CHAPTER 4

THE EFFECT OF HOST-ROOT-DERIVED CHEMICAL SIGNALS ON THE GERMINATION OF PARASITIC PLANTS

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Abstract. The parasitic plants *Orobanche* and *Striga* spp. are holo- and hemiparasites, which largely depend on a host plant to obtain their nutrients and water. The seeds of these parasites can only germinate in the presence of a chemical compound that is exuded from the roots of their host. These compounds are called germination stimulants and so far several of these compounds have been identified in the exudates of hosts (and false hosts) of several *Orobanche* and *Striga* species. The germination stimulants play an important role in fine-tuning of the lifecycle of the parasites to that of their hosts. In this chapter we describe the processes that play a role in this interaction, for example how the germination stimulants are produced by the host and how they are perceived by the parasite. Also we discuss the possible importance of the germination stimulants in determining host specificity.
Keywords: *Orobanche*; *Striga*; carotenoids; dormancy; host specificity; sensitivity

INTRODUCTION

Underground chemical signalling and parasitic plants

Chemical signalling between individuals of one species but also between individuals of different species plays an essential role in biology. Although plants cannot talk, listen or see, they communicate extensively, using secondary metabolites to convey messages (see Chapters 2 and 6, Degenhardt et al. 2003; Dicke and Hilker 2003). Although the concept of communication of plants is perhaps less easy to imagine underground, underground signalling too is of great importance for plants (Bais et al. 2004). Examples are the colonization by nitrogen-fixing bacteria (rhizobia) and the attraction of insect-parasitic nematodes by insect-attacked roots (Limpens and Bisseling 2003; Rasmann et al. 2005). In all these signalling processes, the specificity of the interaction is very important and delicately regulated. Predators are attracted to plants attacked by their prey and rhizobia respond to the roots of legumes. In non-beneficial underground interactions chemical cues produced by the

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host plant are also of great importance and also here the specificity is often amazing (Hirsch et al. 2003). An exciting example of plant–plant underground communication is the recognition by the parasitic plants *Orobanche* and *Striga* spp of chemical signals exuded by the roots of suitable host plants. The parasitic broomrapes and witchweeds can only survive on the roots of a host and must obtain most of their resources from them. The seeds of the parasitic plants are tiny, and after germination they must attach themselves to a host root within days or otherwise they will die (Butler 1995). Parasitic plants have evolved a graceful strategy to deal with this requirement: their germination depends unconditionally on compounds that are produced by the roots of their hosts in extremely low concentrations. These stimulants are collectively called strigolactones. The strigolactones belong to the chemical class of the isoprenoids, to which many of the known biologically active plant communication signals belong. Much is known about the biosynthesis of isoprenoids in above-ground plant organs; by contrast we know surprisingly little of this process in the root system. Until recently, the significance of the strigolactones for the plant itself has remained elusive (why do plants produce these compounds when they are obviously disadvantageous, since they cause parasitism?). The fact that they have persisted despite the supposedly strong counter-selection suggests that they are essential for the plant. Indeed, an intriguing recent study has shown that the strigolactones are used by arbuscular mycorrhizal fungi for their colonization process (the strigolactones are the branching factor that is required for mycorrhizal mycelia to become infective), and this most likely answers the question why plants still produce strigolactones (Akiyama et al. 2005; Matúšová et al. 2005).

Broomrapes (*Orobanche* spp.) and witchweeds (*Striga* spp.) (both *Scrophulariaceae*) can heavily infest crops with a large negative impact on agriculture in many countries. *Orobanche* spp. are holoparasites that are completely lacking chlorophyll and for their growth and development are completely dependent on their host for the supply of water and nutrients. *O. cumana* Wallr. parasitizes sunflower in eastern Europe around the Black Sea, in Spain (Akhtouch et al. 2002), and recently the pest was reported to spread widely in Israel (Aly et al. 2001). *O. ramosa* and *O. aegyptiaca* parasitize a wide range of hosts, such as tomato, potato, eggplant, tobacco, carrot, lettuce and many others (Press et al. 2001). *O. crenata* is a widespread parasite of legumes all around the Mediterranean (Press et al. 2001). *Striga* spp. belong to the hemiparasites with lower photosynthetic activity and basically behave as holoparasites (Parker and Riches 1993). They are serious pests in the African continent. Hosts of *S. hermonthica*, *S. asiatica*, *S. aspera* and *S. forbesii* include grain cereals such as maize, sorghum, millet and upland rice (Press et al. 2001). *S. gesnerioides* is a parasite of cowpea, and causes extensive damage in sub-Saharan dry areas, particularly West-Africa (Press et al. 2001).

Life cycle of Striga spp. and Orobanche spp.

The life cycles of *Striga* and *Orobanche* spp. are essentially similar; both start with the germination of the seed that is induced by compounds exuded by the roots of

their hosts (Figure 1). After germination, the radicle grows towards the host root and forms a haustorium. The haustorium is formed by the swelling of the radicle tip with a hairy structure with which the parasite attaches itself to the host root (Hood et al. 1998). The establishment of a xylem connection, tubercule formation, shooting and seed production are the next steps in the life cycle (Figure 1). In many of these steps chemical communication occurs between the host plant and the parasite. This starts with the secretion of secondary metabolites from the roots of the host (and some non- or false hosts) that induce the germination of the seeds of the parasite. After germination, additional host-derived secondary metabolites play a role in the plant–parasite interaction. The orientation of the parasite's radicle growth towards the host root has been postulated to be directed by the concentration gradient of the germination stimulant (Dube and Olivier 2001) or by other host-root-derived compounds. Host-produced allelochemicals may interfere with the interaction between host and parasite. In sunflower, for example, coumarins were shown to be responsible for the inhibition of germination and necrosis of *O. cernua* after germination (Serghini et al. 2001). Attachment to the root of the host plant and the host–parasite xylem connection is mediated by a haustorium, of which the formation

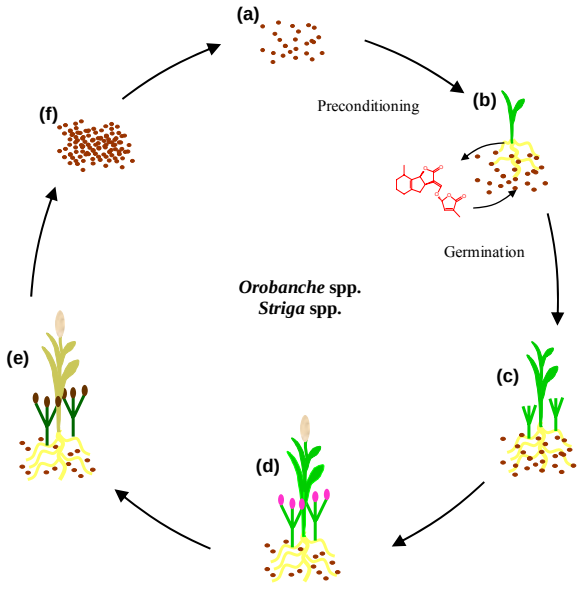

Figure 1. *Life cycle of parasitic plants Orobanche spp. and Striga spp. (a) the seeds are buried in the soil; (b) they become sensitive to the germination stimulants exuded by the roots of the host plant and may germinate; (c) the germinated seeds form a haustorium by which they attach themselves to the host root, establish a xylem connection and emerge; (d) parasitic plants flower; (e) they produce mature seeds and end up in a new generation of seeds in soil; (f) in the next season the cycle starts again (a)*

is initiated by host-derived secondary metabolites, notably phenolics (Keyes et al. 2001; Yoder 2001; Hirsch et al. 2003). Finally, after haustorium formation the penetration of intrusive cells into the host root xylem is realized, probably with the involvement of hydrolytic enzymes produced by the parasite (Losner-Goshen et al. 1998). Successful establishment of a xylem connection is also dependent on the host and can be terminated by host-produced toxins (Goldwasser et al. 1999; Labrousse et al. 2001; Serghini et al. 2001). Indeed, the resistance of some sorghum cultivars is based on the induction of necrosis at the attachment site on the root (Mohamed et al. 2003).

Germination stimulants

As described above the first involvement of chemical signalling in the life cycle of the parasitic plant is the induction of germination by germination stimulants. For *Striga* spp. several germination stimulants were identified from host and non-host plants. Most of them are known as strigolactones (Figure 2). The first identified germination stimulant was strigol; it was isolated from the non-host plant cotton (Cook et al. 1972). Recently, Yoneyama and co-workers isolated and characterized from cotton root exudates also strigyl acetate, which induces germination of *O. minor* (Sato et al. 2005). Germination stimulants in maize and sorghum were identified as strigol (Siame et al. 1993) and sorgolactone (Hauck et al. 1992). Alectrol was identified in the root exudate of cowpea (Muller et al. 1992). Alectrol and orobanchol were isolated and identified from the root exudate of red clover (Yokota et al. 1998) (Figure 2). The same group reported on the isolation of four novel strigolactones from the root exudate of tomato, and the presence of a novel strigol isomer in the root exudate of sorghum (Yoneyama et al. 2004). There are also several synthetic compounds inducing germination of parasitic plants (Reizelman and Zwanenburg 2002). Among them is the strigol analogue GR24, a very potent synthetic stimulant, which induces germination of many *Orobanche* and *Striga* spp. and is widely used as a positive control in most laboratory experiments (Figure 2).

It is obvious that the germination stimulants play a crucial role in the life cycle of parasitic plants and could also be an important target for the design of new control strategies for agriculturally important parasitic plants. Nevertheless, little is known about how these compounds are produced by the host, how they are perceived by the parasite and how selective this process of host recognition is. Here we will review our own work and that of others pertaining to these three subjects.

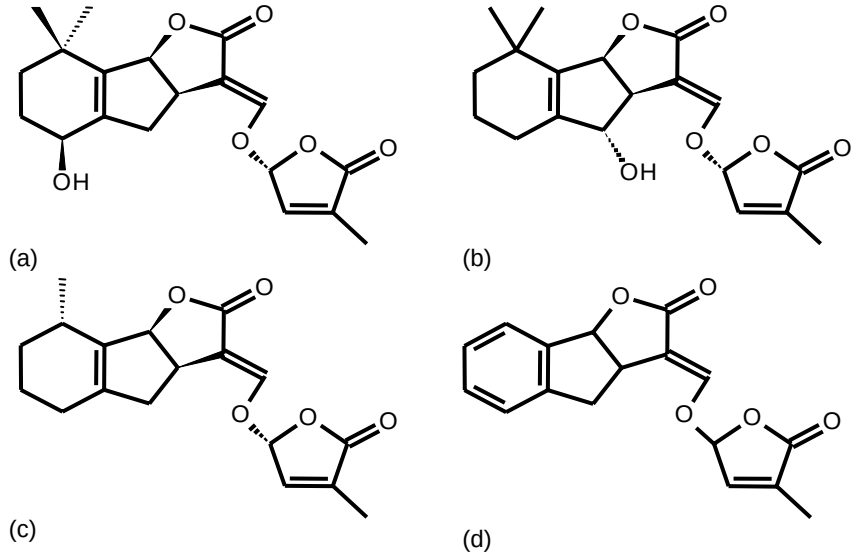

Figure 2. *Structure of strigolactone germination stimulants. (a) (+)-strigol; (b) orobanchol; (c) sorgolactone;(d) synthetic germination stimulant GR24*

PERCEPTION OF GERMINATION STIMULANTS

Germination stimulants are exuded from the roots of host plants in very low quantities. For example, seedlings of cotton produce about 14 pg of strigol per plant per day (Sato et al. 2005). Considering these extremely low amounts it is important that we are aware that the seeds used in studies on natural germination stimulants are sensitive to the stimulants and that this sensitivity is not a static parameter. Indeed, the availability of the synthetic germination stimulant GR24, of which in principle relatively large concentrations can be used (compared with the predicted concentrations of naturally occurring germination stimulants), has more or less obscured the importance (and variability) of sensitivity in a number of studies. To become responsive to the germination stimulants the seeds of *Orobanche* and *Striga* spp. require a moist environment for a certain period of time at a suitable temperature. This treatment is described as preconditioning or conditioning and is comparable to what is called (warm) stratification in seeds of non-parasitic plants or release of dormancy (Matúšová et al. 2004). During preconditioning, seeds become metabolically active (Mayer and Bar Nun 1997). The temperature used during preconditioning strongly affects the responsiveness to chemical stimulants. Seeds of *O. crenata* are able to germinate after preconditioning from 5°C to 30°C (Van Hezewijk et al. 1993). However, preconditioning at sub-optimal temperatures results in a lower sensitivity to the germination stimulant, which does not increase even after prolonged preconditioning (Van Hezewijk et al. 1993; Matúšová et al. 2004).

Preconditioning at an optimal temperature (e.g., about 20°C for *O. cumana* and 30°C for *S. hermonthica*) releases dormancy within 2-3 weeks and increases the sensitivity to GR24 by several orders of magnitude (Figure 3). After reaching maximum sensitivity, prolonged preconditioning induces secondary dormancy, i.e., decreased sensitivity of *O. cumana* and *S. hermonthica* to GR24 (Figure 3) (Matúšová et al. 2004). A similar trend was observed for *O. ramosa* (Gibot-Leclerc et al. 2004). It is important to note that the rapid changes in sensitivity during prolonged preconditioning are particularly visible at low concentrations of GR24. At higher concentrations, GR24 usually induces high germination, regardless of the preconditioning period. Parasitic plant seeds are highly sensitive to the germination stimulant for a short period of time only, and then enter into secondary dormancy relatively quickly. These large changes in sensitivity to germination stimulants are suggestive of a safety mechanism that ensures that seeds can respond to the germination stimulants produced by their host only during a restricted period of the year (assuming – and this is quite likely – that the hosts continue to produce strigolactones throughout further development). This is of great ecological importance as the parasitic plants require a long enough period of time to reproduce, and germination during the later stages of host development would not allow this. The similar pattern of increasing and decreasing sensitivity to GR24 that we observed with *S. hermonthica* seeds preconditioned for a prolonged period of time under field conditions suggests that the mechanism observed is indeed not just a laboratory phenomenon but is of ecological significance (Matúšová et al. 2004).

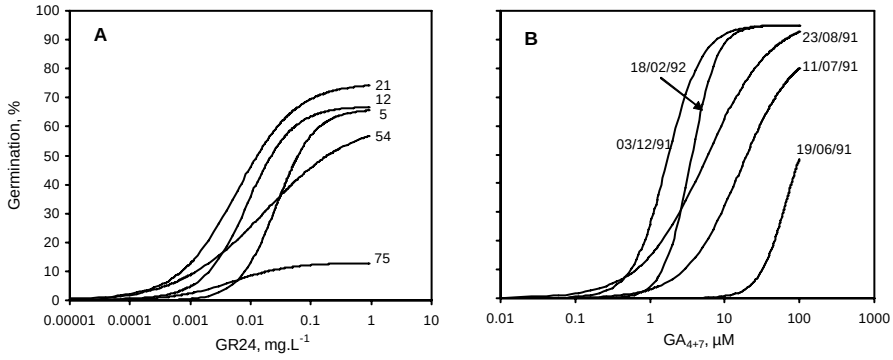

Figure 3. *A. Dose–response curves showing the effect of the preconditioning period on the sensitivity of Striga hermonthica to the germination stimulant GR24. Numbers indicate days of preconditioning at 30°C. B. Changes in gibberellin GA_{4+7} dose–response curves of Arabidopsis thaliana as a consequence of burial in the field. Dates indicate the date that seeds were exhumed and their germination tested in a range of gibberellin concentrations. Burial date: 19 June 1991 (Derkx and Karssen 1994).*

Interestingly, these changes in the sensitivity of the parasitic plant seeds to germination stimulants display a similarity to the dormancy (sensitivity) changes of seeds of non-parasitic wild plants to, for example, light (position), nitrate (growth

conditions), and gibberellin (Derkx and Karssen 1993a; 1993b; 1994; Matúšová et al. 2004). For non-parasitic plants, this mechanism ensures that seeds will germinate and grow under favourable conditions only. Apparently, parasitic plants have adapted this mechanism to recognize suitable growing conditions also, i.e., the presence of a suitable host, by responding to a typical host/plant-produced metabolite. Indeed, the shift in the GR24-response curves and the shift in the gibberellin-response curves during dormancy relief in *Arabidopsis*, as reported by Derkx and Karssen (1994), are quite similar (Figure 3). Gibberellins and a putative gibberellin receptor play a crucial role in the germination of non-parasitic wild-plant seeds, even though changes in the sensitivity to gibberellins was hypothesized not to be the mechanism responsible for the changes in dormancy in the seeds of *Arabidopsis* and *Sisymbrium officinale* (Derkx and Karssen 1993a; 1994). According to a model proposed by Hilhorst and Karssen (1988), gibberellin biosynthesis and sensitivity to gibberellin in these seeds are controlled by a receptor that is activated by nitrate and red light (Hilhorst 1993; Hilhorst and Karssen 1988; Vleeshouwers et al. 1995). The structure of the strigolactone parasitic-plant germination stimulants and the gibberellins is fairly similar, and it is not unlikely that their respective receptors have a common origin (Matúšová et al. 2004). A gibberellin receptor in non-parasitic plant seeds was postulated by (Hilhorst et al. 1996; 1986). The involvement of a receptor in germination-stimulant recognition has been postulated (Wigchert and Zwanenburg 1999) and is supported by the dose–response curves (Figure 3) (Matúšová et al. 2004).

BIOSYNTHETIC ORIGIN OF GERMINATION STIMULANTS

Germination stimulants are exuded from the roots of host plants in very low concentrations, which makes the isolation and characterization of these compounds quite difficult. Moreover, the big losses during the isolation process and instability of these compounds (Sato et al. 2005) are reasons why large volumes of root exudates are still needed for their characterization. For the same reasons, also the study of the biosynthesis of these compounds is difficult. The strigolactone germination stimulants were isolated from a wide variety of plant species and induce germination of a range of parasitic plant species. Nevertheless, they are strikingly similar and are obviously derived from the same biosynthetic pathway. The strigolactones are usually defined to be sesquiterpene lactones (Butler 1995; Yokota et al. 1998), but there is also some structural similarity to higher-order terpenoids/isoprenoids such as abscisic acid and other compounds, which are derived from the carotenoid pathway (Parry and Horgan 1992; Tan et al. 1997; Bouwmeester et al. 2003).

Isoprenoids are biosynthesized from isopentenyl diphosphate (IPP) and the isomeric dimethylallyl diphosphate (DMAPP) via two independent pathways: the cytosolic mevalonic-acid (MVA) pathway and the plastidic, non-mevalonate, methylerythritol-phosphate (MEP) pathway. The plastidic MEP pathway produces IPP and DMAPP for the biosynthesis of monoterpenes, diterpenes, carotenoids, the plant hormones gibberellins and abscisic acid and the side chains of chlorophylls,

plastoquinones and phylloquinones. Sesquiterpenes, sterols and triterpenes are produced from the cytosolic MVA pathway.

Elucidation of germination-stimulant biosynthetic pathway

To determine the biosynthetic origin of the germination stimulants produced by plants we used two approaches: (1) the use of specific inhibitors of isoprenoid pathways and (2) the use of defined mutants in predicted biosynthetic pathways. Inhibitors were applied to seedlings only during a number of days to ensure normal plant development. Because of the very low concentrations at which the germination stimulants are active an analytical method to study the consequences of our treatments on germination-stimulant formation could not be used. Instead we used a germination bioassay as a very sensitive and useful detection method to analyse production of germination stimulants even in single seedlings.

The isoprenoid-pathway inhibitors mevastatin (inhibitor of the cytosolic MVA pathway) and fosmidomycin (inhibitor of the plastidic MEP pathway) only had a minor effect on germination-stimulant formation, possibly because of the exchange of IPP that has been shown to occur between the two pathways, particularly upon the use of these inhibitors (Hemmerlin et al. 2003). However, the carotenoid-pathway inhibitor fluridone reduced root-exudate-induced germination by about 80% compared with control maize seedlings, suggesting that the germination stimulants produced by maize are derived from the carotenoid pathway (Figure 4) (Matúšová et al. 2005). Therefore, we decided to analyse the induction of germination by a series of carotenoid mutants from the Maize Genetics COOP Stock Center, Urbana, Illinois. The root exudates of several maize carotenoid mutants *lw1, y10*, *al1*, *al1y3, vp5* and *y9* (Figure 4) were tested for induction of *S. hermonthica* seed germination. The seedlings of all mutants induced lower germination of *S. hermonthica* seeds in comparison to their corresponding wild-type phenotype siblings (Matúšová et al. 2005). The carotenoid biosynthesis inhibitor fluridone blocks the activity of phytoene desaturase, which corresponds to the maize *vp5* locus (Li et al. 1996; Hable et al. 1998). Both fluridone-treated maize and the *vp5* mutant root exudates induced significantly lower germination of *S. hermonthica*. Also treatment with the herbicide amitrole that blocks lycopene cyclase in maize seedlings (Dalla Vecchia et al. 2001) resulted in lower germination of *S. hermonthica* seeds than induced by control seedlings. The results in germination bioassays with root exudates of amitrole-treated plants suggest that the germination stimulants are derived from the carotenoid pathway below lycopene (Figure 4) (Matúšová et al. 2005).

Below this point in the carotenoid pathway there are unfortunately only few well-characterized mutants available and one putative inhibitor of the enzyme *9-cis*-epoxycarotenoid dioxygenase (NCED), naproxen (Lee and Milborrow 1997; Schwartz et al. 1997) (Figure 4). The formation of the germination stimulant of maize was reduced by the use of naproxen. Bioassays with maize *vp14*, a mutant of NCED, confirmed the result obtained with naproxen. Also *vp14* induced lower

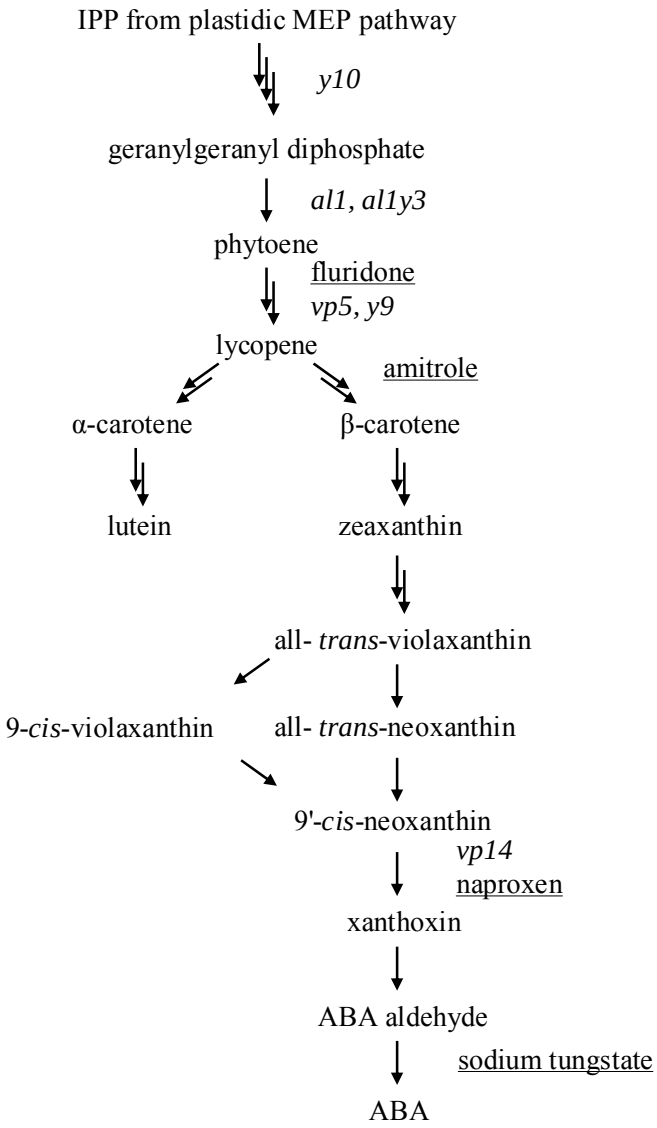

Figure 4. *Schematic representation of the carotenoid and abscisic-acid biosynthetic pathway. Carotenoid maize mutants (italics) and inhibitors (underlined) at different steps in the pathway are indicated*

germination. This suggests that carotenoid cleavage is involved in germination-stimulant biosynthesis, which is to be expected as the C40 carotenoids need to be cleaved in order to lead to the C14 (excluding the D-ring) strigolactones. The action

of NCED leads to the formation of abscisic acid (ABA), and we tested whether ABA is a precursor of the germination stimulants. However, plants supplied with low concentrations of ABA induced much lower *S. hermonthica* germination, whereas treatment with the inhibitor of ABA-aldehyde oxidation, sodium tungstate, did not have any effect on *S. hermonthica* germination (Figure 4) (Matúšová et al. 2005). This shows that the germination stimulants are neither derived from intermediates below ABA aldehyde nor from ABA itself. The reduction of root-exudate-induced germination by ABA is most probably due to feedback inhibition by the exogenously applied ABA on the carotenoid pathway (Matúšová et al. 2005). In conclusion, the germination stimulants are derived from the carotenoid pathway through the action of a carotenoid-cleavage enzyme, possibly NCED. The cleavage may occur in several steps of the pathway and is expected to lead to the production of a C15 aldehyde, which we have postulated can be converted to the strigolactones in a number of enzymatic steps (Matúšová et al. 2005).

Germination of *S. hermonthica* is also induced by cowpea and sorghum root exudates (Gurney et al. 2002; Rugutt and Berner 1998). In cowpea root exudate the strigolactone alectrol has been identified (Muller et al. 1992), in sorghum exudates sorgolactone (Hauck et al. 1992) and hydroquinone (Chang et al. 1986). The root exudates of fluridone-treated cowpea induced about 80% less germination of *S. hermonthica* than those of non-treated cowpea. Interestingly, also the germination of *O. crenata* seeds with fluridone-treated-cowpea root exudate was less than that induced by the control. Fluridone treatment of sorghum seedlings almost completely blocked subsequent exudate-induced germination of *S. hermonthica* seeds (Matúšová et al. 2005). These results show that the germination stimulant(s) of *S. hermonthica* exuded from the roots of cowpea and sorghum is (are) also derived from the carotenoid pathway. Also the cowpea-produced germination stimulant of *O. crenata* is derived from the carotenoid pathway. The germination stimulant(s) of *O. crenata* produced by its legume host(s) have not been identified yet, but our results suggest that this species also responds to a strigolactone germination stimulant. With regard to sorghum, Keyes and co-workers have claimed that the phenolic sorgoleone is the sorghum germination stimulant of *Striga* spp. (Keyes et al. 2001), but our results suggest that the natural sorghum germination stimulant is a strigolactone, such as sorgolactone. We have proven the carotenoid origin of germination stimulants for two parasitic plant species in three mono- and dicotyledonous hosts. At the same time, Yoneyama and co-workers have demonstrated strigolactones – known ones as well as new (tentatively) identified ones – in the root exudates of other plant species such as red clover and tomato (Yoneyama et al. 2004; Yokota et al. 1998), suggesting that carotenoid-derived germination-stimulant formation occurs in a variety of plant species.

ROLE OF GERMINATION STIMULANTS IN HOST SPECIFICITY

From the work by Yoneyama and coworkers (2004) on the identification of strigolactone germination stimulants it has become clear that there is a large structural diversity in the strigolactones (Yoneyama et al. 2004). Although the

biological activity of the strigolactones resides mainly in the D ring (Mangnus and Zwanenburg 1992), an interesting question is whether the small changes in the remainder of the molecule have an effect on receptor binding in the parasitic plant seeds, and hence on host–parasite specificity. Of course host–parasite recognition/selectivity occurs at different stages of the life cycle also after germination (also see above). For example, the haustorial initiation and development up to attachment are very similar for host and non-host plants, but development following attachment differs for host (successful) and non-host (not successful) species (Hood et al. 1998). Nevertheless, the recognition of the germination stimulant is a crucial moment in the life cycle of the parasitic plants. Here, a strong selection pressure is present that should ensure that the seeds of the parasites only germinate in the presence of a true host and thus may complete their life cycle. Nevertheless, a number of examples suggest that the specificity may not be very high. Alectrol, for example, is inducing germination of *S. gesnerioides* (Muller et al. 1992), but it was also identified in red clover as a germination stimulant for *O. minor* (Yokota et al. 1998). Wigchert and Zwanenburg (1999) induced germination of the seeds of *O. crenata* – which normally parasitizes legumes – with sorgolactone, one of the germination stimulants identified in sorghum, and the root exudate of cowpea induces germination of *S. hermonthica*, which is known to parasitize monocotyledons. Finally, the synthetic strigolactone analogue GR24 (Figure 2) induces germination of many parasitic plant seeds regardless of parasite or host plant species.

On the other hand, there are examples of a certain degree of host specificity. Not all host plant species induce germination of all parasitic plant seeds. Also, not all synthetic germination stimulants induce germination of all parasites to the same extent (Mwakaboko 2003). We have compared the induction of germination of *S. hermonthica* batches collected from maize and sorghum by the exudates of maize (host), cowpea (non-host) and the synthetic germination stimulant GR24 (Table 1). Maize root exudates induced 36% germination of *S. hermonthica* seeds collected from maize. Cowpea root exudates induced 51% germination, and 0.001 $mg.l^{-1}$ of GR24 induced 44% germination of the same *S. hermonthica* seeds. The highest germination (62%) was induced by 0.1 $mg.l^{-1}$ GR24 (Table 1). In contrast, *S. hermonthica* seeds collected from sorghum germinated to 37% in response to the maize root exudate, to 22% in response to the cowpea exudate and to 49% in response to 0.001 $mg.l^{-1}$ of GR24, whereas the maximum germination in response to 0.1 $m.l^{-1}$ GR24 was 96%. *S. hermonthica* collected from another sorghum field responded to maize and cowpea root exudates by very low germination (15 and 14%, respectively), even though germination in 0.001 and 0.1 $mg.l^{-1}$ GR24 was as high as 28% and 89%, respectively (Table 1). The slightly different response of the two sorghum-collected *S. hermonthica* batches may be due to the fact that different sorghum varieties may have different root exudate compositions.

Table 1. *Germination of Striga hermonthica induced by root exudates of maize, cowpea and the synthetic germination stimulant GR24. Numbers are averages of 6 individual replicates ± SE).*

	Germination (%) induced by				
			GR24, mg.l^{-1}		
Origin of *Striga hermonthica* seeds	maize	cowpea	0.001	0.01	0.1
Maize (Kenya)	36 ± 2	51 ± 1	44 ± 7	49 ± 7	62 ± 3
Sorghum (Sudan)	37 ± 4	22 ± 2	49 ± 5	82 ± 8	96 ± 1
Sorghum (Mali)	15 ± 2	14 ± 1	28 ± 7	56 ± 2	89 ± 2

These results also show that even if parasitic plant seed populations are able to germinate up to 100% (with GR24), they still can respond quite differently to the root exudates of host (or non-host) plants. We found similar differences in host specificity in several populations of *O. ramosa* collected from tomato, tobacco and rapeseed. Most of *O. ramosa* populations germinated to about 80% in a low (0.001 mg.l^{-1}) concentration of GR24 (maximum germination, in 0.1 mg.l^{-1} GR24, 90-95%). However, the same tomato root exudates induced high germination of *O. ramosa* collected from tomato and tobacco fields but almost no germination of *O. ramosa* parasitizing rapeseed (data not shown). On the other hand, the *O. ramosa* collected from rapeseed germinated up to 90% after induction with the hairy-root exudates of *Arabidopsis*. It is obvious that there is some specificity in induction of germination by tomato (Solanaceae) or *Arabidopsis* (Brassicaceae) root exudates, depending on the host parasitized by the parent plant. However, Gurney et al. (2002) showed that host specificity is more complex and is also determined during later stages of the host–parasite interaction. In general, the seeds of *Orobanche* or *Striga* can germinate in the presence of several germination stimulants, but to a different extent. The germination in the presence of different host exudates gives the parasite an advantage of greater diversity of resources and ensures the survival of the parasite if the 'true' host is no longer present in the surrounding environment (Watling and Press 2001). The enormous amount of seeds produced by single plants of *Orobanche* or *Striga* spp. provides the best guarantee for the individual's contribution to future generations even if the most preferred host is not present (anymore).

CONCLUSION

This review summarizes what is known about the importance of the strigolactone germination stimulants in the interaction between host plants and the parasitic *Orobanche* and *Striga* spp. During preconditioning large changes in sensitivity of the parasitic plant seeds to the germination stimulants occur and there is an

interesting analogy between these changes in sensitivity in parasitic plant seeds and the changes in sensitivity to other environmental and internal factors in their non-parasitic counterparts (Figure 3). These changes in dormancy may have ecological significance in restricting germination to the right period of the year. The selectivity of the response of parasitic plant seeds to specific germination stimulants may be one of the factors that determine host–parasite specificity. Finally, we have shown that the strigolactone germination stimulants are derived from the carotenoid biosynthetic pathway. This is a major breakthrough, and intriguing in view of the accumulation in mycorrhized roots of apocarotenoids. Hopefully, the knowledge that we are generating about the germination stimulants will help to propose the most effective strategies to eliminate the parasite without a harmful impact on the host plant.

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