* led to the isolation of two new phytotoxic trisubstituted nonenolides named herbarumins I and II (**29** and **30**, Fig. 3 Table 1 ESI), respectively. Herbarumin I (**29**) and II (**30**) caused significant inhibition of radicle growth of seedlings of prince's feather (*Amaranthus hypochondriacus*), and the activity level observed for **29** clearly revealed its potential as a herbicidal agent.[64] Reinvestigation of the same fungus led to the isolation of another monosubstituted nonenolide named herbarumin III (**32**, Fig. 3 Table 1 ESI). Herbarumins I-III interacted with bovine-brain calmodulin and inhibited the activation of the calmodulin-dependent enzyme cAMP phosphodiesterase.[65]

2.4 Oxazatricyclcalkalenones and complex carbon skeleton compounds

Four new oxazatricycloalkenones, named phyllostictines A-D (**35**-**38**, Fig. 4, Table 1 ESI), were isolated from the fungal culture filtrates of *Phyllosticta cirsii*, a fungal pathogen isolated from *C. arvense* L. and proposed as biocontrol agent of this noxious perennial weed. The absolute configuration of the secondary hydroxylated carbon C-15 of phyllostictine A (**35**) was determined by applying an adavanced Mosher's method.[66] **35** was characterized by a

pentasubstituted β-lactam and an hexasubstituted trihydrofurane ring included into a ten membered macrocyclic ring. Phyllostictines B (**36**) and C (**37**) differed from **35** for both the functionalities and ring size, while phyllostictine D (**38**) also diffred for the presence of a tetrasubstituted δ-lactam ring instead of the β-lactam ring.[67] Tested by leaf-puncture assay on the host plant **35** proved to be highly toxic. At first, the phytotoxicity appears to decrease when both the dimension and the conformational freedom of the macrocyclic ring change, as in phyllostictines **36** and **38**, and it is totally lost when also the functionalization of the same ring was heavily modified, as in **37**. Beside its phytotoxic properties, phyllostictine A has no antifungal activity, an interesting antibiotic activity only against Gram + bacteria, and a noticeable zootoxic activity when tested at high concentrations. The integrity of the oxazatricycloalkenone system appears to be an important feature to preserve these activities.[67] Phyllostictines A-D are the first fungal metabolites belonging to the oxazatricycloalkenone group described as natural compounds with interesting biological activities.[67] **35** was further studied to develop a rapid analytical method to estimate its content in culture preparations and to assess the phytotoxic effect for potential weed control on tobacco protoplasts by flow cytometric analysis, and on *C. arvense* L. protoplasts, by fluorescence microscopy.[68]

Further purification of the organic extract of culture filtrates of the same fungus provided two other metabolites, named phyllostoxin and phyllostin (**39** and **40**, Fig. 4, Table 1 ESI), characterized as a new pentasubstituted bicyclo-octatrienylacetic acid ester and a new pentasubstituted hexahydrobenzodioxine carboxylic acid methyl ester, respectively.[69] Tested on punctured *C. arvense* L. leaves, **39** proved to be highly phytotoxic, causing rapid and large necrosis, whereas **40** had no phytotoxicity in this bioassay. These results further support the focused approach of finding novel metabolites with herbicidal properties by looking at the

culture extracts of weed fungal pathogens.[69] From the same fungus was later isolated scytolide (**41**, Fig. 4, Table 1 ESI), a natural analogue of **40** differing for the presence of an exomethylene group instead of the secondary methyl group of the dioxine ring. The same compound was also previously obtained from *Scytalidium uredinicola*.[70] The X-ray crystal structure of **40** was successively determined together with the relative configuration of the four chiral centers C3/C4a/C8/C8a, which resulted to be *R*/*S*/*R*/*S*.[71] The 3*S*,4a*R*,8*S*,8a*R* absolute configuration was assigned to naturally occurring (-)-**40** by computational analysis of its optical rotatory dispersion (ORD), electronic circular dichroism (ECD), and vibrational circular dichroism (VCD) spectra, while 4a*R*,8*S*,8a*R* absolute configuration was assigned to (-)-scytolide only by ECD and VCD, because in this case ORD analysis turned out to be unsuitable for absolute configuration assignment.[72] This study shows that in the case of flexible and complex natural products only a concerted application of more than a single chiroptical technique permits unambiguous assignment of absolute configuration.[72]

Among fungal phytotoxin with an original but complex structure there is also a new phytotoxic trisubstituted naphthofuroazepinone, named drazepinone (**42**, Fig. 4, Table 1 ESI) isolated from the liquid culture of *Drechslera siccans*.[73] *D. siccans* was proposed as mycoherbicide for the control of *Lolium perenne* L., also known as perennial ryegrass, is regarded as an environmental weed in different countries. Natural compounds containing the naphthoazepin skeleton had not been previously reported, and those having furoazepine are described only as synthetic derivatives with important pharmacological activity.[74,75] Therefore, **42** is the first natural compound presenting all three features joined in a new and interesting bioactive fungal metabolite. Assayed at 2 μg μL^{-1} solution the metabolite proved to have broad-spectrum herbicidal properties, without antibacterial and antifungal activities, and low zootoxicity.

Applied to wounded leaves, the toxin caused necrosis on almost all the species tested. Necrosis severity ranged from very wide, as in the case of *Urtica dioica* L., to small ones as those observable applying the toxin to *Setaria viridis* L. and *L. perenne* L. leaves. The necrosis on *E. helioscopia* L. and *Mercurialis annua* L. leaves and *C. album* L. were also interesting.[73]

2.5 Benzochromanones

Two new polycyclic ethanones, named alternethanoxins A and B (**43** and **44**, Fig. 5, Table 1 ESI), were isolated from solid culture of *Alternaria sonchi,* a fungal pathogen isolated from *S. arvensis* L. and proposed as a biocontrol agent of this noxious perennial weed. The *R* absolute configuration at the hemiacetalic C-6 of alternethanoxin A (**43)** was assigned by applying the above cited advanced Mosher's method.[66] Alternethanoxin B (**44)** differed from A (**43)** for the opposite *S* stereochemistry at C-6 and for the presence of a tetrasubstituted furan ring which was joined to the benzochromene ring system. Tested by leaf disk-puncture assay on the fungal host plant and several non host plants, alternethanoxins A and B were shown to be phytotoxic, whereas they did not possess antimicrobial activity up to 100 μg $disk^{-1}$.[76]

Recently, three new polycyclic ethanones, named alternethanoxins C-E (**45**-**47**, Fig. 5, Table 1 ESI), were isolated from the organic extract of the same fungal culture filtrates. Thus, alternethanoxin C (**45**) and D (**46**) appeared closely related to **43** while alternethanoxin **47** to **44** although they showed different functionalities upon the benzochromene ring system, in particular alternethanoxin C showing hydrolytic opening of the hemiacetalic ring.[77] Assayed on leaf segments of *S. arvensis* L. and *E. repens* L., **43** and **45** showed phytotoxic activity inducing notable necrotic lesions. Alternethanoxins C and D possess notable antimicrobial activity when

tested against *Bacillus subtilis* (MIC 10 μg disc^{-1}) and *C. tropicalis* (MIC 25 μg disc^{-1}), while **43** and **44** had low activity against these microbes, and **45** was inactive.[77]

2.6 Spirotoxins

Papyracillic acid (**55**, Fig. 6, Table 1 ESI) is a pentasubstituted 1,6-dioxa-spiro[4,4]non-3-en-one obtained for the first time from *Ascochyta agropyrina* var. *nana* isolated from naturally diseased leaves of *E. repens* L. and proposed as a potential biocontrol pathogen of this species. *E. repens* L. is a widespread perennial weed throughout cold temperate regions all over the world and is managed only by chemical herbicides[78,79] because it easily spreads by seed and rhizomes and produces phytotoxic metabolites suppressing the growth of other plants.[80] **55** is a fungal metabolite already described for its antimicrobial, nematocidal and cytotoxic activities,[81] but not yet for its interesting phytotoxicity.[80] Tested by leaf disk-puncture assay, papyracillic acid at the concentration of 1 mg mL^{-1} was phytotoxic both for the host plant and a number of non host plants. It was active against bacteria (*Xanthomonas campestris* and *B. subtilis*) and the fungus *C. tropicalis* at 6 μg disk^{-1}.

Other interesting spirotoxin were produced growing in liquid culture *P. semeniperda*, the mycohercide proposed for the biological control of *B. tectorum* (see paragraph 2.2). They were identified as a novel spirocyclic γ-lactam, named spirostaphylotrichin W (**54**, Fig. 6, Table 1 ESI), and the well known and closely related spirostaphylotrichins A, C, D, R and V, as well as triticone E (**48**-**50**, **52**, **53** and **51**, Fig. 6, Table 1 ESI). **54** was characterized using essentially spectroscopic methods and comparing its data with those reported for the other above cited spirostaphylothrichin. Its relative configuration was assigned using NOESY experiments and by comparison with data of spirostaphylotrichin V (**53**) and triticone E (**51**). The

spirostaphylotrichins isolated from *P. semeniperda* differed between them for the functionalities and stereochemistry of both the cyclohexenone and γ-lactam rings. In a *B. tectorum* coleoptile bioassay at concentration of 10^{-3} M, spirostaphylotrichin A (**48**) proved to be the most active compound, followed by spirostaphylotrichins C (**49**) and D (**50**). Spirostaphylotrichin W (**54**) and V (**53**) showed mild toxicity while spirostaphylotrichin R (**52**) and **51** were not active. When tested on host and non host plants by leaf puncture bioassay, spirostaphylotrichins **48-50** caused the appearance of necrotic spots while the other compounds were inactive.[82]

The two new phytotoxic napthtoquinones spiroketals palmarumycin EG_1 and preussomerin EG_4 (**56** and **63**, Fig. 6, Table 1) together with the already known preussomerins EG_1-EG_3 (**60-62**, Fig. 6, Table 1 ESI), palmarumycin CP2, CP_{17} and CP_{19} (**57-59**, Fig. 6, Table 1, Fig. 9 ESI), and ergosta-4,6,8(14),22-tetren-3-one, were isolated from the mycelium of the endophytic fungus *Edenia gomezpompae*. Preussomerins and palmarumycins were evaluated for their ability to inhibit the seed germination, root elongation and seedling respiration of *A. hypochondriacus* L., *S. lycopersicum* L. and *E. crusgalli* L. at 100 μg L^{-1}. The compounds exhibited a significant phytotoxicity and were potent on germination and root elongation inhibitors but less active on seedling respiration inhibitors. However, this effect appeared concentration dependent.[83]

The action mechanism on the photosynthesis reactions of preussomerins EG1 (**60**) and EG4 (**63**), as well as palmarumycins CP17 (**58**) and CP2 (**57**) were investigated. They showed to inhibit the ATP synthesis in freshly lysed spinach thylakoids from water to methylviologen (MV), and also to inhibit the non-cyclic electron transport in the basal, phosphorylating and uncoupled conditions from water to MV. Therefore, they act as Hill reaction inhibitors. The results suggested that the four naphthoquinone spiroketals have two interactions and inhibition site on PSII electron transport chain. The first one involves the water splitting enzyme inhibition and the

second one the acceptor site of PSII in a similar way to that of herbicide Diuron, which was studied by polarography and corroborated by fluorescence of the chlorophyll *a* of PSII.[83]

2.7 Aromatic Compounds

Interestingly, when *A. agropyrina* var. *nana* was grown in liquid culture it produced metabolites other than papyracillic acid (**55**), the main of which was characterized as a new 6-monosubstituted salycilic aldehyde, named agropyrenol (**64**, Fig. 7, Table 1). Two other minor metabolites were isolated from the same culture and characterized as a trisubstituted naphthalene carbaldehyde and a pentasubstituted 3*H*-benzofuranone and named agropyrenal and agropyrenone (**65** and **66**, Fig.7, Table 1 ESI), respectively.[84] Assayed on leaves of several weedy plants, i.e. *M. annua* L., *C. album* L. and *S. viridis* L., **64** proved to be phytotoxic, causing the appearance of necrotic lesions, **65** was less active, while **66** was inactive. None of these compounds showed antibiotic, fungicidal or zootoxic activity.[84]

Among the aromatic compounds produced by fungi proposed for the control of weeds there is also the 6-hydroxymellein (**68**, Fig. 7, Table 1 ESI) isolated as reported at paragraph 2.9 from *Phoma chenopodiicola* and proposed for control of *C. album* L.[85]

2.8 Terpenes

Monoterpenes

A new phytotoxic geranylcyclohexentriol, named phomentrioloxin (**75**, Fig. 8, Table 1 ESI),[86] was purified from the liquid culture of a *Phomopsis* sp. isolated from naturally infected *Carthamus lanatus* L. ssp. *lanatus* (saffron thistle plants).[87] *Phomopsis* sp. was proposed in Australia as potential mycoherbicides against the dangerous host plant.[88] In particular, the relative configuration of phomentrioloxin was assigned by X-ray diffractometric analysis which

also supported the structure assigned. The absolute configuration was determined by applying the above cited NMR method[66] to its 1,2-*O,O'*-isopropylidene derivative.

C. lanatus L. is a widespread winter-growing annual weed of both pastures and crops throughout Australia, introduced from the Mediterranean region.[89] It is considered the most economically important thistle species in New South Wales[90,91] and it was one of the weeds targeted by the Australian Cooperative Research Centre for Weed Management Systems.[92] It is declared noxious in all Australian States.[93] Poor results of mechanical[93] and chemical control have made this weed a suitable target for biological control.[90] Recently a teleomorph of the pathogen was classified as *Diaporthe gulyae.*[94]

Tested at different concentrations against host plants of *C. lanatus* L. and some other weeds (*M. annua* L., *C. album* L., *C. arvense* L., *Sonchus oleraceus* L. and *S. viridis* L.), the phomentrioloxin (**75**) caused the appearance of necrotic spots when applied to leaves of both host and non host plants at 6.85 mM. It also causes growth and chlorophyll content reduction of fronds of *Lemna minor* L., and inhibition of tomato rootlet elongation while in preliminary bioassays **75** did not show any antibacterial, fungicidal, or zootoxic activities. Thus, it exhibited only phytotoxic properties and no toxicity to other non target organisms at the tested concentrations.[86]

A more virulent strain of *D. gulyae* showed to produce different new and known metabolites when grown in static liquid culture or in a bioreactor. Two new 1,*O*- and 2,*O*-dehydro derivatives of **75**, named phomentrioloxins B and C (**76** and **77**, Fig. 8, Table 1 ESI) were isolated together with two new α-pyrones (see paragraph 2.9) from its liquid culture filtrates.[95] Phomentrioloxins B (**76**) and C (**77**) showed the same unsaturated geranyl side chain of phomentrioloxin (**75**) but differed for the functionalities of the triolcyclohexene ring. Known

metabolites as 3-nitropropionic, succinic, *p*-hydroxy and *p*-methylbenzoic acids, *p*-hydroxybenzaldehyde, and nectriapyrone were also isolated.[95] Besides nitropropionic acid, the main metabolite responsible for the strong phytotoxicity of the culture filtrates, only **76** proved to cause small but clear necrotic spots on a number of plant species when assayed at 5 mM on punctured leaf disks of weedy and crop plants. All the other compounds were weakly active or inactive.[95]

Sesquiterpenes

Also the already cited pathogen *P. semeniperda* (see paragraph 2.2) produced phytotoxic sesquiterpenes. In fact, in a *B. tectorum* coleoptile bioassay carried out as part of this study, solid culture extracts of the fungus at a concentration equivalent to 10^{-3} M of cytochalasin B (**6**, Fig. 2) exhibited significantly higher toxicity (8-18% of control) than the cytochalasin B standard (34% of control). This suggested the possible presence of other phytotoxic metabolites.[56] Further investigation of the same culture led to isolate a new phytotoxic sesquiterpenoid penta-2,4-dienoic acid, named pyrenophoric acid (**71**, Fig. 8, Table 1 ESI) and characterized as new substituted 3-methylpenta-2,4-dienoic acid.[96] The relative stereochemistry of **71** was assigned using $^{3}J_{H,H}$ couplings NOESY experiments, while its absolute configuration was determined by applying the above cited NMR method.[66] Assayed in a cheatgrass coleoptile elongation test at 10^{-3} M, **71** showed strong phytotoxicity, reducing coleoptile elongation by 51% relative to the control. In a 10^{-4} M mixture, its negative effect on coleoptile elongation was additive with that of cytochalasin B (**6**) demonstrating that the extract toxicity observed in earlier studies was due to the combined action of multiple phytotoxic compounds.[96]

When the fungus was grown on host cheatgrass (*B. tectorum*) seeds, cytochalasins A, B, F and Z3 (**5**-**7**,**11**), as well as deoxaphomin (**12**) and pyrenophoric acid (**71**) were reisolated together

with two new phytotoxic sesquiterpenoid acids, named pyrenophoric acids B and C, and the well known abscisic acid (**72**-**74**, Fig. 8, Table 1 ESI). Pyrenophoric acids B (**72**) and C (**73**) differed from **71** and themselves for the functionalities and stereochemistry of the heptasubstituted cyclohexane ring.

Seedling bioassays with **71** and absisic acid (**74**) demonstrated a hierarchical order of toxicity. All four compounds caused significant 5-day coleoptile and radicle length reductions relative to the control even at the lower concentration, demonstrating their potential role as phytotoxins. Not surprisingly, **74** was by far the most active compound and was able to suppress most germination, to delay the germination of seeds which did germinate, to completely suppress coleoptile elongation, and to greatly reduce radicle length relative to the control. Whereas, among the others only **72** was able to reduce 7-day germination and caused significant germination delay relative to the control. Pyrenophoric acid (**71**) caused more coleoptile growth suppression than pyrenophoric acid C (**73**), but only at the higher concentration. The relative toxicity ranking of the four compounds was therefore abscisic acid >> pyrenophoric acid B > pyrenophoric acid > pyrenophoric acid C. When cytochalasin A (**5**), deoxaphomin (**12**)**,** and cytochalasin F (**7**) were assayed in comparison with cytochalasin B (**6**) on 5-day coleoptile and radicle length at both concentrations (10^{-3} and 10^{-4}M) relative to the control, clearly demonstrated that their toxicity was no additive. When **71** was combined in pairwise tests with each cytochalasin, a synergistic interaction increased toxicity to seedling coleoptiles, especially with **6**, but this synergistic growth suppression effect was not observed for radicles.[46,97]

A new eremophilane-type sesquiterpene, characterized as (3*R*,6*R*)-dihydroxy-9,7(11)-dien-8-oxoeremophilane along with three known analogues, namely, isopetasol, sporogen AO-1 and dihydrosporogen AO-1 (**94** and **95**, Fig. 8, Table 1 ESI), were isolated by bioguided fractionation

of an extract from the solid culture of the coprophilous fungus *Penicillium sp.* G1-a14. Sporogen AO-1 (**94**) and dihydrosporogen AO-1 (**95**) caused significant inhibition of radicle growth against *A. hypochondriacus* (IC_{50} = 0.17 μM for both compounds) and *E. crusgalli* L.(IC_{50} = 0.17 and 0.30 μM, respectively).[98]

Diterpenes

Recently, the fungus *P. chenopodiicola* was proposed as a potential mycoherbicide for the control of *C. album* L. for which was also previously proposed *A. caulina* (see paragraph 2.1). A new phytotoxic unrearranged *ent*-pimaradiene diterpene, named chenopodolin (**70**, Fig. 8, Table 1 ESI), was isolated from the fungal liquid cultures. At a concentration of 2 mg mL^{-1}, the toxin caused necrotic lesions on *M. annua* L., *C. arvense* L., and *S. viride* L.[99]

Sesterterpenes

Drechslera gigantea, isolated in Florida from naturally infected large crabgrass (*Digitaria sanguinalis* L.), showed to produce phytotoxic metabolites in liquid cultures, the main of which being ophiobolin A (**86**, Fig. 8, Table 1 ESI).[100] *Digitaria sanguinalis* is one of the better-known species of the genus *Digitaria* and it is known nearly worldwide as a common weed. It is known by several common names and used as animal fodder. The seeds are edible and used as a grain in Germany and especially Poland, where it is sometimes cultivated.[101] *D. gigantea* is a cosmopolitan fungal pathogen found throughout North and South America, Japan, and other regions.[102,103] Chandramohan and Charudattan (2001)[83 104] and Chandramohan et al. (2002)[84 105] have shown that *D. gigantea* alone and in combination with two other grass pathogens, *Exserohilum longirostratum* and *Exserohilum rostratum*, could be proposed in a bioherbicide

cocktail to control seven different weedy grasses. Ophiobolin A (**86**) is a well known phytotoxic sesterterpene produced by several phytopathogenic fungi of important crops including rice, barley and other cereals, actually all grouped in *Bipolaris genus*. **86** was extensively studied for its several interesting biological activities and its mode of action including its receptor calmodulin, a protein which preserve its structure in different organisms.[106] In fact, ophiobolin A (**86**) inhibited the calmodulin-dependent cyclic nucleotide phosphodiesterase activity with an IC_{50} of 9 μM.[107] The proposed mechanism of action of **86** provided for its reaction with the ε-amino group of lysine residue in calmodulin, giving rise to a conjugated enamine, formed via tautomeric rearrangement of an initially formed Schiff base.[108] The other three minor metabolites isolated proved to be related to **86** and were identified as 6-*epi*-ophiobolin A, 3-anhydro-6-*epi*-ophiobolin A and ophiobolin I[100] (**87**, **88**, **90**, Fig. 8, Table 1 ESI). The first two differed from **86** for the stereochemistry at C-6 of the dicyclopentaoctatricyclic system and the second one also for the dehydratation of the hydroxy group at C-3 and the formation of the double bond between C-3 and C-4, leading to the corresponding α,β-unsaturated lactone. Ophiobolin I (**90**) showed also the reduction of the aldehyde group at C-7 of the octacyclic ring. Assayed on punctured detached leaves of several grass and dicotyledonous weeds, **86** proved to be on average more phytotoxic than the other related compounds.[100] These results demonstrated that structural features important for the phytotoxicity are the hydroxy group at C-3, the stereochemistry at C-6, and the aldehyde group at C-7. The structure of ophiobolin A (**86**) was also confirmed by direct X-ray analysis to better understand the role of the stereochemistry of its carbotricyclic ring system in the affinity with the receptors and the changes in the stereochemistry induced by the modifications of the functional groups.[109] Successively, a new sesterterpene, named ophiobolin E (**91**, Fig.8, Table 1 ESI) was isolated from the same cultures. From the solid culture of the same

fungus, the already known ophiobolins B and J (**89** and **92**, Fig. 8, Table 1 ESI), were isolated together with the new closely related 8-*epi*-ophiobolin J[110] (**93**, Fig. 8, Table 1 ESI). Ophiobolin B (**89**) differed from A (**86**) for the reductive opening of the furane ring while ophiobolin E (**91**) showed the conversion of the cyclopentane ring joined to the furane ring into a cyclopentandiene joined to a dihydropyrane ring. Ophiobolin J (**92**) differed from I (**90**) for the shift of the double bond of the octacyclic ring from C-7-C-8 to C-7-C-6 with the hydroxylation of the C-8 which is epimerized into 8-*epi*-ophiobolin J (**93**). Tested at the concentration of 0.5 mg mL^{-1} on four weedy plants using the leaf-puncture assay, only **89** and **92** proved to be toxic, whereas the two new ophiobolin **91** and **93**, appeared to be inactive on all the tested plant species. In particular, **89** was highly toxic to *Bromus sp.* and *Hordeum marinum* leaves, but less toxic to other two weed species tested, namely *Avena sterilis* L. and *Oryzopsis miliacea* L.. The same range of toxicity, but at a lower level, was observed for **92**.[110] The different levels of phytotoxicity shown by the two **92** and **91** could be attributed to the different conformation assumed by the octacyclic ring, as a consequence of the different position of the double bond, located between C-7 and C-8 in **92**, and between C-6 and C-7 in **90**. Probably, when present, the epimerization of the hydroxy group of C-8, observed for the first time in ophiobolin 8-*epi* J (**93**), imparts the total loss of the activity. The noteworthy structural difference present in ophiobolin E (**91**) could justify the observed inactivity on the tested plants.[110]

2.9 α-Pyrones

Two new phytotoxic trisubstituted α-pyrones, named gulypyrones A and B (**96** and **97**, Fig. 9, Table 1 ESI), were recently isolated from the liquid culture filtrates of *D. gulyae*, the fungus reported above (see paragraph 2.8) for the biocontrol of *C. lanatus* L., together with the already cited monoterpenes phomentrioloxin and phomentrioloxins B and C (**75-77**, Fig. 8). Gulypyrones

A (**96**) and B (**97**) differed between them and from nectriapyrone, an α-pyrone previously isolated as the main phytotoxin produced by *Pestalotiopsis guepinii* pathogen of halzenut,[111] for the functionalities of the α-pyrone ring and, in particular, for the side chain linked to the oxygenated carbon involved in the lactone bond. Assayed at 5 mM on punctured leaf disks of weedy and crop plants, **96** caused leaf necrosis on *H. annuus* L. plantlets while **97** was inactive.[95] The *S* absolute configuration of the hydroxylated secondary carbon of the 2-hydroxy-1-methylpropyl side chain at C-6 of **96** was determined by applying the above cited NMR method.[66]

2.10 Furopyranes

Further investigations of the same organic extract of the above cited pathogen *P. chenopodiicola* led to isolate three new tetrasubstituted furopyrans, named chenopodolans A-C (**98**-**100**, Fig. 10, Table 1 ESI), and a well known fungal metabolite identified as the (-)-(*R*)-6-hydroxymellein (**68**, Fig. 7, Table 1 ESI). The *R* absolute configuration of the hydroxylated secondary carbon of the side chain of chenopodolan A was determined by applying the above cited NMR method.[66] Assayed by leaf puncture on host and non host weeds chenopodolans A (**98**) and B (**99**), and the 11-*O*-acetylchenopodolan A showed a strong phytotoxicity. Chenopodolan C (**100**) was inactive. These results showed that the nature of the side chain is an important feature for their phytotoxicity.[85]

3 Miscellaneous toxins

Tenuazonic acid (**101**, Fig. 11, Table 1 ESI) is a natural tetramic acid derivative isolated from several phytopathogenic fungi species of *Phoma*, *Pyricularia* and *Alternaria*, particularly from

Alternaria alternata, *A. longipes* and *A tenuissima*.[112-115] It reduces root and shoot length of seedlings.[116-118] Qiang et al.[119] found that **101** produced by *A. alternata* isolated from the invasive plant croftonweed (*Ageratina adenophora*) is highly phytotoxic to a broad spectrum of plants. Tenuazonic acid (**101**) causes a brown leaf spot disease in many plants, and then quickly kills the seedlings of monocotyledon and dicotyledon weeds, including *A. adenophora*. Mode of action studies revealed **101** is a novel natural photosynthesis inhibitor that mainly interrupts photosystem II (PSII) electron transport beyond primary quinone acceptor by competing with secondary quinone acceptor (QB) for the QB-niche in the D1 protein.[120] It causes plant cell necrosis by direct oxidative damage through the chloroplast-derived reactive oxygen species (ROS) burst produced by the inhibition of photosynthetic electron transport.[121] Tenuazonic acid (**101**) was patented given its unusual herbicidal activity and its structural simplicity.[122] Recently, its effect as photosynthetic inhibitor *in vivo* was evaluated by the JIP-test based on fast chlorophyll *a* fluorescence transient kinetics, to determine whether differential binding to the target protein D1 in PSII is a tolerance mechanism in some plants.[120]

Cyperin (**102**, Fig. 11, Table 1 ESI) is a natural diphenyl ether phytotoxin produced by several fungal plant pathogens such as *Preussia fleischhakii*, *Phoma sorghina*, and *Ascochyta cypericola*,[123-125]. The latter two species are pathogens of purple nutsedge (*Cyperus rotundus* L.) and pokeweed (*Phytolacca Americana* L.), respectively. **102** showed to be phytotoxic to *C. rotundus* and several other plant species in a detached leaf assay.[124,125] It was also phytotoxic to *Arabidopsis thaliana* L. and inhibited growth in a dose-dependent manner, with an IC_{50} of 38.4 μM. The effect was most evident on root length, but the leaves were also small and chlorotic at the higher concentrations.[126] Due to its structural similarities with diphenyl ether herbicides (i.e. acifluorfen and oxyfluorfen) its mode of action was studied and revealed that at high

concentrations, this metabolite inhibits protoporphyrinogen oxidase, a key enzyme in porphyrin synthesis. Cyperin (**102**) action is not light dependent, causing loss of membrane integrity in the dark. Dayan et al., 2008[127] report that **102** inhibits Arabidopsis (*A. thaliana* L.) enoyl (acyl carrier protein) reductase (ENR) as the more potent triclosan, a synthetic antimicrobial diphenyl ether. **102** and triclosan were stabilized by the π–π stacking interaction between one of their phenyl rings and the nicotinamide ring of the NAD^+. Moreover the side chain of tyrosine seems involved in hydrogen bonding with a phenolic hydroxy group of **102**, thus contributing to the virulence of the pathogens by inhibiting ENR and destabilizing the membrane integrity of the cells surrounding the point of infection.[127]

A new polyketide derivative *O*-methylated SMA93 (**103**, Fig. 11, Table 1 ESI) and five known compounds SMA93, rhodolamprometrin, radicin (**104**, Fig. 11, Table 1 ESI), dehydroallogibberic acid, and 3-methyl-6,8-dihydroxyisocoumarin were isolated by bioguided fractionation of the ethyl acetate extract of the culture filtrate of *Fusarium proliferatum* ZS07, a fungus residing in the gut of long-horned grasshoppers (*Tettigonia chinensis*). Assayed on the radicle growth of *A. retroflexus* seeds *O*-methylated SMA93 (**103**) and radicin (**104**) showed good phytotoxic activity in the concentration of 100 μg mL^{-1}, with inhibition rates of 83.0 and 65.2%, respectively.[128]

Recently, a new 1,4-disubstituted pentanone (**105**, Fig. 11,Table 1 ESI) was isolated from culture filtrate of *P. herbarum* FGCC#54 and exhibited high phytotoxicity against *Parthenium hysterophorus* L.*,* followed by *Lantana camara* L.*, Hyptis suaveolens* L. and *Sida acuta* detached leaves.[129]

The two main phytotoxic and antifungal phthalic acid butyl isobutyl ester (**106**, Fig. 11, Table 1 ESI) and the known radicin (**104**) were isolated from the culture of *Curvularia sp*. FH01, a

fungus residing in the *Atractomorpha sinensis*. The two metabolites exhibited significant phytotoxic activity against the radical growth of *E. crusgalli* L. with their IC_{50} values of 61.9 and 5.9 μg mL^{-1}, respectively, which were comparable to that of 2,4-dichlorophenoxyacetic acid (2.0 μg mL^{-1}) used as a positive control.[130]

Vulculic acid (**107**, Fig. 11, Table 1 ESI) was isolated as the main phytotoxic metabolites from the fungus *Nimbya* (=*Alternaria*) *alternantherae*, discovered and confirmed to be highly damaging to alligatorweed (*Alternanthera philoxeroides*), an agressive weed in many part of the world.[131] When it was isolated for the first time from *Penicillium* sp., it was characterized as 2-acetyl-3,4-dihydroxy-5-methoxyphenyl-acetic acid. Several isolates of *Nimbya alternantherae* from Brazil, USA, and Puerto Rico were compared and no differences in virulence were observed, although a lower dew requirement was demonstrated for the Brazilian isolates. In a host-range study, *N. alternantherae* infected 6 plant species from a total of 42 species belonging to 23 families.[132,133]

The mode of action of vulculic acid (**107**) on the photosynthetic apparatus of *Alternanthera philoxeroides*, was recently investigated via the photochemical activity and SDS-PAGE of protein on thylakoid membranes, fast chlorophyll *a* fluorescence transient measurements, and the JIP-test.[134] The results demostrated that **107** is a photosynthetic inhibitor with multiple action sites. The main targets are the light harvesting complex and the oxygen evolving complex on the PSII donor side. Compound **107** blocks electron transport beyond QA and on the PSI acceptor side by digesting major PSI and PSII proteins.[134]

4 Phytotoxins produced by fungi for the control of parasitic plants

Orobanche and *Phelipanche* species (the broomrapes) are obligate root parasitic plants, some of which represent serious weed problems causing severe yield reduction of many important crops.[4,135-139] A parasitic plant derives some or all of its nutritional requirements from another living plant. They germinate only by stimulation with host root exudates and produce a germ tube that, when attaches to the host root, develops a haustorium penetrating the root and forms a tubercle. This is followed by the most damaging phase, with the parasitic withdrawal of water, nutrients and photosynthates from the host due to the long underground phase. Plant emergence occurs only when most of the damage has already been produced.[4,138]

Difficulties in controlling parasitic weeds are due to their physiological traits and life cycle.[4,137] Preventing early growth stages, such as seed germination, host attachment, and tubercle development, could be a strategy to interfere successfully with the parasite, resulting in its management. Taking into account that the germination of parasitic plants seeds depends on the presence of chemical signals exuded by the roots of the host plant in the nearby, an alternative approach for the management of parasitic weeds is the so called ‘‘suicidal germination’’. This latter consists in the induction of seed germination by the application of a fungal metabolite to the soil, mimicking a host germination stimulant but in absence of the host plant, so that the seeds will die resulting in a reduction of the soil seed bank.[140] Another strategy is the application of fungal phytotoxins with hormone-like activity which could inhibit parasitic seed germination or germ tube elongation, so preventing their attachment to the host plant.[7]

4.1 Phytotoxins inhibiting seed germination

Orobanche ramosa is a widespread parasitic weed of many Solanaceae species, such as tobacco or tomato, legumes, cabbage etc., and parasites many other species, including ornamentals and

weeds. It is distributed mainly in the Mediterranean area, central Europe, northern Africa, and the Middle East.[141] It is responsible for both qualitative and quantitative damage to crops by interfering with water and mineral intake and by affecting photosynthate partitioning

4.1.1 Trichotechenes

Seven compounds were isolated from *Myrothecium verrucaria* identified as verrucarins A, B, M and L acetate, roridin A, isotrichoverrin B and trichoverrol B (**108**-**114**, Fig. 12, Table 1 ESI) together with the main metabolite identified as verrucarin E, a disubstituted pyrrole not belonging to the trichothecene group. Neosoloaniol monoacetate (**115**, Table 1, Fig. 12 ESI), was the main metabolite produced by *Fusarium compactum*. Both *M. verucaria* and *F. compactum* were isolated from diseased *O. ramosa* plants collected in southern Italy to find potential biocontrol agents of this parasitic weed. Both fungi grown in liquid culture produced metabolites that inhibited the germination of *O. ramosa* seeds at 1-10 μM. All the trichothecenes proved to be potent inhibitors of *O. ramosa* seed germination and possess strong zootoxic activity when assayed on *A. salina* brine shrimps. Verrucarin E is inactive on both seed germination and zootoxic assay. The trichothecenes are a family of tetracyclic sesquiterpenoid substances produced by several fungal species. More than 100 compounds are known and cause a wide variety of biological effects owing to the diversity of chemical structures within the group. Although inhibition of seed germination of many plant species (i.e., broccoli, carrot, radish, and turnip) by macrocyclic trichothecenes has already been reported),[142,143] this was the first report of the inhibitory effect of these metabolites to parasitic plant seeds. All the compounds assayed showed the presence of an epoxy group, which plays an important role in the biological activity of some classes of naturally occurring compounds.[144,145] However, these toxins are powerful mammalian mycotoxins, and the risks of introducing mammalian toxic compounds into the

environment must be carefully determined as well as the "real" fate of those metabolites in the soil. Many toxins are not selective, being able to cause the same toxic effects both on host and non host plants. For this reason, even the toxicity to crop plants has to be determined. Considering the efficacy of some phytoxins at very low concentrations (10^{-6}-10^{-7}M), their quick degradation after inhibition of seed germination could avoid toxic effects. From a practical point of view, the toxins could be introduced by drip irrigation systems, in very low amounts near the host roots as most of the crop plants parasitized by broomrape are irrigated. This should minimize environmental risks, reducing the amount applied and avoiding toxin dispersal.[146]

4.1.2 Miscelleneous fungal metabolites

Other metabolites isolated from *Fusarium* spp. together with ophiobolin A (**86**, Fig. 8) and phyllostictine A (**35**, Fig. 4) were than tested on the germination of *O. ramosa* seeds.[7] Seven out of the 18 already known toxins assayed caused 100% inhibition of seed germination when tested at 100 μM. At 10 μM the seven most active metabolites were still highly active, causing the complete inhibition of germination, whereas the other compounds had negligible or no effects. The strongest toxins, neosolaniol and diacetoxyscirpenol (**115** and **117**, Fig. 12, Table 1 ESI) and T-2, HT-2 (**118** and **119**, Fig. 12, Table 1 ESI), caused almost complete inhibition of seed germination at 1 μM. Some of the latter were still very active at 0.1 μM, causing complete failure of germination and suggesting that they are true inhibitors. This was further supported by the observation that toxin removal by seed washing did not result in germination. Roridin A (**112**, Fig. 12) and neosolaniol monoacetate (**116**, Fig. 12) were both able to cause 100% inhibition of seed germination at 1 μM. Ophiobolin A (**86**, Fig. 8) was very efficacious, causing 100% inhibition when tested at 100 μM. Its efficacy was much lower at10 μM (around 10%), and nil at

even lower concentrations. Phyllostictine A (**35**, Fig. 4) caused around 28% inhibition of germination at 100 μM, whereas at lower concentrations its effect was almost negligible or nil.[7]

Ten phytotoxins were tested against *Cuscuta campestris* (dodder) at concentrations below 1 mM: phyllostictine A (**35**, Fig. 4), ophiobolin A (**86**, Fig. 8), fusicoccin A (**78**, Fig. 8, Table 1 ESI) and five of its derivatives (**79**, **82**-**85**, Fig. 8, Table 1 ESI) (for detail on fusicoccin source and isolation see below). Only dideacetylfusicoccin (**79**, Fig. 8) significantly reduced the percentage of dodder seed germination compared with the control (around 28% reduction). Four toxins, **86**, **78** and two of its derivatives (**84** and **85**, Fig. 8), greatly inhibited growth of the parasite seedlings. Assayed at ten-fold lower concentration, **86** was still very active, causing almost total inhibition of the seedling growth, whereas at 100-fold dilution it had a lower effect. Besides their effect in reducing the seedling length of the parasite, the three toxins ophiobolin A (**86**), fusicoccin A (**78**), and dideacetylfusicoccin (**79**) caused necrosis and browning of almost all the treated seedlings, leading to their death.[7]

4.2 Phytotoxins for 'suicidal' germination

4.2.1 Diterpenes

Fusicoccin A (**78**, Fig. 8), the major toxin of *Fusicoccum amygdali* [147,148] the causative fungal agent of peach and almond canker, is able, together with its deacetyl aglycone (**82**, Fig. 8) and the structurally related compound cotylenin A and its aglycone (cotylenol, the aglycone of all cotylenins) (**80** and **81**, Fig. 8, Table 1 ESI), produced by *Chladiosporum* spp. 507-7w,[149-152] to induce seed germination of *Striga hermonthica* and *O. minor*.[153] A SAR study was carried out, testing 25 compounds at 10 and 100 μM: fusicoccin A (**78**), its aglycone (**82**), several fusicoccin A derivatives, and natural analogues and cotylenol. Some natural fusicoccin A analogues and derivatives were more active than **78**, and the stimulatory properties were modulated by chemical

modifications, essentially in the functionalities and/or the conformation of the carbotricyclic diterpenoid ring. Among the glucosides, the most active compound was dideacetylfusicoccin A (**79**), which could be of practical interest because it can be easily prepared and in high yields by fusicoccin A (**78**). The importance of the presence of a free primary hydroxy group at C-19 was evident. The same SAR were observed testing the fusicoccin aglycones. Among these, the most active (58%) proved to be the isopropylidene derivative **83**, followed by the fusicoccin aglycone (16%). In fact, both compounds have a free hydroxygroup at C-19.[140] The effect of some fusicoccin A derivatives and ophiobolin A (**86**) (Fig. 8) was also evaluated on seed germination of nine different *Orobanche* species, namely, *O. aegyptiaca*, *O. ramosa*, *O. crenata*, *O. cumana*, *O. densiflora*, *O. foetida*, *O. gracilis*, *O. hederae*, and *O. minor*. The results obtained showed that the stimulation of seed germination was species-dependent and also affected by the concentration of the stimulant. Among **86**, **78**, and its seven derivatives, tested in the concentration range of 10^{-4}-10^{-7} M, the highest stimulatory effect was observed for **86** and the hexacetyl and pentacetyl isomers of 16-*O*-demethyl-de-*tert*-pentenylfusicoccin A (**84** and **85**, Fig. 8) prepared by chemical modification of the fusicoccin A, while the other fusicoccin derivatives appeared to be essentially inactive. The most sensitive species appeared to be *O. aegyptica*, *O. cumana*, *O. minor*, and to a lesser extent, *O. ramosa*.[154]

4.2.2 Miscellaneous toxins: oxazatricyclic alkalenones, cyclohexenepoxides, benzofuranes, nonenolides, chalcones and chromanones, cytochalasans, diterpenes

The effect of metabolites belonging to different classes of natural compounds on broomrape seed germination and radicle development (on *O. crenata*, *O. cumana*, *O. minor*, and *P. ramosa*) was recently assayed *in vitro*.[139] The diterpene pimamaradienes sphaeropsidin A (**69**, Fig.8, Table 1

ESI), and chenopodolin (**70**, Fig. 8), the cyclohexenepoxides sphaerospidone, *epi*-sphaeropsidone and *epi*-epoformin, (**120-122**, Fig. 13, Table 1 ESI) and the pentasubstituted benzofuranone cyclopaldic acid (**67**, Fig. 7, Table 1 ESI) were purified from the culture filtrates of *Diplodia cupressi*[155,156] *P. chenopodiicola*,[99] *Diplodia quercivora*,[157] and *Seiridium cupressi*,[158] respectively. The nonenolides pinolidoxin (**27**, Fig. 3, Table1 ESI), pinolide (**33**, Fig. 3, Table 1 ESI), herbarumin II (**30**, fig. 3), 2-*epi*-herbarumin II (**31**, Fig. 3, Table 1 ESI), and putaminoxin (**28**, Fig. 3, Table 1 ESI) were isolated from the culture filtrates of *Didymella pinodes*[159] and *P. putaminum*.[60] The chalcone and chromanone cavoxin and cavoxone (**123** and **124**, Fig. 14, Table 1 ESI), chenopodolan C (**100**, Fig. 10), and 6-hydroxymellein (**68**, Fig. 7), were isolated from the culture filtrates of *Phoma cava*[160] and *P. chenopodiicola*, respectively.[85] Cytochalasins A and B, and deoxaphomin, (**5**, **6** and **12**, Fig. 2) were isolated from the solid culture of *P. seminiperda.*[46]

Among the metabolites tested, *epi*-sphaeropsidone (**121**) and cyclopaldic acid (**67**) induced broomrape germination in a species-specific manner. *epi*-Epoformin (**122**), sphaeropsidin A (**69**) and cytochalasans (**5**,**6,12**) inhibited germination of GR24-treated broomrape seeds. The growth of broomrape radicle was strongly inhibited by **69** and compounds belonging to cyclohexene epoxide and cytochalasan classes. Broomrape radicles treated with **121** developed a layer of papillae hampering the contact of the parasite to the host, while radicles treated with cytochalasans or with **69** turned necrotic.[139]

5 Structure activity relationship (SAR) studies on promising phytotoxins

5.1 Chenopodolin SAR study

Five key derivatives were prepared by chemical modification of chenopodolin (**70**) functionalities to carry out a SAR study, assaying their phytotoxic and antimicrobial activities compared to the parent toxin.

By routine acetylation, **70** was converted into the 3-*O*-acetyl derivative (**125**, Fig. 15) while treated with diluted ammonia, into the corresponding 1-*O*-deacetyl derivative (**126**, Fig. 15). By reaction with *p*-I-benzosulphonyl chloride in dry methylene chloride and triethylamine, **70** was converted into the corresponding ester **127** while by catalytic hydrogenation it was converted in its 6,*O*,15,16-tetrahydro- and 6,*O*,7,8,15,16-hexahydro-derivatives (**128** and **129**, Fig. 15). Applied by puncture to detached leaves of *M. annua* L., *C. arvense* L., and *S. viride* L., only 1-*O*-deacetyl derivatives caused necrosis, but less than (2−3 mm) **70** which caused 4−5 mm diameter necrosis. None of the compounds showed any antibacterial or antifungal activity when assayed on microorganisms up to 100 μg/disc. These results showed that the hydroxy group at C-3, the α,β-unsaturated ketone at C-6, and probably the vinylic group at C-13 are important features for the activity. The acetyl group at C-1 does not affect the phytotoxicity.[99] The structural features important for the activity are red drawn in Fig. 15.

5.2 Nonenolides and cytochalasins SAR study

A SAR study was conducted assaying 15 natural analogues and derivatives of nonenolides and cytochalasins, for their toxicity against the perennial weeds *C. arvense* L. and *S. arvensis* L.[161] The toxic nonenolides (stagonolide, pinolidoxin, and putaminoxin: **17**, **27** and **28**) and cytochalasins (deoxaphomin, cytochalasins A, B, F, T, Z2 and Z3: **12**, **5**-**8**, **10** and **11**) were isolated from phytopathogenic *Stagonospora*, *Phoma* and *Ascochyta* spp. The pinolidoxin derivatives (7,8-*O*,*O*'-diacetyl- and 7,8-*O*,*O*'-isopropylidene-pinolidoxin) and the cytochalasin B derivatives (21,22-dihydro-, 7-*O*-acetyl- and 7,20-*O*,*O*'-diacetyl-cytochalasin B) were obtained

by chemical modifications of the corresponding toxins. Among the 15 phytotoxic compounds tested stagonolide (**17**) was the most phytotoxic to *C. arvense* L. leaves and deoxaphomin demonstrated the highest herbicidal effect to *S. arvensis* L. leaves. The tested phytotoxic nonenolides were stronger inhibitors of photosynthesis in *C. arvense* L. leaves than cytochalasins A (**5**) and B (**6**). Although the photometric observations allowed to suppose that both **17** and **6** did not inhibit electron transport, **17** had a much lower effect on membrane permeability in *C. arvense* L. leaves than **6**. These results indicate different modes of action of phytotoxic nonenolides and cytochalasins. Stagonolide (**17**) seems to inhibit selectively the photosynthesis in *C. arvense* L. The study of its mode of action looks interesting because most of the known herbicides affecting photosynthesis inhibits electron transport in photosystem II and, further, causes membrane damage.[162-164]

Analysis of SAR showed the importance of specific functional groups and of the ring conformational freedom for the toxicity of nonenolides. The presence of the hydroxy group at C-7 and the functional group at C-20, as well as the conformational freedom of the macrocyclic ring appeared important structural features of toxic cytochalasins.[161]

5.3 Papyracillic Acid SAR study

The crystalline papyracillic acid (**55**) was used for the preparation of six derivatives to carry out a SAR study aimed at finding a derivative with increased phytotoxicity and specificity. **55** was converted in its methyl ester (**134**, Fig. 16) by reaction with an ethereal solution of diazomethane, while treatment with methanol and a catalytic amount of trifluoracetic acid yielded the corresponding 3-*O*-methyl acetal (**133**, Fig. 16). By acetylation carried out with acetic anhydride and sodium acetate at high temperature, **55** was converted in three monoacetyl derivatives (**130**-**132**, Fig. 16), two of them were *Z*/*E* diastereomers (**132**/**131**). Finally, by

catalytic hydrogenation the toxin was converted into the corresponding 5,9-dihydroderivatives (**135**, Fig. 16). Assayed on *E. repens* L. at 1 mg mL^{-1} the 3-*O*-acetal and the three monoacetyl derivatives were phytotoxic, but significantly less active than **55**. *C. arvense* L. leaves were sensitive to the methyl ester, the 3-*O*-acetal, the monoacetyl and the *Z*-monoacetyl derivatives but at less than papyracillic acid (**55**). The methyl acetal and the two monoacetyl derivatives (*Z*- and *E*-diastereomers) showed antibacterial activity against *B. subtilis* and *X. campestris,* while only the methyl acetal inhibited growth of the fungus *C. tropicalis*, at 50 μg $disk^{-1}$, but to a lesser extent than papyracillic acid. Since the monoacetyl *E*-diastereomer derivative had phytotoxic activity not associated to antimicrobial activity, it seems to be interesting to be further evaluated. These results showed that the butenolide ring is an important feature imparting phytotoxicity. In fact, the papyracillic acid methyl ester, which showed the opening of the hemiacetalized 1,6-dioxospiran system, was essentially inactive on the host and hemp plants and poorly active on *C. arvense* L., whereas the other derivatives of papyracillic acid had this moiety unaltered. The inactivity of the dihydroderivative of papyracillic acid, in which the butenolide ring is unaltered, showed the importance for the phytotoxicity of the exocyclic methylene group at C-5. Furthermore, the heptasubstituted trihydrofuran seems to be unessential, considering the activity of the three monoacetyl derivatives, in which it is opened and converted into differently C-4 and C-9 substituted butenolides. These latter derivatives also lack of the exocyclic methylene group at C-5 but display the presence of an exocyclic double bond belonging to the different side chain at C-4. Probably, the different functionalities and stereochemistry of this latter justified the little difference observed in the phytotoxicity of the three monoacetyl derivatives. The reduced activity of the papyracillic acid acetal suggested a role of the hemiacetalic hydroxy group at C-7.[80] The structural features important for the activity are red drawn in Fig. 16.

5.4 Agropyrenol SAR study

Six agropyrenol (**64**) derivatives were prepared by chemical transformation of the functionalities present in agropyrenol and tested on non-host weedy and agrarian plants, fungi, Gram+ and Gram- bacteria, as well as brine shrimp larvae. **64** was converted by acetylation into the corresponding 3',4'-*O,O'*-diacetyl derivative (**136**, Fig. 17), while treatment with dry acetone and dried cuprum sulphate yielded the corresponding 3',4'-*O,O'*-isopropylidene derivative (**137**, Fig. 17). By oxidation with manganese dioxide in methylene choride, agropyrenol was converted into the 4',*O*-didehydro derivative (**138**, Fig. 17), while by reduction with sodium borontetrahydride, its aldehyde moiety was converted in the corresponding primary alcohol (**139**, Fig. 17). Finally, by catalytic hydrogenation it was converted in the 1',2'-dihydroderivative (**140** Fig. 17), which in turn, by usual acetylation yielded the 6,3',4'-*O,O',O''*-triacetyl derivative (**141**, Fig. 17). Assayed by leaf puncture on detached leaves, **136** and **137** caused severe to medium necrosis to weedy dicot plants. The activity showed by the diacetyl derivate of agropyrenol was not unexpected, as it was probably hydrolyzed into agropyrenol (**64**) at physiological pH according to the "lethal metabolism".[165] A similar mechanism of hydrolysis could also convert **137** into **64** as the acetonide is stable at basic pH but hydrolyzes in acid conditions. **136** and **137** derivatives of agropyrenol (**64**) also exhibited a strong reduction of germination (48 and 60 % reduction), a severe depletion of rootlet growth (69 and 90% reduction), and an effective reduction in the chlorophyll content of *L. minor* fronds (31 and 67% reduction). Both the double bond and the diol system of the 3,4-dihydroxypentenyl side chain as well as the aldehyde group at C-1 of the phenolic ring of agropyrenol proved to be important for the phytotoxicity. These results suggest that the two derivatives, which are less polar than agropyrenol, could be absorbed and pass across the cell membranes of the tested plants, before

converting into agropyrenol. The lesser polar 3′,4′-*O,O′*-isopropylidene derivatives also showed significant zootoxic and slight antimicrobial activities.[166] The structural features important for the activity are red drawn in Fig. 17.

5.5 Phomentrioloxin SAR study

Seven phomentrioloxin derivatives (Fig. 18) were prepared by chemical transformation of its functionalities and tested on host (*C. lanatus* L.) and non host weedy (*C. album* L.*, M. annua* L.*, S. oleareus* L.) and some agrarian plants, fungi, Gram + and Gram - bacteria, and on brine shrimp larvae. Phomentrioloxin (**75**) was converted in 1-*O*-acetyl, 1,2-*O,O'*-diacetyl and 1,2,4-*O,O',O''*-triacetyl derivatives (**142**-**144**, Fig. 18) by acetylation, while reaction with dry acetone and dried cuprum sulphate yielded the corresponding 1',2'-*O,O'*-isopropylidene derivative (**145**, Fig. 18). Oxidation with manganese dioxide in methylene chloride yielded the 4,*O*-didehydro derivative (**146**, Fig. 18), while catalytic hydrogenation yielded a derivative (**147**, Fig. 18) showing the saturation of the geranyl side chain. Finally, the toxin oxidation with sodium periodate gave the corresponding dialdehyde which was stabilized by reduction with sodium borontetrahydride affording the corresponding 1,2-diolderivative **148**. The hydroxy groups at C-2 and C-4 appeared to be important features for the phytotoxicity, as well as an unchanged cyclohexentriol ring. In fact, when the 2-hydroxy or the 2- and 4-hydroxy groups were acetylated, a total loss of activity was observed. The role of these two groups (2- and 4-hydroxy) was confirmed by the reduced activity observed when the hydroxy group at C-2, together with that at C-1, was ketalized and by the total loss of activity when the hydroxy group at C-4 was oxidized. The hydroxy group at C-1 seems not to be important for activity. In fact, when it was acetylated this derivative proved to be as toxic as phomentrioloxin. The reduced activity showed

by 1′,1′,2′,2′,3′,8′,6′,7′-octahydrophomentrioloxin (**147**), indicated that the unsaturation of the geranyl side chain also plays a role in imparting phytotoxicity. Finally, the lack of activity of derivative 2-methoxy-5-(3-methylene-oct-6-en-1-ynyl)-hex-4-ene-1,3,6-triol also meant that the presence of a unchanged cyclohexenetriol ring is important for activity.[86,167] The structural features important for the activity are red drawn in Fig. 18.

6 Perspectives

Although numerous fungal metabolites with potential herbicidal activity have been isolated, as also highlighted in this review, their tests in greenhouses and in open field are still very limited. The same happens for their transfer to industry for large scale production and their application into practice as commercial products. These delays are due to the lack of funding to this research field by both Local Authorities and/or private companies including those specialized in the field, which do not believe the industrial development of novel natural herbicides still economically competitive with the cheaper traditional chemical herbicides currently in use. One of the first limitation is the availability of phytotoxins, which are produced, except in rare cases, mostly in relatively low amount in fungal culture filtrates. This prevents the possibility to carry out ecotoxicology experiment required by current regulations in many countries and especially in the European Union. Given the low yields in production the industrial scale-up of phytotoxins requires an optimization of the production in fermenter choosing the best fungal producer strain, the best culture medium, and a simple purification method with little to none use of organic solvents. The growth in the fermenter, widely used with the bacteria even for the large scale production of antibiotics, requires the development of new technologies to avoid the adhesion of the mycelia masses to the walls and the poles of the bioreactor. All these tests were carried out

with the mixture of *A. caulina* toxins (see paragraph 2.1) within a project supported by the Italian State Railways and the Governatorate of Regione Lombardia. The total or semi-synthesis and, in particular, the enantioselective synthesis, could represent an alternative to overcome these problems. However, the stereostructural complexity of phytotoxins make difficult to fullfill the industry requirements to develop a few steps process with high yields and possibly using environmentally friendly ragents. Another problem to be overcome is the correct formulation of the natural herbicide to find the best surfactant and adjuvant to provide a slow and gradual release of the active ingredient in the field. Some tests were done again with the mixture of *A. caulina* toxins (see paragraph 2.1).[168] However, an important and effective alternative could be the employment of nanoparticles. They have been already successfully applied in medicine to deliver ophiobolin A (**86**) as anticancer against Rhabdomyosarcoma, the most common soft tissue sarcoma in children. The anticancer efficacy of **86** is significantly increased loading with the phytotoxin nanoparticles of polystyrene-coated mesoporous silica.[169] This new technology could be extended in agriculture finding nanoparticles suitable to deliver it as a natural and safe herbicide.

7 Conclusions

Politics and economy are driving modern agriculture towards crop production systems that are healthier, safer, and friendlier to the environment as consumers demand pesticide-free products and environmentally-safe cultural practices. Taking into account these perspectives, the richest sources of natural compounds present in nature and their ecology, assume higher and higher importance and can increase the possibility of finding natural herbicides with new scaffolds and modes of action, fundamental factors overcoming resistance in weeds to conventional, synthetic herbicides. Being the result of co-evolution of the producing organism and its biotic

environment, these compounds can have high target selectivity, with potentially reduced risks for humans and non-target organisms. Furthermore, they can have a shorter environmental half-life than synthetic compounds, thus reducing potential environmental impact. The tremendous structural diversity and the promising herbicidal potential of many of these natural products reported in the current review will prompt a continued interest in developing fungal metabolites as natural safe herbicides.

Electronic Suppoting Information (ESI)

A Table 1 reporting the IUPAC name, the fungal source, the target weed and references for each compound was available at XXXXX

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Figure Legend

Fig. 1 Non proteinogenic amino acids: ascaulitoxin, *trans*-4-aminoproline, ascaulitoxin aglycone and ascosonchine (**1**-**4**).

Fig. 2 Cytochalasans: cytochalasins A, B, F, T, Z1, Z2 and Z3 (**5**-**11**), dexoxaphomin (**12**), phomachalasins A, B, C and D (**13**-**16**).

Fig. 3 Nonenolides: stagonolide (**17**), stagonolides B-I (**18**-**25**), modialide A (**26**), pinolidoxin (**27**), putaminoxin (**28**), herbarumins I, II and III (**29**, **30** and **32**), 2-*epi*-herbarumin II (**31**), pinolide (**33**) and tetrasubstituted nonenolide (**34**).

Fig. 4 Oxazatricyclocalkalenones and complex carbon skeleton compounds: phyllostictines A-D (**35**-**38**), phyllostoxin (**39**), phyllostin (**40**) scytolide (**41**) drazepinone (**42**).

Fig. 5 Benzochromanones: alternethanoxins A-E (**43**-**47**).

Fig. 6 Spirotoxins: spirostaphylotichins A, C, R, and V and W (**48**-**50**, **52** and **54**), triticone E (**51**), papyracillic acid (**55**), palmarumycin EG_1, CP_2, CP_{17},CP_{19} (**56**-**59**) preussomerin EG_1, EG_2, EG_3,EG_4 (**60**-**63**).

Fig. 7 Aromatic compounds: agropyrenol, agropyrenal and agropyrenone (**64**-**66**), cyclopaldic acid (**67**), 6-hydroxymellein (**68**).

Fig. 8 Terpenes: sphaeropsidin A (**69**), chenopodolin (**70**), pyenophoric acid (**71**), pyrenophoric acids B and C (**72** and **73**), abscisic acid (**74**), phomentrioloxin (**75**), phomentrioloxins B

and C (**76** and **77**), fusicoccin A (**78**), dideacetylfusicoccin A (**79**), cotylenol (**80**), cotylenin A (**81**), fusicoccin A deacetylaglycone (**82**), 8,9-isopropylidene of fusicoccin A deacetylaglycone (**83**), isomers of fusicoccin A (**84** and **85**), ophiobolins A and B, I, E, J and 8-*epi*-J (**86** and **89-93**), 6-*epi*-ophiobolin A (**87**), 3-anhydro-6-*epi*-ophiobolin A (**88**), sporogen AO-1 (**94**), dihydropsporogen AO-1 (**95**).

Fig. 9 α-Pyrones: gulypyrones A and B (**96** and **97**).

Fig. 10 Furopyrans: chenopodolans A-C (**98-100**).

Fig. 11 Miscelaneous toxins: tuenazoic acid (**101**), cyperin (**102**), O-MeSMA93 (**104**), radicin (**105**), 1,4-disubstituted pentanone (**106**), phthalic acid butyl isobutyl ester (**106),** vulculic acid (**107**).

Fig 12 Trichotechenes; verucarins A, B, L acetate, M (**108-111**), roridin A (**112**), isotrichoverrin B (**113**), isotrichoverrol (**114**), neosolaniol (**115**), nesolaniol monacetate (**116**), diacetoxyscirpenol (**117**), T_2 (**118**) HT_2 (**119**).

Fig 13 Cyclohexen epoxides: sphaeropsidone (**120**), *epi*-sphaeropsidone (**121**), *epi*-epoformin (**122**).

Fig 14 Chalcones and chromanones: cavoxin (**123**), cavoxone (**124**).

Fig. 15 Derivatives of chenopodolin (**125-129**) for SAR studies.

Fig. 16 Derivatives of papyracillic acid (**130-135**) for SAR studies.

Fig. 17 Derivatives of agropyrenol (**136-141**) for SAR studies.

Fig. 18 Derivatives of phomentrioloxin (**142-148**) for SAR studies.