Strigolactones: ecological significance and use as a target for parasitic plant control

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Abstract

Parasitic weeds cause severe damage to important agricultural crops. Although some promising control methods against these parasitic plants have been developed, new strategies continue to be relevant in integrated approaches. The life cycle for root parasitic weeds is intimately associated with their host and is a suitable target for such new control strategies, particularly when directed at the early stages of the host–parasite interaction. Here, the authors focus on knowledge of the germination stimulants – strigolactones – for the root parasitic plants Striga and Orobanche spp. and discuss their biosynthetic origin, ecological significance and physiological and biochemical regulation. In addition, the existing and possible new control strategies that are based on this knowledge, and that could lead to more efficient control methods against these root parasitic weeds, are reviewed.

Keywords: strigolactones; germination stimulants; root parasitic plants; control strategies

1 INTRODUCTION

Parasitic weeds from the genera Striga and Orobanche spp. are difficult to control because they are intimately associated with the host root, and most of their life cycle occurs underground (Fig. 1). This fact makes the diagnosis of infection difficult, and normally irreversible damage has already been caused to the crop. To date, a series of control strategies, pre- and post-attachment, against root parasites have been developed (see this issue). However, new control methods and integrated strategies using different methods are required for effective long-term control of these parasitic weeds. Since the root parasites affect their host from the moment they attach to it and exert the greatest damage prior to their emergence, the development of new control strategies should preferably focus on the initial steps in the host–parasite interaction, of which germination is the first. Although Striga and Orobanche spp. parasitize different hosts in different parts of the world, their life cycles are very similar and involve seed germination in response to root host stimuli called strigolactones. Strigolactones are signalling molecules that play a double role in the rhizosphere as host detection signals for arbuscular mycorrhizal (AM) fungi and root parasitic plants.

In addition to their important role as rhizosphere signalling compounds, it has recently been demonstrated that strigolactones also act as a new hormone class that inhibits shoot branching in plants and hence regulates above-ground plant architecture. Here, the authors review knowledge on the root parasitic seed germination stimulants – strigolactones – as well as the regulation of their production, and describe their ecological significance. In addition, the possibilities of using this knowledge to develop new root parasitic weed control strategies is assessed.

2 ECOLOGICAL SIGNIFICANCE OF STRIGOLACTONES

2.1 Strigolactones are germination stimulants for root parasitic plants

The interaction between host and root parasitic plant begins with the exudation of secondary metabolites – the strigolactones – from the roots of the host, which induce germination of the seeds of the parasites. These signalling molecules are produced and exuded by the plants in very low, nano- and picomolar, concentrations. The strigolactones are instable in a watery environment, and it has been suggested that the induction of germination is receptor mediated. All the strigolactones characterized so far have similar chemical structures. The structural core of the molecules consists of a tricyclic lactone (ABC part) that connects via an enol ether bridge to a butyrolactone group (the D-ring) (see Fig. 2). All known strigolactones possess one or two methyl substituents on the cyclohexyl A-ring and various combinations of hydroxyl or acetate substituents around the A- and B-rings, whereas the C- and D-rings remain constant, except for the enol ether bridge, for which an epi-orientation has also recently been reported. It has been suggested that the biological activity of the strigolactones resides in this enol ether bridge. It has been proposed that 5-deoxystrigol, which does not have any hydroxyl...
substituent, is the precursor of all the other strigolactones through further chemical and/or enzymatic modification.\textsuperscript{13,16}

Strigolactones have been detected in the root exudates of a wide range of mono- and dicotyledonous plant species. Different plant species and even different varieties of one crop species produce different strigolactones and/or mixtures of these signalling compounds.\textsuperscript{5,14} To date, nine strigolactones have been structurally elucidated,\textsuperscript{15} but several new strigolactones are currently being isolated and characterized from different plant species.\textsuperscript{13,14} Because of all the potential modifications to the A- and B-rings, over a hundred strigolactone derivatives have been predicted to exist in the plant kingdom.\textsuperscript{13,17}

### 2.2 Strigolactones are hyphal branching factors for AM fungi

As mentioned above, strigolactones were identified as germination stimulants for the parasitic \textit{Striga} and \textit{Orobanche} spp. An intriguing question was why plants would produce compounds that have such negative consequences for the plants themselves. The answer to this question came only few years ago when it was demonstrated that these secondary metabolites are involved in signalling between plants and the symbiotic AM fungi.\textsuperscript{3} AM fungi are obligate symbionts that must be associated to a host plant to complete their life cycle.\textsuperscript{4,6,18} Their spores can germinate spontaneously and undergo an initial asymbiotic stage of hyphal growth, which is limited by the amount of carbon storage in the spore. If the fungal hyphae do not encounter a symbiotic partner, the hyphae cease growth and retract. When there is a host plant root in the vicinity of the germinating spore, signalling molecules released by the roots into the rhizosphere – strigolactones\textsuperscript{5} – reach the hyphae, and the fungus responds to this with increased growth and intensive hyphal branching. This intensive branching is expected to increase the probability of the fungi encountering a host root and establishing symbiosis.\textsuperscript{5,19} In spite of this apparently important function, it is not yet fully understood how essential strigolactones are for the establishment of the symbiosis and/or whether they also play a role in subsequent steps of the interaction.

The symbiosis between plants and AM fungi is widely distributed throughout the plant kingdom.\textsuperscript{18} This interaction is considered to be older than 400 million years, and it is suggested that it has played an important role in the evolution of aquatic into terrestrial plants.\textsuperscript{4} During the symbiosis, the AM fungi obtain carbohydrates from their host plant, and at the same time plants obtain water and mineral nutrients (mainly phosphorus and nitrogen) from their fungal partners, enabling them to perform better under various stressful conditions.\textsuperscript{4,6} Therefore, the ability to develop AM symbiosis is advantageous to the plants, and could explain why strigolactones are produced in spite of the risk of being abused by root parasitic plants. In this three-way interaction it is likely that the parasitic plants have evolved the capacity to perceive the presence of host plants by taking advantage of the already existing signalling between plants and AM fungi.

### 2.3 Strigolactones are a hormone class that inhibits shoot branching

Strigolactones are present in root exudates of many mono- and dicotyledonous plant species,\textsuperscript{5} including Arabidopsis, a non-mycotrophic plant genus.\textsuperscript{20} Moreover, they have also been detected in the plant, notably the shoots.\textsuperscript{14,21} Just recently, it has been shown that strigolactones – or their derivatives – also act as a new class of plant hormones.\textsuperscript{7,8} Excessive shoot-branching mutants of pea \textit{[rms (ramosus) mutants]}, rice \textit{[htd (high-tillering dwarf) mutants]} and Arabidopsis \textit{[max (more axillary shoots) mutants]} proved not to produce strigolactones. Application of the synthetic strigolactone GR24 to these mutants could rescue the wild-type branching phenotype. It was concluded that strigolactones inhibit shoot branching by preventing the outgrowth of leaf axillary buds. Like other plant hormones, the strigolactones require a receptor in order to exert their activity, they are active at very low concentrations and they can be transported in the plant over some distance.\textsuperscript{7,8} The fact that strigolactone biosynthesis is promoted under low phosphate and nitrogen conditions (see below)\textsuperscript{15,21–23} leads the authors to propose that, under inorganic phosphate (and possibly other nutrient) deficiency, plants increase the biosynthesis of strigolactones to reduce shoot branching and at the same time maximize the symbiotic interaction with AM fungi to facilitate the uptake of mineral nutrients.\textsuperscript{7,8}

### 3 BIOSYNTHETIC ORIGIN OF STRIGOLACTONES

The strigolactones were originally thought to be sesquiterpene lactones. To verify the biosynthetic origin of the strigolactones, the authors studied the effect of chemicals inhibiting biosynthesis of different isoprenoids on the production and exudation of the germination stimulants by the roots of host and non-host plants.\textsuperscript{16} In parallel to this biochemical approach, several mutants in the predicted biosynthetic pathways were examined for germination stimulant production. Based on these two different approaches, it was shown that the ABC part of the strigolactones is derived from the carotenoids by oxidative cleavage of one or several unidentified carotenoid substrates through the action of carotenoid cleavage dioxygenase enzymes, thus making the strigolactones members of the apocarotenoids.\textsuperscript{5,16,23} The carotenoid-cleaving enzymes form a small family composed of...
Figure 2. Schematic representation of the biosynthesis of strigolactones. The known biosynthetic pathway for carotenoid-derived secondary metabolites is shown by black arrows. The dotted arrows indicate possible interactions between different components in the scheme. Open arrows show several conditions or strategies that may modify the production of strigolactones (+, positive effect on strigolactone production; —, negative effect on strigolactone production). Enzymes: NCED, 9-cis epoxycarotenoid dioxygenase; CCD, carotenoid cleavage dioxygenase. Hormones: ABA, abscisic acid.

nine different members in Arabidopsis and 12 in rice, which cluster into six different groups. Carotenoid cleavage dioxygenases (CCDs) are involved in the formation of important apocarotenoids such as flavour volatiles and cyclohexenone and mycorradicin derivatives, which compose the yellow pigment that accumulates in the host roots on AM colonization (Fig. 2). Recently it was discovered that two other enzymes from this family, CCD7 and CCD8, which were already proposed to be responsible for the biosynthesis of shoot-branching inhibiting signal, are directly involved in the biosynthesis of strigolactones (Fig. 2). Indeed, pea, rice and Arabidopsis mutants for these genes produce markedly less strigolactones than the corresponding wild types, and, in pea, ccd8 mutations reduced mycorrhizal symbiosis, which could be restored with exogenously applied strigolactone. In addition, root exudates from ccd7 and ccd8 induced less AM fungal hyphae branching and less germination of Orobanche seeds. Similarly in rice, the orthologous mutants were less infected by Striga hermonthica (Del.) Benth.

The other class of carotenoid cleavage dioxygenase enzymes, the 9-cis-epoxycarotenoid dioxygenases (NCEDs), are involved in the production of the plant hormone abscisic acid (ABA), which is derived from cis-neoxanthin (Fig. 2). ABA-deficient mutants viviparous14 (vp14) in maize and notabilis in tomato, with a null mutation in the genes ZmNCED and LeNCED1 respectively, induced less germination of S. hermonthica and Orobanche ramosa L. seeds respectively. In addition, this reduction in germination stimulatory activity in tomato correlated closely with a reduction in the exudation of strigolactones, as judged by LC-MS/MS analysis. This suggests that NCED1 is involved in strigolactone biosynthesis, either directly, for example by producing the substrate for CCD7 and/or CCD8 enzymes, or indirectly through ABA action (Fig. 2).

Nutrient deficiency, mainly of phosphate and nitrogen, promotes strigolactone biosynthesis (see below). Using a microarray study on tomato grown under control and phosphate-starved conditions, however, showed no increase in the expression of LeNCED1 under phosphate starvation. This could imply that NCED1 does not control the biosynthesis of the strigolactones or that the enzyme is regulated post-transcriptionally. Alternatively, ABA itself may exert a regulatory role on strigolactone production. Then, the reduced production of strigolactones in the mutants notabilis and vp14 is due to the reduced ABA content rather than directly to reduced NCED activity. Hence, it is clear that NCED(s) are involved in the biosynthesis of strigolactones,
although further research is required to determine whether their involvement in this biosynthetic pathway is direct or indirect.

4 ENVIRONMENTAL EFFECTS ON STRIGOLACTONE PRODUCTION

As mentioned, one of the primary roles of AM fungi in the symbiotic relationship with plants is the supply of mineral nutrients, mainly phosphate and nitrogen. In many areas of the world, the concentration or availability of these essential mineral nutrients in the soil is low, which negatively impacts plant growth and fitness. Phosphorus is one of the least available of all essential nutrients in soils because of its low mobility. Moreover, the majority of the applied phosphorus may be fixed in the soil owing to the interaction with other ions and may hence become unavailable to plants. Similarly, mineral nitrogen becomes limited owing to its loss through volatilization and leaching. AM symbiosis can be of great importance for the plant partner in helping with the uptake of these essential minerals.

Root exudates produced by plants grown under phosphate limited conditions are more stimulatory to AM fungi than exudates produced under adequate phosphate nutrition. Moreover, phosphate and nitrogen deficiency have a significant stimulatory effect on the production and exudation of strigolactones (Fig. 2). The response in strigolactone production and exudation to nutrient availability differs between families of plant species. Legumes, which can establish symbiosis with Rhizobia and acquire nitrogen from root nodules, only respond to phosphate deficiency with enhanced strigolactone production, whereas in non-leguminous plant species both phosphate and nitrogen starvation enhance the production of strigolactones. Interestingly, if sorghum is inoculated with AM fungi, less germination and emergence of Striga hermonthica, indicating that the production and/or secretion of strigolactones is reduced on AM colonization.

5 STRIGOLACTONES AS A TARGET FOR PARASITIC WEED CONTROL

Many different control strategies have been developed to reduce the dramatic effect caused by root parasitic plants on crops. In general, control strategies against root parasitic plants may be classified into two different types: control methods targeting preattachment and control methods targeting post-attachment life cycle stages (Fig. 1). These strategies include both traditional and more sophisticated techniques such as sanitation and hand weeding, crop rotation, improving soil fertility, soil treatments by fumigation and solarization, biological control, the use of selective herbicides, the use of herbicide-resistant maize, suicidal germination, and breeding for resistance.

Root parasites start to affect host development immediately after attachment and cause important damage prior to parasite emergence. Therefore, control strategies based on the preattachment stage of the host–parasite interaction are preferred. Germination is the first critical step in the interaction (Fig. 1), which makes it an important target for improved control measures. One advantage of this approach is that the host signalling molecules responsible for this activity — strigolactones — are common to both Striga and Orobanche spp. Indeed, several control strategies based on the germination stimulants have already been developed (see below) (Fig. 3). A disadvantage of this approach may be that strigolactones are also signalling molecules for AM fungi and, as was recently discovered, also play a role in the regulation of shoot architecture. It is clear that the consequences for AM fungi and plant architecture of any parasitic weed control strategy targeting the strigolactones should first be carefully evaluated.

5.1 Control through enhanced germination

5.1.1 Trap and catch crops

One of the strategies to control parasitic weeds is the use of trap and catch crops in monoculture or in intercropping (Fig. 3). This strategy consists in the use of non-host species that produce germination stimulants, inducing massive suicidal germination of the parasite. These crops can be resistant in a later stage of the parasite life cycle (trap crops) or harvested before the seeds of the parasite are shed (catch crops). The effectiveness of catch and trap crops could be increased by the selection of cultivars overproducing germination stimulants (breeding) or through molecular engineering of such overproduction (Fig. 3). The latter can potentially be achieved by overexpression of one or more of the rate-limiting enzymes from the strigolactone biosynthetic pathway, such as CCD7, CCD8 or NCED(s). In addition to the benefit of catch and trap crops with enhanced production of strigolactones, the overproduction of these signalling compounds could also improve arbuscular mycorrhizal colonization, with

![Figure 3](www.interscience.wiley.com/journal/ps)
corresponding improvement in plant growth and fitness, and hence increased (trap) crop yields.

5.1.2 Synthetic germination stimulants

Another approach for the induction of suicidal germination is the use of chemicals. Synthetic germination stimulants such as Nijmegen 1, which could be potential control agents for parasitic weeds, have been developed (Fig. 3).\textsuperscript{11,14} The synthetic strigolactone analogues GR24 and Nijmegen 1 are both active at very low concentrations, indicating that they could be used to induce suicidal germination by treating the soil before the crop is sown. However, one of the limitations of this approach is that the synthetic germination stimulants should be inexpensive enough for farmers in the developing world to use them. In addition, these synthetic compounds should be much more stable than the natural strigolactones to increase their effectiveness.

5.2 Control through reduced germination

Other control strategies to avoid root parasitic weed infection may be based on the opposite strategy, aimed at reducing the germination of parasitic plant seeds (Fig. 3).

5.2.1 Chemical inhibitors

As strigolactones are derived from the carotenoids,\textsuperscript{16,23} herbicides that inhibit carotenoid biosynthesis, such as fluridone, norflurazon and clomazone, could be used to reduce strigolactone production and ultimately germination of parasitic seeds (Figs 2 and 3). Fluridone and norflurazon are bleaching herbicides that block the enzyme phytoene desaturase which represents the second dedicated enzymatic step in carotenoid biosynthesis and catalyses the conversion of phytoene to phytofluene.\textsuperscript{45} Fluridone application – even in concentrations that do not cause chlorophyll bleaching – to maize, sorghum, cowpea, rice and tomato strongly reduces \textit{in vitro} germination of \textit{S. hermonthica} and \textit{O. ramosa} seeds by the exudates of the treated plants.\textsuperscript{16,23,46} Moreover, for tomato it has been shown that this reduction in germination correlated with a reduction in the production of the strigolactones orobanchol, solanacol and dihydro-orobanchol isomers.\textsuperscript{23} The use of these inhibitors in concentrations as low as 0.01 or 0.1 \textmu M can reduce parasitic weed seed germination by about 60–80%. These data show that treatments with such herbicides may be an effective and relatively cheap method for reducing the germination of parasitic seeds, by reducing strigolactone production by the host plant, and hence reducing parasitic weed infestation, either alone or in combination with other control strategies. Preliminary experiments in pots show that treatments with these inhibitors also reduce Striga infection \textit{in situ}. Considering that some of these chemical inhibitors are used on a large scale as herbicides, and that very low concentrations are required to reduce strigolactone formation, this control strategy is promising and may even be accessible to poor regions like the African continent.

5.2.2 Soil fertility

As described above, plants grown under nutrient-deficient conditions, and particularly under phosphate and nitrogen limited conditions, are more active in stimulating germination of root parasitic plant seeds, and this increase is due to an increase in the production of strigolactones (Fig. 2).\textsuperscript{21–23} Indeed, \textit{Striga} spp. are most problematic on soils with low fertility, particularly those that are low in nitrogen.\textsuperscript{1} In many areas of the world, the concentration or availability of this essential mineral nutrient in the soil is limited, and this fact has a significant impact on plant growth and health. Obviously, the use of fertilizers would improve soil fertility and improve plant fitness and crop yield. However, at the same time it would reduce strigolactone production by the host plant and hence reduce infection by parasitic plants (Fig. 3). Indeed, the application of phosphate to phosphate-deficient fields of clover and skeleton weed significantly reduced the population of clover broomrape.\textsuperscript{32} The application of phosphate to tomato plants grown in pots suppressed infection by \textit{Orobanche aegyptiaca} (Pers.), and tomato plants grown under sufficient phosphate conditions produced fewer strigolactones and, as a consequence, less active root exudates for \textit{O. ramosa} seed germination.\textsuperscript{23,32,47} Also, exudates of rice plants grown under phosphate or nitrogen limited conditions induced more germination of \textit{S. hermonthica} than the exudates of control plants having normal phosphate and nitrogen supply (Jamil M \textit{et al.}, unpublished data). Also, in sorghum, the application of nitrogen resulted in a reduction in \textit{S. hermonthica} germination.\textsuperscript{48} It seems, therefore, that the use of fertilizers may be a useful method for reducing strigolactone production by the host plants and hence avoiding or reducing parasitic weed infection. Before using fertilizer application as a control strategy, it should be considered that the response of strigolactone production/exudation to nutrient availability differs between plant species. Therefore, fertilizer rate and composition should be carefully optimized, depending on crop species, soil fertility and possibly also parasitic weed species.

5.2.3 Mycorrhiza

Considering that strigolactones also are host detection signals for AM fungi, it is perhaps not unexpected that AM fungal inoculation of maize and sorghum leads to a clear reduction in \textit{S. hermonthica} infection.\textsuperscript{69} Indeed, although the analytical evidence has not yet been given, there is biological evidence that the reduction in \textit{S. hermonthica} infection on AM colonization is caused, at least partly, by a reduction in the production of strigolactones.\textsuperscript{28,40} The presence of AM fungal hyphal networks and the likely better mineral nutrient availability in the natural ecosystem could well keep strigolactone exudation by wild host plants within limits that do not induce large amounts of parasitic plant seeds to germinate and infect their host (Fig. 3). In addition to the effect of AM symbiosis on strigolactone production, this symbiosis also confers resistance to biotic stresses to the host plant by inducing defence-related genes,\textsuperscript{50,51} which could also partially explain why, after AM symbiosis, plants are infected to a lesser extent by \textit{Striga} spp. Altogether, the positive effect of AM colonization on plant fitness (facilitating the uptake of mineral nutrients by plants) and their effect in reducing strigolactone production and on the induction of plant defence genes make AM fungi a suitable and promising tool for controlling parasitic plants. Further research on the AM fungi–host–parasitic plant relationship is required to develop an optimal parasitic weed control strategy using AM fungi.

5.2.4 Breeding

Genetic variation for low \textit{Striga} germination stimulant (LGS) production in sorghum has been described and was used to breed for \textit{Striga}-resistant varieties and introduce them into high-yielding sorghum cultivars in several African countries.\textsuperscript{52} Genetic variation for the induction of \textit{O. aegyptiaca} germination has also been described in tomato,\textsuperscript{53} making breeding for LGS feasible and attractive. In addition, it has recently been shown that different
cultivars of tomato produce/exude largely different amounts of strigolactones.\textsuperscript{54} The tomato mutant \textit{high pigment-2} (\textit{hp-2}\textsuperscript{59}), which is an important mutant line introgressed into commercial tomato cultivars because it has enhanced levels of carotenoids including lycopene, was found to be less susceptible to \textit{O. aegytiaca} infection than the corresponding wild-type background, and this reduced susceptibility correlated well with a lower production of strigolactones.\textsuperscript{24}

Several other mutants in tomato and maize, such as \textit{notabilis} and \textit{vp14} respectively, also produce less strigolactones than their corresponding wild types.\textsuperscript{16,23} Overall, these results indicate that effective control strategies should be based preferably on the function and physiological and biochemical regulation of the molecular basis of the biosynthetic pathway for these signalling molecules, as well as of the host–parasite interaction. To date, no single approach applied alone has led to a long-term solution to any problem in any agricultural crop. Therefore, an integrated approach using several strategies, such as, for example, the control of germination of the seeds in combination with other control methods such as smart herbicide treatments and selection for crop varieties that inhibit development and/or growth or cause a reduction in seed production of the parasite, should lead to a better and durable control and hence provide solutions to the important problems in agriculture that are caused by parasitic plants.

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