# Parasitic weed management by using strigolactones-degrading fungi

Angela Boari[a], Biancamaria Ciasca[a], Rocío Pineda-Martos[b], Veronica MT Lattanzio[a], Koichi Yoneyama[c], Maurizio Vurro[a]*

[a]Institute of Sciences of Food Production, C.N.R., via Amendola 122/O, 70126 Bari, Italy;
[b]Agroforestry and Plant Biochemistry and Proteomics Research Group, Department of Biochemistry and Molecular Biology, University of Córdoba-CeiA3, Severo Ochoa Building, Campus Universitario de Rabanales, Ctra. Madrid-Córdoba km 396, 14071 Córdoba, Spain;
[c]Center for Bioscience Research and Education, Utsunomiya University, 350 Mine-machi, Utsunomiya 321-8505, Japan.

*Corresponding author: phone: +39.0805929331; fax: +39.0805929374; e-mail: maurizio.vurro@ispa.cnr.it

**Running title**: SL degrading fungi

**Keywords**: Strigolactones; *Fusarium*; *Trichoderma*; *Botrytis*; Parasitic weed management; Biodegradation

**Abstract**

BACKGROUND

Seed germination is a key phase of the parasitic plant life cycle which is stimulated by the secondary metabolites, mainly strigolactones (SLs), secreted by the host roots. Interventions during this stage would be particularly suitable for parasitic weed management practices since blocking these chemical signals would prevent seed germination and thus parasite attack.

Four fungal strains with different ecological functions were considered for their possible capability to metabolize SLs, i.e.: *Fusarium oxysporum, F. solani*), biocontrol agents of *Phelipanche ramosa*; *Trichoderma harzianum*, a potential biopesticide; *Botrytis cinerea*, a phytopathogenic fungus. Four different SLs (i.e. the natural strigol, 5-deoxystrigol (5DS) and 4-deoxyorobanchol (4DO), and the synthetic analogue GR24) were added to fungal cultures, followed by the determination of the SL content by liquid chromatography-tandem mass spectrometry.

RESULTS

Differences were observed among microorganisms, treatments and SLs used. *Trichoderma harzianum* and *F. oxysporum* were the most capable to reduce the SL content; considering the whole set of fungi used, 5DS and 4DO proved to be the most degradable SLs.

CONCLUSIONS

Beneficial microscopic fungi could differently be used for biocontrolling parasitic weeds, acting as a "physiological" barrier, by preventing the germination of their seeds due to the capability of bio-transforming the stimulatory signals.

## 1. INTRODUCTION

Parasitic weeds of the genera *Orobanche* and *Phelipanche* (commonly named broomrapes), and *Striga* (witchweeds) are responsible for enormous yield losses of several crops all around the World (Parker, 2009). Traditional weed management methods including among others, the use of herbicides, soil fumigation and solarization, mechanic, physical or biological control, can provide only a modest or even a null control. Difficulties in controlling parasitic weeds are due to the large amount of seeds produced that can remain viable, even in the absence of a host, for many years. The seed germinates only if it is stimulated by host root exudates and produces a germ tube that, if it attaches to the host root, develops a haustorium that penetrates the root and forms a tubercle. This is followed by the withdrawal of nutrients, water, and photosynthates from the host by the parasite. Furthermore, broomrapes have a long underground phase, so that when they emerge most of the damage has already been produced (Book parasitic orobanchaceae).

The interaction between parasitic weeds and their indispensable hosts begins with the secretion of secondary metabolites (germination stimulants) from the roots of the host that induce the germination of the parasite's seeds. Most important germination stimulants identified so far are strigolactones (SLs) which are derived from carotenoids [1-4]. Interestingly, SLs are produced by both the host and non-host plants, and do not just act as germination stimulants but also as compounds responsible for the induction of hyphal branching and/or spore germination in arbuscular mycorrhizal (AM) fungi, obligate symbionts that help the plant by improving their uptake of inorganic phosphate and other minerals [5,6]. More recent studies have shown that SLs work as phytohormones inhibiting shoot branching [7,8] and regulating root architecture and root hair development [9-11], secondary growth [12], and senescence [13,14]. SLs have been reported to be present in the root exudates of a wide range of different plant species, and thus it would not be surprising if these compounds would act as signals also for microorganisms other than AM fungi, that could be both beneficial (e.g. ectomycorrhizal fungi, biocontrol agents, biofertilizers, resistance inducers) as well as phytopathogenic ones. Recently, the involvement of SLs in rhizobacterial colonization in legumes has also been reported [15,16], further supporting this latter hypothesis. These aspects could be highly interesting from a practical point of view, allowing novel approaches for parasitic plant management. For example, if beneficial

microorganisms were able to metabolize SLs and rhizosphere competent, meaning that they could grow along the root system of crop plants and could avoid signal recognition by the seeds of the parasitic plants, preventing parasite germination and successive attachments to the host root, and then resulting in natural crop protective agents.

The aim of the present article is to obtain first indications on the capability of some beneficial and phytopathogenic fungi to detect the presence of SLs and possibly to biologically degrade/metabolize them.

## 2. EXPERIMENTAL METHODS

### 2.1. Microorganisms

To perform the biological assay described in the subsequent section, one strain each of the following fungal species was chosen: *Botrytis cinerea* (De Bary) Whetzel, *Fusarium oxysporum* Schlect. emend. Snyd. & Hans. (strain FT2), *F. solani* (Mart.) Sacc. (strain ET4) and *Trichoderma harzianum* Rifai.

The strains are stored in the mycological collection (Fungi Culture Collection) of the Institute of Sciences of Food Production (ISPA), National Research Council (CNR) in Bari, Italy; and labelled as ITEM 5154, 5107, 5106 and 908, respectively. Strains were maintained and routinely grown on Potato Dextrose Agar (PDA) plates.

For an easier reading of text and tables, in the current article the fungal strains were named by using the initials of their scientific names, and thus: BC for *B. cinerea*, FO for *F. oxysporum*, FS for *F. solani* and TH for *T. harzianum*.

### 2.2. Strigolactones

To perform the biological assay below described, the following SLs were used: the natural (+)-strigol (ST), (±)-5-deoxystrigol (5DS), (±)-4-deoxyorobanchol (4DO), and GR24 (mixture of four stereoisomers). ST was a generous gift from Emeritus Professor Kenji Mori (The University of Tokyo, Japan). 5DS, 4DO, and GR24 were kindly provided by Prof. Kohiki Akiyama (Osaka Prefecture University, Japan). They were dissolved in dimethylsulfoxide (DMSO) at a concentration of 0.1 µg $\mu L^{-1}$, and stored at –20°C until use.

### 2.3. Biological assay

Conidial suspensions were prepared by surface scraping the fungal colonies actively growing on PDA plates. The collected material was suspended in a few ml of a modified Wickerham's medium [17] and gently shaken to disperse the conidia. Suspensions were then filtered through sterile filter gauze to remove mycelium and debris, and the conidia concentration was determined by using of a hemocytometer (Thoma chamber), by adjusting the final concentration to $10^7$ conidia $ml^{-1}$. Five ml of suspension were used to inoculate 250 ml Erlenmeyer flasks containing 100 ml of Wickerham's medium mentioned above. Inoculated flasks were kept on a benchtop incubation shaker (Certomat IS, Sartorius) at 25 ± 1°C at 120 rpm for three days. Then, three different experiments were performed, by adding the SL solution (10 µl, containing 1 µg of SL) to 25 ml of: (i) actively growing liquid fungal culture, culture substrate and fungus (CSF); (ii) liquid culture filtrate (CF) after removal of the mycelium by filtration through filter paper (Whatman, Grade 4); and (iii) non-inoculated medium (used as control), culture substrate (C). Samples were placed in sterile polypropylene conical tubes (Falcon® 50-mL centrifuge tubes) and left to incubate on an orbital shaker at room temperature (25°C) at 100 rpm for 90 min. Each experiment was carried out in triplicate.

**2.4. Extraction**

Each sample prepared as described above (2.3) was extracted according to the following procedure. After incubation, the whole sample volume was collected, filtered and extracted with ethyl acetate (3 x 25 mL, VWR International SAS, Fontenay-sou-Bois, France). Extracts were combined, washed twice with an equivalent volume of 0.2 M $K_2HPO_4$ buffer (Sigma Aldrich, USA), and dried over anhydrous $Na_2SO_4$. After filtration, the organic solvent was evaporated under reduced pressure by using a Rotavapor (R-200 Büchi). Dried extracts were stored at –20ºC then reconstituted in 400 µL of methanol (HPLC grade, Mallinckrodt Baker, Milan, Italy) / water (ultrapure water produced by a Milli-Q system, Millipore, Bedford, MA, USA) (60:40) and filtered through regenerated cellulose filters (HPLC syringe filters, 0.20 µm, Alltech, Deerfield, IL, USA) just prior to liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Mycelia obtained after filtration of the CSF were first re-suspended on 0.2 M $K_2HPO_4$ buffer (25 mL) followed by homogenization by using a homogenizer (ULTRA-TURRAX, IKA-Werke GmbH & Co., Staufen, Germany), then filtered and finally extracted with ethyl acetate as described above.

### 2.5. LC-MS/MS analysis of strigolactones

LC-MS/MS analyses were performed on a QTrap MS/MS system, from Applied Biosystems (Foster City, CA, USA) equipped with an electrospray ionization (ESI) interface and an 1100 series micro-LC system comprising a binary pump and a microautosampler from Agilent Technologies (Waldbronn, Germany).

The analytical column was a Zorbax C18 Eclipse XDB (100 mm x 4.6 mm, 3.5 µm particles) (Agilent Technologies, Waldbronn, Germany). The flow rate of the mobile phase was 300 µL/min, while the injection volume was 10 µL. The column effluent was directly transferred into the ESI interface without splitting. The column oven was set at 40°C. Eluent A was water and eluent B was MeOH. A gradient elution was performed by changing the mobile phase composition as follows. The proportion of eluent B was linearly increased from 60% to 100% in 30 min. The column was re-equilibrated with 60% eluent B for 10 min.

For LC-MS/MS analyses, the ESI interface was used in positive ion mode, with the following settings: temperature (TEM) 300°C; curtain gas (CUR), nitrogen, 30 psi; nebulizer gas (GS1), air, 10 psi; heater gas (GS2), air, 30 psi; ionspray voltage +4500 V. The mass spectrometer operated in MRM (multiple reaction monitoring) mode, by monitoring two transitions for each compound, with a dwell time of 100 ms. Selected transitions were: 369.1 > 272.1 and 369.1 > 257.1 for ST, 353.2 > 256.2 and 353.2 > 241.2 for 5DS and for 4DO, 299.1 > 185.2 and 299.1 > 157.1 for GR24.

### 2.6. Statistical analysis

Data were subjected to the analysis of the variance according to a complete randomized experimental design with three replications. Furthermore, comparisons of the main effects and interactions were made by using the SNK (Student-Newman-Keuls) method, with $P = 0.01$.

As the data obtained by LC-MS/MS analysis of the samples were expressed as area of the chromatographic peak they were then expressed as reduction of SL content by comparison with the relevant control, prepared as described above.

## 3. RESULTS AND DISCUSSION

In order to evaluate the capability to recover SLs when using the set-up procedure, and to determine the amount of SLs that could be degraded due to “natural degradation”, pure SLs were dissolved in 400 µL of methanol/water (60:40) and immediately analyzed in comparison with the controls, i.e. the samples obtained by adding the SLs to the non-inoculated medium, extracted as described above. SL content in the controls proved to be in the range between 83 and 96% of the “pure” injected compounds, with a relative standard deviation lower than 5%. This means that the “natural” degradation of the SLs was modest and thus not affecting the general results.

In the case of TH, a strong reduction in SL content was observed for 5DS, GR24 and 4DO, by 87.3, 82.8 and 75.5%, respectively, when using the whole culture broth (CSF). A quite modest and significantly different reduction occurred in the case of ST (Figure 1).

In the case of FO, the SL content decreased by 79.2 and 70.4% for 5DS and 4DO, respectively, when SLs where added to CSF (Figure 1); a much lower reduction occurred for GR24 and ST (55.9 and 25.0, respectively) (Figure 1), and almost negligible when SLs were added to the culture filtrates after mycelium removal (CF) (data not shown). A quite similar trend was observed for BC, with over 70% reduction for both 5DS and 4DO, and much lower for the other two SLs. Very similar results, but at lower levels, were also obtained when using FS (Figure 1), which caused a reduction over 50% in 5DS and 4DO contents, and a modicum one for the other two compounds. In case of the use of the culture filtrates, the reduction effects were modest or negligible (data not shown). Furthermore, SLs in the extracts from mycelia were not detectable at all, or detected as negligible traces (less than 1%) (data not shown).

Considering the effects of all the strains on the total pool of SLs, on average they were able to reduce it by over 50% in case of the whole culture (CSF), significantly different by the less than 2% reduction when using CF (data not shown). If considering the effectiveness of each fungus on the whole set of compounds, the best proved to be TH, with a total capability to reduce their content above 70% (Figure 2), followed by FO (around 57%), BC (44%) and FS (around 37%). When considering the use of CF, the effectiveness dropped down to almost negligible values.

Interesting results appear when analyzing the effects of the strains used (considered as a whole) on each SL. In this case, the most susceptible SLs to “bio-modification” appear to be 5DS and 4DO

(73.5 and 68.9 %, respectively) followed by GR24 and ST, at significantly lower levels (Figure 3). Negligible are the effects obtainable when using the CFs.

This could not be surprising. Indeed, GR24 is a synthetic stimulant and thus, although it is universally used by scientists for experiments involving germinated seeds of parasitic weeds, it is feasible that some microorganisms could not metabolize and transform it. ST is a natural SL isolated from the root exudates of cotton (*Gossypium hirsutum* L.), a non-host of *Striga* [18,19]. Later on, ST was also identified in the root exudates of real *Striga* hosts, i.e. sorghum [*Sorghum bicolor* (L.) Moench], maize (*Zea mays* L.) and common millet (*Panicum miliaceum* L.) [20]. 5DS was originally identified as a branching factor, an essential signal for root colonization by AM fungi [5], and later on identified as a germination stimulant of *Orobanche* and *Striga* seeds, too [21]. 5DS is a stereoisomer (diastereomer) of 4DO, which was initially identified as *ent*-2'-*epi*-5-deoxystrigol [8,22] that induces hyphal branching in germinating spores of the AM fungus *Gigaspora margarita*, and is a stimulant of the seed germination of *Orobanche* and *Striga*. Thus, it seems to be feasible that both 5DS and 4DO could be the compounds more easily detected and metabolized also by other fungi. Further studies are in progress in order to identify the possible degradation products. However, a possible metabolic pathway seems to be different from the hydroxylation, as this could lead to the production of strigol or orobanchol (for 5DS and 4DO, respectively).

Considering the hypothesis to use beneficial organisms as potential “bioherbicides” with a novel biocontrol mechanism, four different fungal strains were chosen as follow:

(i) The two strains of *Fusarium* (FO and FS) were isolated by some of the authors in previous studies aimed at finding fungal strains pathogenic to *Orobanche*, to be used directly in the field as biocontrol agents against *P. ramosa*. In lab [23] and field (unpublished) experiments, they proved to be very effective in causing necrosis of broomrape tubercles when applied to the parasite.

(ii) The strain of *T. harzianum* (TH) used in the current studies was previously isolated from olives and proved to be a very interesting biopesticide to control soil-borne plant pathogens, e.g. *Pythium* spp., *Sclerotinia* spp., *Rhizoctonia* spp. and *Fusarium* spp. Moreover, strains of this species proved to act as biofertilizers, stimulating the root system of several crops and increasing the plant vigour, by releasing some phytohormone-like compounds as well as by favouring the solubilisation of

mineral nutrients [24,25]. More recently, the strain used in these experiments has been subjected to the EU procedures necessary to register it as a commercial bio-agrochemical.

(iii) The strain of *B. cinerea* (BC) was isolated from *Fragaria* sp. in one of the author's labs. The fungus is known to be an air-borne plant pathogen with a necrotrophic lifestyle attacking over 200 crop hosts worldwide, including important protein, oil, fibre and horticultural crops. It can cause soft rotting of all aerial plant parts, and rotting of vegetables, fruits and flowers post-harvest. *Botrytis cinerea* produces a range of cell-wall-degrading enzymes, toxins and other low-molecular-weight compounds such as oxalic acid.

Thus, the results seem to be in agreement with our hypothesis, that some fungi other than AM ones could detect and metabolize SLs, at the physiological rates produced by the host roots. Indeed, the most effective fungus in our experiments proved to be TH, which is a species that could commonly be found in the soil and in the rhizosphere. Some strains are also rhizosphere competent [26], meaning that they can grow along the root system as soon as it grows. In our case, if our strain were able to metabolize SLs and grow along the host roots, it could offer an active protective system by the "aggression" of the parasitic plants, by interrupting (preventing) the perception of the stimulants thus blocking seed germination.

An analogous action could occur for the *Fusarium* strains. In this case, two different protective mechanisms could be combined in one strain: the pathogenic one, which is the reason of the identification of the strains, i.e. the capability to produce necrosis to the developing tubercles and stems of the parasites; and the interruption of the perception of SLs as stimulants of the seeds of the parasite. This could mean an increased efficiency of the mycoherbicide effectiveness of the two strains.

The lower efficacy of BC seems to be in line with the expectations. Indeed, this fungus is usually living on the aerial plant tissues and organs. Although there are many studies that report SLs could be transported from roots to shoots, although not through the xylem [27], to inhibit bud outgrowth, it would be feasible that the strain is not able to metabolize SLs as the shoot and leaf tissues it usually colonizes do not excrete SLs.

A not negligible aspect could be the capability of the fungi to metabolize the different stereoisomers of the same compound. Indeed, GR24 was used as the mixture of the four stereoisomers; 5DS and 4DO were racemic mixtures (mixture of two enantiomers), whereas ST was the only one used as an optically pure form. In our case, relationships between metabolic biodegradation capability of fungi, and SL stereochemistry were not so evident, or at least they do not appear to be the main factor, considering that the mixture of four stereoisomers (GR24) and the optically pure one (ST) proved both to be metabolized in a lesser extent compared to 4DO and 5DS.

Finally, another weak point in the proposed approach was that the mycelia used in our experiment could work as a “physical” trap for SLs, as a sort of filter able just to remove the compound from the culture. In order to adjure this risk, mycelia obtained by the experiments described above were extracted and analysed (see material and methods), confirming the negligible presence (lower than 1% of the SL amount detected in the control samples) or absence of SLs (data not shown).

To date, SLs have not been detected from microorganisms or macro organisms such as plant feeders. Moreover, no information is available about the fate of the SLs when they are released from the roots, e.g. how they are further metabolized by the soil microorganisms. The knowledge of other biological "functions" of SLs and their metabolites in the environment and ecosystems could open completely novel fields of research and of practical applications, from pest management to pharmaceutical applications [28].

Proteomic and genetics approaches will be used in the future for identifying the enzymes in the microorganisms involved in the metabolism of SLs, and the genes activated by SLs and/or their metabolites.

**Acknowledgements**

This study stems from the STREAM project, “STRigolactones Enhances Agricultural Methodologies” funded as EU COST Action-COST FA1206. R.Pineda-Martos was awarded with a grant for a Short Term Scientific Mission (FA1206-26595) from the COST Action FA1206. Authors are particularly grateful to Dr. Mariano Fracchiolla, University of Bari, for the statistical analysis. We thank Emeritus Professor Kenji Mori (The University of Tokyo, Japan) and Professor Kohki Akiyama (Osaka

Prefecture University, Japan) for their kind gifts of SL standards. A part of works was supported by Italy-Japan Bilateral Collaboration Scheme (CNR, JSPS).

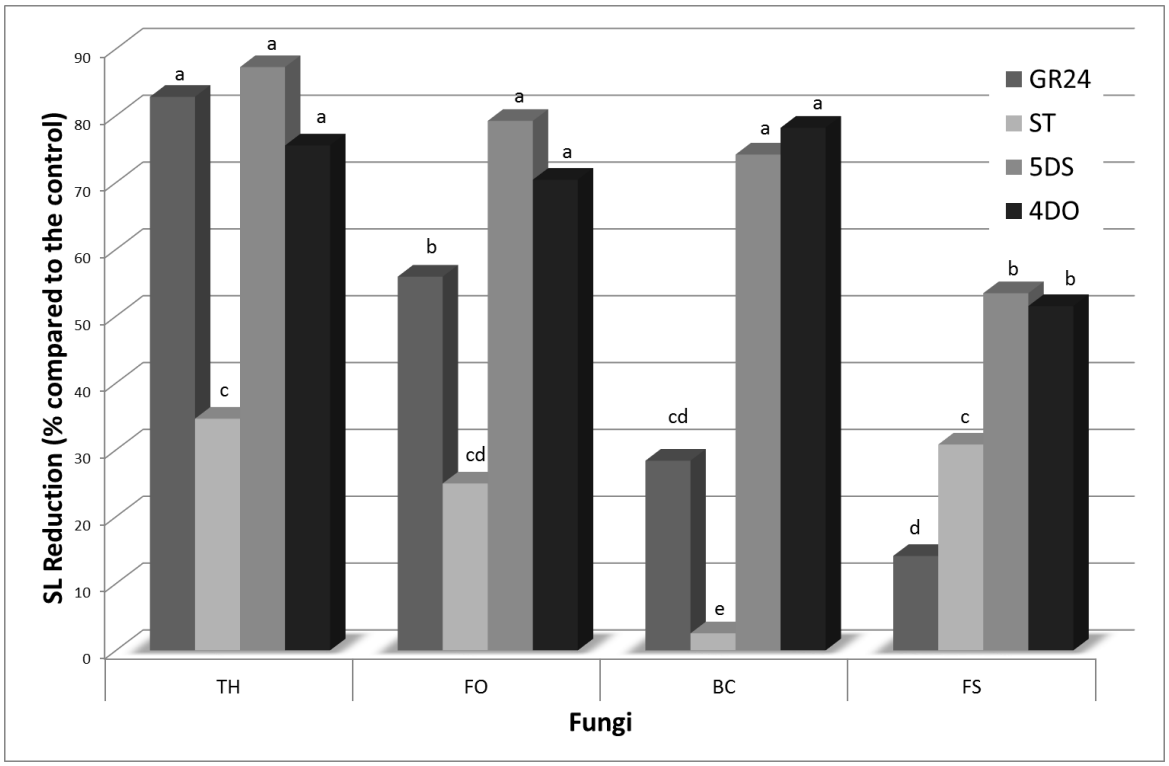
## References


1. Yoneyama K, Awad AA, Xie X, Yoneyama K and Takeuchi Y, Strigolactones as germinating stimulants for root parasitic plants. *Plant Cell Physiol* **51**:1095-1103 (2010).
2. Xie X, Yoneyama K and Yoneyama K, The strigolactone story. *Annu Rev Phytopath* **48**:93-117 (2010).
3. Al-Babili S and Bouwmeester HJ, Strigolactones, a novel carotenoid-derived plant hormone. *Annu Rev Plant Biol* **66**:161-186 (2015).
4. Lopez-Obando M, Ligerot Y, Bonhomme S, Boyer FD and Rameau C, Strigolactone biosynthesis and signaling in plant development. *Development* **142**:3615-3619 (2015).
5. Akiyama K, Matsuzaki K and Hayashi H, Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* **435**:824-27 (2005).
6. Besserer A, Puech-Pagès V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais JC, Roux C, Bécard G and Séjalon-Delmas N, Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. *PLoS Biol* **4**:e226 (2006).
7. Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pagès V, Dun EA, Pillot JP, Letisse F, Matusova R, Danoun S, Portais JC, Bouwmeester H, Bécard G, Beveridge CA, Rameau C and Rochange SF, Strigolactone inhibition of shoot branching. *Nature* **455**:189-194 (2008).
8. Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, Kyozuka J and Yamaguchi S, Inhibition of shoot branching by new terpenoid plant hormones. *Nature* **455**:195-200 (2008).
9. Kapulnik Y, Delaux PM, Resnick N, Mayzlish-Gati E, Wininger S, Bhattacharya C, Sejalon-Delmas N, Combier JP, Bécard G, Belausov E, Beeckman T, Dor E, Hershenhorn J and Koltai H, Strigolactones affect lateral root formation and root-hair elongation in *Arabidopsis*. *Planta* **233**:209-216 (2011).
10. Ruyter-Spira C, Kohlen W, Charnikhova T, van Zeijl A, van Bezouwen L, de Ruijter N, Cardoso C, Lopez-Raez JA, Matusova R, Bours R, Verstappen F and Bouwmeester H, Physiological effects of the synthetic strigolactone analog GR24 on root system architecture in *Arabidopsis*: another belowground role for strigolactones? *Plant Physiol* **155**:721-734 (2011).

11. Koltai H, Strigolactones are regulators of root development. *New Phytol* **190**:545-549 (2011).
12. Agusti J, Herold S, Schwarz M, Sanchez P, Ljung K, Dun EA, Brewer PB, Beveridge CA, Sieberer T, Sehr EM and Greb T, Strigolactone signaling is required for auxin-dependent stimulation of secondary growth in plants. *Proc Natl Acad Sci USA* **108**:20242-20247 (2011).
13. Snowden KC, Simkin AJ, Janssen BJ, Templeton KR, Loucas HM, Simons JL, Karunairetnam S, Gleave AP, Clark DG and Klee HJ, The *Decreased apical dominance1/Petunia hybrida CAROTENOID CLEAVAGE DIOXYGENASE8* gene affects branch production and plays a role in leaf senescence, root growth, and flower development. *Plant Cell* **17**:746-759 (2005).
14. Yamada Y, Furusawa S, Nagasaka S, Shimomura K, Yamaguchi S and Umehara M, Strigolactone signaling regulates rice leaf senescence in response to a phosphate deficiency. *Planta* **240**:399-408 (2014).
15. Soto MJ, Fernández-Aparicio M, Castellanos-Morales V, García-Garrido JM, Ocampo JA, Delgado MJ and Vierheilig H, First indications for the involvement of strigolactones on nodule formation in alfalfa (*Medicago sativa*). *Soil Biol Biochem* **42**:383-385 (2010).
16. Foo E and Davies NW, Strigolactones promote nodulation in pea. *Planta* **234**:1073-1081 (2011).
17. Mulè G, Susca A, Stea G and Moretti A, Specific detection of the toxigenic species *Fusarium proliferatum* and *F. oxysporum* from asparagus plants using primers based on calmodulin gene sequences. *FEMS Microb Lett* **230**:235-240 (2004).
18. Cook CE, Whichard LP, Turner B, Wall ME and Egley GH, Germination of witchweed (*Striga lutea* Lour.): isolation and properties of a potent stimulant. *Science* **154**:1189-1190 (1966).
19. Cook CE, Whichard LP, Wall ME, Egley GH, Coggon P, Luhan PA and McPhail AT, Germination stimulants. II. The structure of strigol-a potent seed germination stimulant for witchweed (*Striga lutea* Lour.). *J Am Chem Soc* **94**:6198-6199 (1972).
20. Siame BP, Weerasuriya Y, Wood K, Ejeta G and Butler LG, Isolation of strigol, a germination stimulant for *Striga asiatica*, from host plants. *J Agric Food Chem* **41**:1486-1491 (1993).
21. Awad AA, Sato D, Kusumoto D, Kamioka H, Takeuchi Y and Yoneyama K, Characterization of strigolactones, germination stimulants for the root parasitic plants *Striga* and *Orobanche*, produced by maize, millet and sorghum. *Plant Growth Regul* **48**:221-227 (2006).

22. Xie X, Yoneyama K, Kisugi T, Uchida K, Ito S, Akiyama K, Hayashi H, Yokota T, Nomura T and Yoneyama K, Confirming stereochemical structures of strigolactones produced by rice and tobacco. *Mol Plant* **6**(1):153-163 (2013).
23. Boari A and Vurro M, Evaluation of *Fusarium* spp. and other fungi as biological control agents of Broomrape (*Orobanche ramosa*). *Biol Control* **30**:212-219 (2004).
24. Altomare C, Norvell WA, Bjorkman T and Harman GE, Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. *Appl Environ Microbiol* **65**:2926-2933 (1999).
25. Harman GE, Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology* **96**:190-194 (2006).
26. Harman GE, Development and benefits of rhizosphere competence fungi for biological control of plant pathogens. *J Plant Nutr* **15**: 835-843 (1992).
27. Xie X, Yoneyama K, Kisugi T, Nomura T, Akiyama K, Asami T and Yoneyama K, Strigolactones are transported from roots to shoots, although not through the xylem. J Pestic Sci: in press (2015).
28. Pollock CB, McDonough S, Wang VS, Lee H, Ringer L, Li X, Prandi C, Lee RJ, Feldman AS, Koltai H, Kapulnik Y, Rodriguez OC, Schlegel R, Albanese C, Yarden RI, Strigolactone analogues induce apoptosis through activation of p38 and the stress response pathway in cancer cell lines and in conditionally reprogrammed primary prostate cancer cells. *Oncotarget* **5**:1683-98 (2014)
29. Parker C, Observations on the current status of *Orobanche* and *Striga* problems worldwide. *Pest Man Sci* **65:**453–459 (2009).
30. Joel DM, Gressel J & Musselman LJ, Parasitic Orobanchaceae. Parasitic Mechanisms and Control Strategies. Springer-Verlag Berlin Heidelberg, Germany (2013).

Figure 1. Reduction of strigolactone content (percentage compared to the control) in fungal cultures




Fungi: TH, *Trichoderma harzianum*; FO, *Fusarium oxysporum*; BC, *Botrytis cinerea*; FS, *Fusarium solani*.
Strigolactones: GR24; ST, strigol; 5DS, 5-deoxystrigol; 4DO, 4-deoxyorobanchol.
See Materials and Methods for details.

Figure 2. Reduction in SL content (percentage compared to the control) in the fungal culture (CSF) and culture filtrate (CF) of four fungal strains. The four SLs analysed are here considered as a whole.

| Fungus* | CSF | CF |
|---|---|---|
| TH | 70.1 a | 7.6 d |
| FO | 57.6 b | 3.7 d |
| BC | 44.5 c | -14.0 e |
| FS | 37.5 c | 7.7 d |

*Fungi: TH, *Trichoderma harzianum*; FO, *Fusarium oxysporum*; BC, *Botrytis cinerea*; FS, *Fusarium solani*.
Values with different letters differ significantly by the SNK method, with $P = 0.01$
See Materials and Methods for details

Figure 3. Reduction in SL content (percentage compared to the control) in the fungal culture (CSF) and culture filtrate (CF). The four fungi analysed are here considered as a whole.

| SL* | CSF | CF |
|---|---|---|
| 5DS | 73.5 a | 1.0 d |
| 4DO | 68.9 a | -1.5 d |
| GR24 | 45.3 b | 1.6 d |
| ST | 21.9 c | 3.9 d |

*Strigolactones: 5DS, 5-deoxystrigol; 4DO, 4 deoxyorobanchol; GR24; ST, strigol.
Values with different letters differ significantly by the SNK method, with $P = 0.01$.
See Materials and Methods for details.