

## Molecules of Interest

## Strigol: Biogenesis and physiological activity

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## Abstract

The role played by molecules of the strigolactone family in stimulating the germination of seeds of parasitic weeds of the genera *Striga*, *Orobanche* and *Alectra* has never been clearly elucidated. The biogenesis of these unusual terpenoid lactones, originally identified in minute quantities in the root exudates of a small number of host plants and two or three “false hosts”, also remains obscure. These lactones, as the chemical signals which initiate the life cycle of *Striga*, are consequently at the forefront of the *Striga* research effort. This paper reviews recent key discoveries relating to the biosynthesis and mode of action of strigolactones, and summarises the evidence suggesting that these molecules may be far more widely distributed and have a greater physiological significance than has hitherto been appreciated.

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## 1. Introduction

The mysterious destruction of cereal crops in parts of subtropical and tropical Africa and Asia by parasitic weeds of the genus *Striga* (Orobanchaceae, ex Scrophulariaceae (Olmstead et al., 2001)) led to their receiving the nickname of “witchweed” (Parker and Riches, 1993). Seeds of these hemiparasites infest two-thirds of the arable land of Africa (Press et al., 2001) and constitute the biggest single biological cause of crop damage in the continent. Combating the ravages of *Striga*, most of whose damage is inflicted before the parasite shoots break the surface, is thus a major issue in international development and poverty alleviation (Press et al., 2001; Oswald, 2005).

*Striga* is one of several families of parasitic weeds whose life cycles appear to have evolved to take advantage of the secretion of certain chemicals from the roots of the hosts. Holoparasites of the genera *Orobanche* and *Alectra*, which prey on dicotyledonous plants including legumes and mem-

bers of the Solanaceae and Brassicaceae, behave in an analogous manner.

Recognition of chemical cues by dormant seeds of the parasitic weeds in the soil is required to stimulate germination. The nature of these chemical cues was first demonstrated by Cook et al. (1966) who reported the isolation of (+)-strigol (**1**) from the roots of cotton (*Gossypium hirsutum* L.), a non-host plant which nevertheless has a strong stimulatory effect on *Striga* seed germination. It was later shown (Siame et al., 1993) that strigol was the main germination stimulant secreted by a number of the most commercially important *Striga* hosts, including maize (*Zea mays* L.) and millet (*Pennisetum* spp.). It has also been identified in the root exudates of other cereals (e.g. *Sorghum bicolor* [L.] Moench) and several dicotyledonous plants including *Menispermum dauricum* DC. (Yasuda et al., 2003). The quantities produced by the roots are tiny. Cotton seedlings grown hydroponically at 25 °C showed average strigol production levels of ~15 pg/plant/day, with peak daily production of ~30 pg/plant around days 5–7 (Sato et al., 2005). This corresponds to a concentration of ca.  $3 \times 10^{-10}$  M in the seedling root exudates. However strigol has been shown to be an effective germination stimulant at even lower levels than this: 50% germination of

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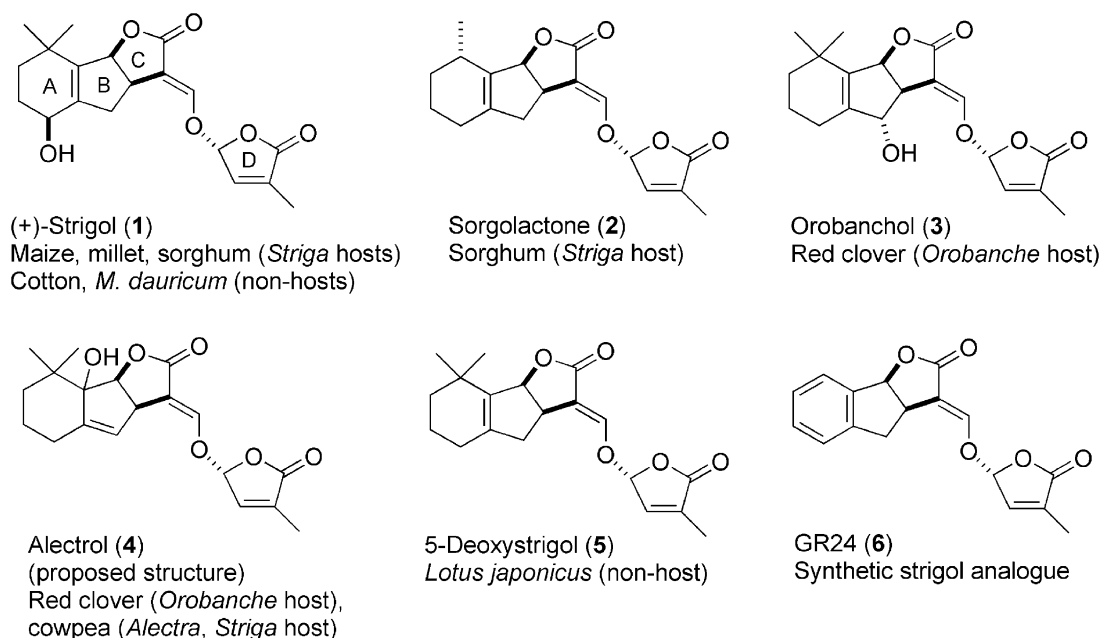


Fig. 1. The strigolactone family. Captions denote plants from which the strigolactones have been isolated.

seeds of *Striga lutea* Lour. (= *Striga asiatica* [L.] Kuntze) was achieved by aqueous solutions of (+)-strigol at  $10^{-11}$  M (Cook et al., 1972).

Strigol is a member of a family of compounds collectively known as the strigolactones. It is now known that these metabolites are secreted by the roots of numerous mono- and dicotyledonous plants, and it is speculated that they may be very widely distributed across the plant kingdom (Matusova et al., 2005). The common features of this class of compounds are clearly discernible by  $^1\text{H}$  NMR and mass spectrometric analysis (Cook et al., 1972; Hauck et al., 1992; Sato et al., 2005) (Fig. 1). The basic structural unit is a tricyclic lactone which connects via an enol ether bridge to a butyrolactone (the D-ring) which is necessary for the biological activity of these compounds (Mangnus and Zwanenburg, 1992; Wigchert and Zwanenburg, 1999). The naturally occurring strigolactones all possess at least one methyl substituent on the cyclohexyl A-ring, and variable hydroxylation around the A- and B-rings. Sorgolactone (2), orobanchol (3) and alectrol (4) have all been shown to possess germination stimulant activity for seeds of *Orobanche* and *Alectra* as well as *Striga* spp. (Hauck et al., 1992; Yokota et al., 1998). 5-Deoxystrigol (5), recently identified as a metabolic product of *Lotus japonicus* L. root cultures, appears to play another key ecological role. Akiyama et al. (2005) have identified this compound as the “branching factor” responsible for the key events in colonisation of *Lotus* roots by arbuscular mycorrhizal (AM) fungi.

## 2. Biogenesis of strigolactones

Classically the strigolactones have been described as sesquiterpene lactones. However, little was known about

their biogenesis until very recently, when a series of studies by Matusova et al. (2005) established that (+)-strigol is in fact a product of the carotenoid biosynthetic pathway. Germination of *Striga hermonthica* (Del.) Benth. induced by root exudates of maize and cowpea (*Vigna unguiculata* [L.] Walp.) was strongly inhibited when the host plant seedlings were grown in the presence of fluridone, an inhibitor of carotenoid biosynthesis. Other possible sources of the inhibitory effect, such as secretion of new inhibitory metabolites, were excluded by a rigorous series of control experiments. Furthermore, seedlings of maize carrying mutations in genes of carotenoid metabolism were also found to cause significantly lower stimulation of *S. hermonthica* germination than a comparable biomass of non-mutant maize. One of these mutant strains, *vp14*, bears a disrupted 9-*cis*-epoxycarotenoid dioxygenase (NCED) gene, which is responsible for the oxidative cleavage of carotenoids in the biosynthesis of abscisic acid (ABA). Matusova et al. have proposed that the strigolactones are thus a product of the carotenoid metabolic pathway and that they derive from the action of an enzyme of the NCED family (Fig. 2). The substrate specificities of NCEDs have not been thoroughly investigated and it is possible that the immediate precursor of the strigolactone ABC-rings may be any one of several carotenoids.

Also investigated in this study were the effects of inhibitors of the early stages of terpene biosynthesis in the host plants on *Striga* germination stimulation. Both mevastatin (which inhibits the cytosolic formation of the terpene precursor isopentenyl pyrophosphate (IPP) via the mevalonic acid pathway) and fosmidomycin (which inhibits the formation of IPP via the 1-deoxyxylulose 5-phosphate pathway in the plastids) caused a small reduction in the propensity of maize roots to stimulate *Striga* germination.

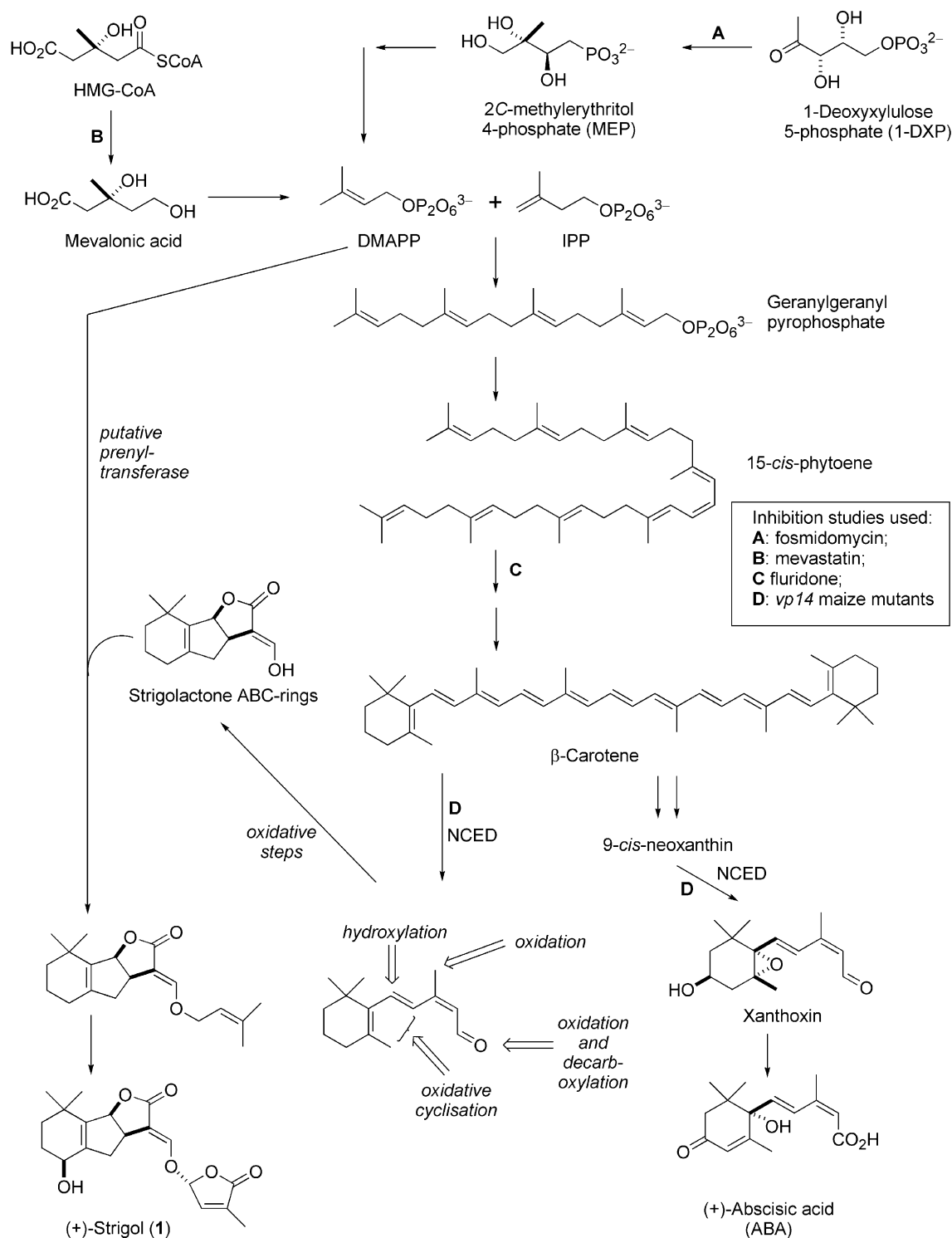


Fig. 2. Proposed outline biosynthesis of strigol based on the findings of Matusova et al. (2005).

The relatively small effect of fosmidomycin was ascribed to cross-talk between the cytosolic and plastidic pathways for IPP formation, in which IPP units needed for carotenoid biosynthesis in the plastids could be supplemented by import of cytosolic IPP. It was suggested that the small but unexpected suppressive effect of mevastatin on germination stimulation might be accounted for by there being

a cytosolic component to strigolactone biosynthesis. Thus, Matusova et al. proposed that the carotenoid-derived strigolactone ABC-portion might be exported to the cytosol prior to coupling with a D-ring precursor derived from the cytosolic terpene biosynthetic pathway, such as dimethylallyl pyrophosphate (DMAPP), as shown in Fig. 2. A series of oxidative steps, also occurring in the cytosol, would

then complete the biosynthesis, in an analogous manner to that of the gibberellins and ABA.

### 3. Mode of action of strigol

The details of how strigol and its analogues stimulate the germination of *Striga* and *Orobanch* seeds have not yet been elucidated. An extensive series of structure–activity studies using synthetic analogues of strigol has been carried out by Zwanenburg and co-workers, and from these it has been established that the biological activity of the strigolactones resides in the lactone–enol ether–D-ring portion of the molecule (Mangnus and Zwanenburg, 1992; Wighert and Zwanenburg, 1999). Nucleophilic attack at the enol ether bridge, which would result in covalent modification of the putative strigolactone receptor as shown in Fig. 3, has been postulated as providing the first chemical signal in the cascade which leads to germination (Mangnus and Zwanenburg, 1992). A wide range of substitution patterns can be tolerated around the A- and B-rings without abolition of stimulant activity, although it appears that individual species of *Striga* and *Orobanch* have evolved a selectivity for particular substitution patterns according to the main stimulants produced by their primary hosts.

A number of studies have linked strigolactone recognition with the initiation of ethylene biosynthesis in the seeds of parasitic weeds (Logan and Stewart, 1991; Zehhar et al., 2002). Application of ethylene gas to *Striga*-infested soil before crop planting is used in some countries as a *Striga* control measure as it induces “suicidal germination” of the seeds, which subsequently die in the absence of a host (Parker and Riches, 1993). Treatment of *O. ramosa* seeds with inhibitors of ethylene biosynthesis or action resulted in a dramatic suppression of germination stimulated by the synthetic strigol analogue GR24 (6) (Zehhar et al., 2002). Several inhibitors of gibberellin biosynthesis, which indirectly affects ethylene biosynthesis, showed a similar suppressive effect on GR24-induced germination of *O. ramosa*. By contrast, the synthetic ethylene precursor 2-chloroethylphosphonic acid was found to stimulate ger-

mination in *O. ramosa* even in the absence of GR24. Similar results have been reported in *S. hermonthica* (Logan and Stewart, 1991). It has been suggested that the strigolactones act as elicitors of ethylene biosynthesis, and that it is ethylene itself which initiates germination (Logan and Stewart, 1991).

### 4. Conclusions

Although some important discoveries have been reported very recently on the biosynthesis and mode of action of strigol, the details of its behaviour at the molecular level remain shrouded in mystery. The carotenoid pathway is now known to play a key role in the biosynthesis, but the nature of the intermediate metabolites and the identity of the enzymes involved have yet to be determined. The recent discovery of a bioactive 5-deoxystrigol (5) (Akiyama et al., 2005) does suggest that the likely immediate precursor of all the strigolactones is an ABC-ring fragment of the form shown in Fig. 2, coupled with a hemiterpenoid D-ring precursor (Matusova et al., 2005), though the nature and location of the enzyme responsible for coupling these fragments is as yet unreported. The strigolactone receptor also remains elusive, despite one reference to the successful purification of a strigolactone binding protein from membrane fractions of *S. hermonthica* seeds (Reizelman et al., 2003).

Intriguingly, a butyrolactone moiety very similar to the D-ring of strigol also exists in another compound recently identified as a germination stimulant. The furo[2,3-*c*]pyrone 7, a combustion product derived from cellulose, stimulates the germination of a range of plant seeds from regions prone to bush fire (Flematti et al., 2004). Its effects on *Striga* or *Orobanch* are not yet reported. The discovery of this germination stimulant does present the possibility that the strigolactone receptor may be a member of a widespread family of seed surface proteins all designed to recognise a butyrolactone moiety. It is unlikely, however, that recognition of 7 by such a receptor would occur through covalent attachment of the type postulated in Fig. 3;

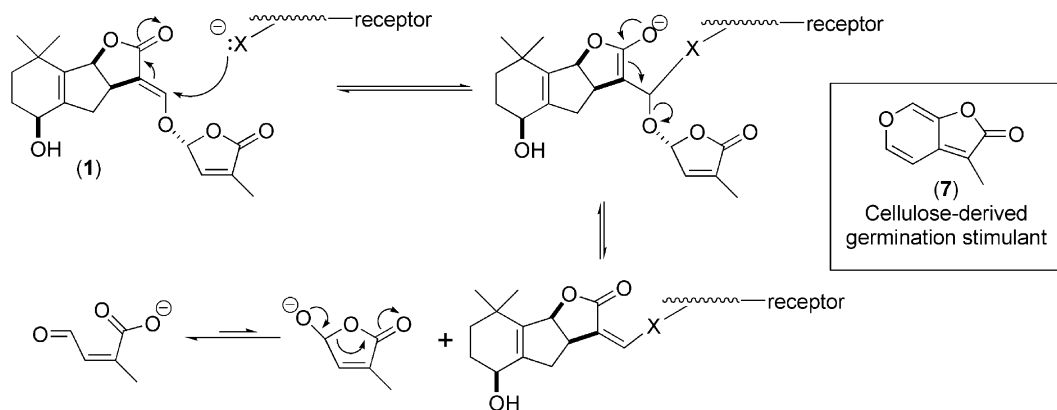


Fig. 3. Proposed mechanism of action of strigol at the strigolactone receptor according to Mangnus and Zwanenburg (1992).

a covalent linkage, if present, would be more likely to form via conjugate addition.

The discovery that a strigolactone is involved in the symbiosis between plant roots and arbuscular mycorrhizal fungi in at least one host plant (Akiyama et al., 2005) also suggests that strigol and its analogues may play a much more significant ecological role than previously appreciated. The benefits of AM fungi for healthy crops and fertile soil are well known, and it has recently been reported that AM fungi may suppress *Striga* emergence in arable fields with a high soil load of *Striga* seeds (Lendzemo et al., 2005). The increasing number of plants which are now known to produce strigol and related compounds also suggests that this molecule may be very widely distributed in nature, and that it may play a variety of roles other than that of a fortuitous signal molecule for parasitic weeds. The likelihood is that the scientific literature will be reporting a great deal more about this molecule in the coming months.

## References

- Akiyama, K., Matsuzaki, K.-I., Hayashi, H., 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* 435, 824–827.
- Cook, C.E., Whichard, L.P., Turner, B., Wall, M.E., 1966. Germination of witchweed (*Striga lutea* Lour.): isolation and properties of a potent stimulant. *Science* 154, 1189–1190.
- Cook, C.E., Whichard, L.P., Wall, M.E., Egle, G.H., Coggon, P., Luhan, P.A., McPhail, A.T., 1972. Germination stimulants. II. The structure of strigol – a potent seed germination stimulant for witchweed (*Striga lutea* Lour.). *J. Am. Chem. Soc.* 94, 6198–6199.
- Flematti, G.R., Ghisalberti, E.L., Dixon, K.W., Trengove, R.D., 2004. A compound from smoke that promotes seed germination. *Science* 305, 977.
- Hauck, C., Müller, S., Schildknecht, H., 1992. A germination stimulant for parasitic flowering plants from *Sorghum bicolor*, a genuine host plant. *J. Plant Physiol.* 139, 474–478.
- Lendzemo, V.W., Kuyper, Th.W., Kropff, M.J., van Ast, A., 2005. Field inoculation with arbuscular mycorrhizal fungi reduces *Striga hermonthica* performance on cereal crops and has the potential to contribute to integrated *Striga* management. *Field Crops Res.* 91, 51–61.
- Logan, D.C., Stewart, G.R., 1991. Role of ethylene in the germination of the hemiparasite *Striga hermonthica*. *Plant Physiol.* 97, 1435–1438.
- Mangnus, E.M., Zwanenburg, B., 1992. Tentative molecular mechanism for germination stimulation of *Striga* and *Orobanchae* seeds by strigol and its synthetic analogues. *J. Agric. Food Chem.* 40, 1066–1070.
- Matusova, R., Rani, K., Verstappen, F.W.A., Franssen, M.C.R., Beale, M.H., Bouwmeester, H.J., 2005. The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanchae* spp. are derived from the carotenoid pathway. *Plant Physiol.* 139, 920–934.
- Olmstead, R.G., dePamphilis, C.W., Wolfe, A.D., Young, N.D., Elisons, W.J., Reeves, P.A., 2001. Disintegration of the Scrophulariaceae. *Am. J. Bot.* 88, 348–361.
- Oswald, A., 2005. *Striga* control – technologies and their dissemination. *Crop Protect.* 24, 333–342.
- Parker, C., Riches, C.R., 1993. *Striga*, the witchweeds, on cereal crops. In: *Parasitic Weeds of the World: Biology and Control*. CAB International, Wallingford, UK, pp. 1–74.
- Press, M.C., Scholes, J.D., Riches, C.R., 2001. Current status and future prospects for management of parasitic weeds (*Striga* and *Orobanchae*). In: Riches, C.R. (Ed.), *The World's Worst Weeds*, BCPC Symposium Proceedings no. 77, British Crop Protection Council, Farnham, UK, pp. 71–88.
- Reizelman, A., Wigchert, S.C.M., del-Bianco, C., Zwanenburg, B., 2003. Synthesis and bioactivity of labelled germination stimulants for the isolation and identification of the strigolactone receptor. *Org. Biomol. Chem.* 1, 950–959.
- Sato, D., Awad, A.A., Takeuchi, Y., Yoneyama, K., 2005. Confirmation and quantification of strigolactones, germination stimulants for root parasitic plants *Striga* and *Orobanchae*, produced by cotton. *Biosci. Biotechnol. Biochem.* 69, 98–102.
- Siame, B.A., Weerasuriya, Y., Wood, K., Ejeta, G., Butler, L.G., 1993. Isolation of strigol, a germination stimulant for *Striga asiatica*, from host plants. *J. Agric. Food Chem.* 41, 1486–1491.
- Wigchert, S.C.M., Zwanenburg, B., 1999. Critical account on the inception of *Striga* seed germination. *J. Agric. Food Chem.* 47, 1320–1325.
- Yasuda, N., Sugimoto, Y., Kato, M., Inanaga, S., Yoneyama, K., 2003. (+)-Strigol, a witchweed seed germination stimulant, from *Menispermum dauricum* root culture. *Phytochemistry* 62, 1115–1119.
- Yokota, T., Sakai, H., Okuno, K., Yoneyama, K., Takeuchi, Y., 1998. Alectrol and orobanchol, germination stimulants for *Orobanchae minor*, from its host red clover. *Phytochemistry* 49, 1967–1973.
- Zehhar, N., Ingouff, M., Bouya, D., Fer, A., 2002. Possible involvement of gibberellins and ethylene in *Orobanchae ramosa* germination. *Weed Res.* 42, 464–469.