Cotylenins and Fusicoccins Stimulate Seed Germination of *Striga hermonthica* (Del.) Benth and *Orobanche minor* Smith

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Several fungal metabolites were examined for their effects on germination of the root parasitic weeds witchweed, *Striga hermonthica* (Del.) Benth, and clover broomrape, *Orobanche minor* Smith. Among these metabolites cotylenins (CNs) and fusicoccins (FCs) at concentrations as low as 10^{-5} M induced high seed germination (>50%) of both parasites. Inhibitors of ethylene biosynthesis [2-(2-aminoethoxyvinyl)glycine (AVG)] and action [silver thiosulfate (STS)] reduced CN- and FC-induced *Striga* germination but not that of *Orobanche*. This suggests that induction of *Striga* germination by CNs and FCs, as is the case with the true natural stimulant "strigol", requires both ethylene biosynthesis and action, while that of *Orobanche* does not.

Keywords: *Cotylenin; fusicoccin; Orobanche minor; seed germination; Striga hermonthica*

INTRODUCTION

Among the parasitic angiosperms, witchweeds (*Striga* spp.) and broomrapes (*Orobanche* spp.) are the two most devastating weeds on several cereal and leguminous crops, respectively (Stewart and Press, 1990). These parasitic weeds produce tiny but large numbers of seeds with prolonged viability and special germination requirements. Seed germination requires pretreatment (conditioning) in a warm moist environment for several days and subsequent exposure to an exogenous germination stimulant (Worsham, 1987; Joel et al., 1995). The first natural *Striga* germination stimulant, strigol, was isolated from cotton, a nonhost plant (Cook et al., 1966, 1972). Strigol, at a very low concentration 10^{-15} M, induces considerable germination of *Striga*. Subsequently, xenognosin or dihydrosorgoleone was isolated from a genuine host plant, sorghum (Lynn et al., 1981; Netzly et al., 1988). In addition, strigol has been found in root exudates of several host crops (Siame et al., 1993). Furthermore, sorgolactone, a compound structurally related to strigol, was isolated from sorghum root exudate (Hauck et al., 1992). The strigol-related natural stimulants sorgolactone and alectrol (Müller et al., 1992) are called strigolactones (Butler, 1995). It is likely that various host plants contain strigolactones, but some of them have not yet been characterized. Alternatively, some host or nonhost plants may contain germination stimulants of totally different chemistry (Ma et al., 1996).

Seed germination of *Striga* is also stimulated by other chemicals including natural and synthetic cytokinins, scopoletin, inositol, methionine, sodium hypochloride, and ethylene (Egley and Dale, 1970; Egley, 1972; Worsham, 1987; Visser, 1989; Logan and Stewart, 1991; Babiker et al., 1993b; Joel et al., 1995). Strigol and strigol analogues were reported to induce ethylene biosynthesis in *Striga* seeds, and both ethylene biosynthesis and action are required for germination (Logan and Stewart, 1991; Babiker et al., 1993a). In contrast with *Striga*, *Orobanche* species appear to have stricter germination requirements. Natural *Orobanche* germination stimulants from host plants have not yet been characterized. So far, strigolactones and their synthetic analogues have been shown to induce *Orobanche* germination (Hauck et al., 1992; Joel et al., 1995; Mori et al., 1997). The germination response of *Orobanche* species to ethylene is still a controversial matter (Parker and Riches, 1993; Joel et al., 1995). Instability of strigolactones in soil and their high synthetic costs preclude their use under practical field conditions.

Under these circumstances, screening of microbial and fungal metabolites for activity as *Striga* and *Orobanche* germination stimulants is imperative and probably a more promising strategy. In the present paper, we report on the induction of *Striga hermonthica* (Del.) Benth and *Orobanche minor* Smith germination by the fungal metabolites cotylenins (CNs) and fusicoccins (FCs) produced by *Cladosporium* sp. 501-7W (Sassa, 1970, 1971; Sassa et al., 1970) and *Fusicoccum amygdali* Del. (Ballio et al., 1964, 1968), respectively. Implication of ethylene biosynthesis and action in the germination of the two species was also included and discussed. In addition, since FCs and CNs are known to antagonize abscisic acid (ABA) in several biological processes (Marrè, 1979), the effects of ABA on *Striga* germination induced by (±)-strigol and cotylenin A (CN-A) were examined.

MATERIALS AND METHODS

Source of Plant Materials and Chemicals. *S. hermonthica* (Del.) Benth seeds were collected in 1992/1993, from under sorghum, at the Gezira Research Station, Sudan, and supplied by Prof. A. G. T. Babiker. *O. minor* Smith seeds were collected locally from under red clover (*Trifolium pratense* L.). Coty-

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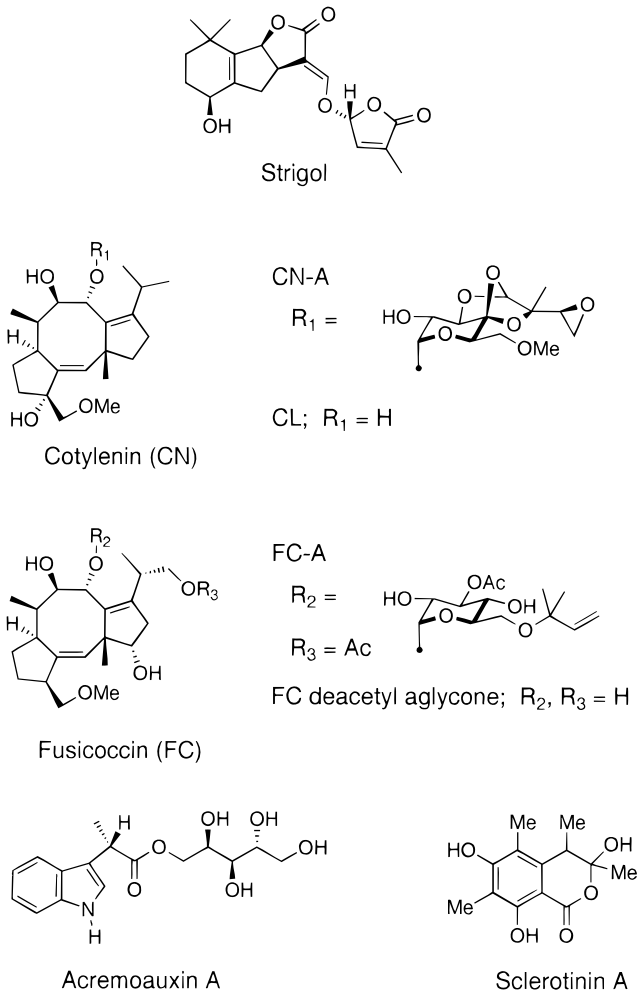

Figure 1. Structures of strigol and fungal metabolites promoting seed germination of *S. hermonthica* and *O. minor*.

lenin A (CN-A) and cotylenol (CL) were obtained from culture broth of *Cladosporium* sp. 501-7W (Sassa, 1971; Sassa et al., 1972). Acremoauxin A and sclerotinin A were isolated as metabolites from *Acremonium roseum* I4267 (Sassa et al., 1989) and *Sclerotinia sclerotiorum* (Sassa et al., 1968) cultures, respectively. Fusicoccin deacetyl aglycon (FC deacetyl aglycon) was a generous gift of Prof. A. Ballio (Universita La Sapienza, Italy). Structures of these compounds are shown in Figure 1. The stereostructure of CN-A was confirmed by an X-ray analysis and its details will be reported elsewhere.

(±)-Strigol was synthesized as described by Brooks et al. (1985) and Dailey (1987). Other compounds, including fusicoccin A (FC-A) and 2-[(2-aminoethoxy)vinyl]glycine (AVG), were purchased from Sigma or Aldrich Chemical Co. Silver thiosulfate (STS) was prepared by mixing equal volumes of 8 mM $AgNO_3$ and 32 mM $Na_2S_2O_3$ (Babiker et al., 1993a,b). (*S*)-Abscisic acid (*S*-ABA) was a generous gift of Dr. Y. Kamuro (BAL Planning Co., Ltd.).

Seed Germination Tests. *Striga* or *Orobanche* seeds, 10 each, were sown on a 5-mm glass fiber disk. Sixty of the disks were placed in a 9-cm Petri dish lined with two layers of filter paper wetted with 5 mL of distilled water for *Striga* and with 10^{-4} M gibberellic acid (GA_3) for *Orobanche*. The Petri dishes were enclosed in polyethylene bags and incubated in the dark for 2−3 weeks at 30 °C for *Striga* and at 26 °C for *Orobanche*. After the conditioning period, the glass fiber disks were blotted to remove excessive water or GA_3 solution. Four disks, each, were transferred to a 5-cm disposable Petri dish containing a sheet of filter paper wetted with 0.7 mL of the respective test solution. All of the test solutions, unless otherwise mentioned, contained 0.1% (v/v) acetone. The dishes, for each treatment, were enclosed in a polyethylene bag (permeable for CO_2, O_2, and ethylene but not for moisture) and were placed in the dark at the same temperature for the conditioning. Seeds of *Striga* and *Orobanche* were examined for germination 2 and 3 days after treatment, respectively. Treatments, each replicated three times, were repeated at least twice. The data presented are from single typical experiments. Seeds treated with (±)-strigol (10^{-7}−10^{-10} M) or distilled water, each containing 0.1% acetone (v/v), were included as controls.

Several fungal metabolites were screened for their effects on seed germination of *Striga*. In the present paper, only those fungal metabolites that induce *Striga* germination at a concentration of 10^{-4} M are reported.

Table 1. Induction of *S. hermonthica* Germination by Fungal Metabolites[a]

	% germination ± SE at a concentration of		
compd	10^{-4} M	10^{-5} M	10^{-6} M
CN-A	72 ± 2.2	66 ± 1.7	1 ± 0.2
CL	60 ± 0.4	79 ± 2.2	6 ± 0.1
FC-A	79 ± 3.2	57 ± 2.7	0
FC deacetyl aglycon	86 ± 2.2	79 ± 2.2	5 ± 1.8
acremoauxin A	18 ± 1.2	0	−[b]
sclerotinin A	18 ± 1.0	0	−
(±)-strigol	(91 ± 2.1)[c]	(45 ± 2.3)[d]	−
control		0	

[a] Data presented are the mean ± SE of one representative experiment. [b] Not measured. [c,d](±)-Strigol was tested at 10^{-8} and 10^{-9} M, respectively.

Table 2. Induction of *O. minor* Germination by Fungal Metabolites[a]

	% germination ± SE at a concentration of		
compd	10^{-4} M	10^{-5} M	10^{-6} M
CN-A	89 ± 1.2	45 ± 1.9	0
CL	91 ± 2.1	79 ± 0.7	0
FC-A	86 ± 1.1	56 ± 1.7	0
FC deacetyl aglycon	80 ± 2.1	80 ± 2.7	0
(±)-strigol	(90 ± 1.3)[c]	(80 ± 2.0)[d]	−[b]
control		0	−

[a] Data presented are the mean ± SE of one representative experiment. [b] Not measured. [c,d](±)-Strigol was tested at 10^{-9} and 10^{-10} M, respectively.

RESULTS

***Striga* Germination.** No or negligible germination was observed in *Striga* seeds incubated in distilled water, while (±)-strigol at 10^{-8} M induced high germination (85−95%).

The fungal metabolites FC-A, FC deacetyl aglycon, CN-A, and CL at 10^{-4} M displayed high activity (60−86% germination) as shown in Table 1. At a concentration of 10^{-5} M, CL and FC deacetyl aglycon induced high germination (∼80%), and CN-A and FC-A exhibited moderate activity. At a concentration of 10^{-6} M, CN-A, FC-A, CL, and FC deacetyl aglycon displayed negligible activity. Acremoauxin A and sclerotinin A, irrespective of concentration, elicited poor germination (1−18%). (±)-Strigol at 10^{-8} M induced 91% germination. The stimulants' activities decreased in the order (±)-strigol ≫ CL, FC deacetyl aglycon > CN-A and FC-A.

***Orobanche* Germination.** Under the set of experimental conditions, in general, *Orobanche* was more sensitive, to all germination stimulants, than *Striga* (Table 2). (±)-Strigol at 10^{-10} M induced >80% germination. The fungal metabolites CN-A, CL, FC-A, and FC deacetyl aglycon at 10^{-4} M effected high germination (80−91%). At 10^{-5} M, CL and FC deacetyl aglycon CL displayed high activity (∼80% germination). CN-A and FC-A induced poor (45%) and moderate (56%) activities,

Table 3. Effects of Ethylene Biosynthesis and Action Inhibitors on *Striga* Germination Induced by CN-A, CL, and (±)-Strigol[a]

treatment	% germination ± SE	
	Striga	*Orobanche*
CN-A 10^{-4} M	84 ± 0.8	84 ± 2.7
CN-A 10^{-5} M	52 ± 1.3	65 ± 1.6
CN-A 10^{-4} M + STS 2 × 10^{-3} M	82 ± 2.7	78 ± 2.6
CN-A 10^{-5} M + STS 2 × 10^{-3} M	21 ± 1.7	47 ± 2.5
CN-A 10^{-4} M + AVG 10^{-4} M	58 ± 2.3	83 ± 1.4
CN-A 10^{-5} M + AVG 10^{-4} M	6 ± 0.3	64 ± 2.4
CL 10^{-5} M	61 ± 2.3	76 ± 2.0
CL 10^{-5} M + STS 2 × 10^{-3} M	21 ± 1.7	55 ± 2.1
CL 10^{-5} M + AVG 10^{-4} M	0	60 ± 2.1
(±)-strigol 10^{-9} M	16 ± 1.8	90 ± 1.3
(±)-strigol 10^{-10} M	0	80 ± 1.3
(±)-strigol 10^{-9} M + STS 2 × 10^{-3} M	3 ± 1.8	83 ± 1.4
(±)-strigol 10^{-9} M + AVG 10^{-4} M	0	85 ± 1.2
control	0	0

[a] Data presented are the mean ± SE of one representative experiment.

Table 4. Effect of ABA on *Striga* Germination Induced by CN-A and (±)-Strigol[a]

treatment	% germination ± SE
CN-A 10^{-4} M	91 ± 1.4
CN-A 10^{-5} M	62 ± 2.2
CN-A 10^{-4} M + ABA 10^{-5} M	84 ± 2.1
CN-A 10^{-4} M + ABA 10^{-6} M	91 ± 1.8
CN-A 10^{-5} M + ABA 10^{-5} M	42 ± 1.2
CN-A 10^{-5} M + ABA 10^{-6} M	56 ± 1.1
(±)-strigol 10^{-9} M	16 ± 0.7
(±)-strigol 10^{-9} M + ABA 10^{-5} M	11 ± 0.3
(±)-strigol 10^{-9} M + ABA 10^{-6} M	5 ± 0.1
ABA 10^{-4} M	0
ABA 10^{-5} M	0
ABA 10^{-6} M	0
control	0

[a] Data presented are the mean ± SE of one representative experiment.

respectively. At 10^{-6} M, all fungal metabolites tested displayed no activity (0% germination). The activity of the stimulants decreased in the order (±)-strigol ≫ CL, FC deacetyl aglycon > CN-A, FC-A.

Effects of Ethylene Biosynthesis and Action Inhibitors. AVG, an inhibitor of ACC synthase, significantly reduced (±)-strigol-, CN-A-, and CL-induced *Striga* germination (Table 3). Germination was similarly curtailed by STS, an inhibitor of ethylene action. The inhibitors were more effective at low stimulant concentration. AVG had no or only little effect on *Orobanche* germination. STS was slightly more inhibitory to *Orobanche* germination than AVG, particularly at low stimulant concentration. Similar results were obtained with FC-A and FC deacetyl aglycon (data not shown).

Effect of *S*-ABA on *Striga* Germination Induced by (±)-Strigol and CN-A. ABA alone at 10^{-4}–10^{-6} M did not affect *Striga* germination as shown in Table 4. However, ABA slightly reduced response of *Striga* seed to CN-A and (±)-strigol.

DISCUSSION

Here we demonstrate stimulation of *Striga* and *Orobanche* seed germination by the fungal metabolites CNs and FCs. It should be noted that there has been no previous report on potent stimulants for *Orobanche* germination, except for strigolactones (Foy et al., 1989; Joel et al., 1995; Mori et al., 1997). Despite the differences in sensitivity between *Striga* and *Orobanche* seeds in their response to the stimulants, the stimulants displayed similar trends of activity. Since CL and FC deacetyl aglycon, which lack the sugar moiety, were more active stimulants than CN-A and FC-A, the aglycons seem to play a pivotal role in the stimulation of germination. Under the experimental conditions, CL and FC deacetyl aglycon were about 1/1000 and 1/10000 as active as (±)-strigol in the stimulation of *Striga* and *Orobanche* germination, respectively.

Ethylene has been shown to stimulate *Striga* germination but not that of *Orobanche*. In fact, inhibitors of ethylene biosynthesis (AVG) and action (STS) reduced *Striga* germination, while their effects on (±)-strigol- and CN-A-elicited *Orobanche* germination were rather small. Slight reductions in *Orobanche* germination in the presence of STS might be due to the heavy metal toxicity. These results imply that both ethylene biosynthesis and action are implicated in the CN- and FC-induced *Striga* germination but not that of *Orobanche*. This conclusion is consistent with various reports on the role of ethylene in *Striga* and *Orobanche* germination (Parker and Riches, 1993; Joel et al., 1995).

FCs and CNs display various physiological effects in plants (Marrè, 1979; Ballio, 1991). They were reported to promote seed germination and stimulate stomatal pore opening by antagonizing ABA (Marrè, 1979). In this study, ABA was found to reduce, slightly, the CN-A-induced *Striga* germination. ABA was inhibitory at the low CN-A concentration, thus suggesting partial recovery at the higher CN-A concentration.

FCs are suggested to bind to a receptor that is involved in the regulation of key enzymes including plasma membrane H^+-ATPase and soluble nitrate reductase, and receptors of FCs have been identified as 14-3-3 proteins, which are known to play an important role in signal transduction of ethylene and phytochrome (De Boer, 1997). The promotive effect of CNs on seed germination of *Monochoria vaginalis*, which requires light and flooded conditions for germination, may be attributed to effects on phytochrome and ethylene signaling pathways (Takeuchi et al., 1995). Although receptors of germination stimulants in *Striga* and *Orobanche* have not been identified, our results suggest that 14-3-3 proteins may be possible candidates. The involvement of ethylene in strigolactone-induced *S. hermonthica* and *S. asiatica* germination may support this supposition (Jackson and Parker, 1991; Babiker et al., 1993a).

FCs were originally isolated as phytotoxic metabolites from the fungus *Fusicoccum amygdali* Del. (Ballio et al., 1964, 1968). Their production was thought to be restricted to fungi. However, it has been reported that higher plants also produce FCs (Muromtsev et al., 1994). FC-A has been identified from root extracts of maize, a host of *Striga*. Therefore, FC-A in maize root exudate might be involved in seed germination of the parasite. In Russia, FC-A is now used as a plant growth regulator with antistress activity (Muromtsev et al., 1994). Since FCs and CNs are much more stable than strigolactones and xenognosins, especially under alkaline conditions (Ballio et al., 1968; Sassa et al., 1975), presowing of nonhost crop seeds treated with FCs or CNs may promote suicidal germination of *Striga* and *Orobanche* seeds and thus provide a good strategy for depleting seed reserves in soil. Furthermore, structural modifications of FCs and CNs may afford practical compounds

that induce suicidal germination of these parasites under field conditions. Studies on the structure−activity relationships of FCs and CNs and on mechanisms of germination are in progress.

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LITERATURE CITED

Babiker, A. G.; Ejeta, G.; Butler, L. G.; Woodson, W. R. Ethylene biosynthesis and strigol-induced germination of *Striga asiatica*. *Physiol. Plant.* **1993a**, *88*, 359−365.

Babiker, A. G. T.; Butler, K. G.; Ejeta, G.; Woodson, W. R. Enhancement of ethylene biosynthesis and germination by cytokinins and 1-aminocyclopropane-1-carboxylic acid in *Striga asiatica* seeds. *Physiol. Plant.* **1993b**, *89*, 21−26.

Ballio, A. Non-host-selective fungal phytotoxins: Biochemical aspects of their mode of action. *Experientia* **1991**, *47*, 783−790.

Ballio, A.; Chain, E. B.; De Leo, P.; Erlanger, B. F.; Mauri, M.; Tonolo, A. Fusicoccin: A new wilting toxin produced by *Fusicoccum amygdali* Del. *Nature* **1964**, *203*, 297.

Ballio, A.; Brufani, M.; Casinovi, C. G.; Cerrini, S.; Fedeli, W.; Pellicciari, R.; Santurbano, B.; Vaciago, A. The structure of fusicoccin A. *Experientia* **1968**, *24*, 631−635.

Brooks, D. W.; Bevinakatti, H. S.; Kennedy, E.; Hathaway, J. Practical total synthesis of (±)-strigol. *J. Org. Chem.* **1985**, *50*, 628−638.

Butler, L. G. Chemical communication between the parasitic weed *Striga* and its crop host. *ACS Symp. Ser.* **1995**, *582*, 158−166.

Cook, C. E.; Whichard, L. P.; Turner, B.; Wall, M. E.; Egley, G. H. Germination of witchweed (*Striga lutea*): isolation and properties of a potent stimulant. *Science* **1966**, *154*, 1189−1190.

Cook, C. E.; Whichard, L. P.; Wall, M. E.; Egley, G. H.; Coggon, P.; Luhan, P. A.; McPhail, A. T. Germination stimulants. II. The structure of strigol—a potent seed germination stimulant for witchweed (*Striga lutea* Lour.). *J. Am. Chem. Soc.* **1972**, *94*, 6198−6199.

Dailey, O. D., Jr. A new synthetic route to (±)-strigol. *J. Org. Chem.* **1987**, *52*, 1984−1989.

De Boer, B. Fusicoccin—a key to multiple 14-3-3 locks? *Trends Plant Sci.* **1997**, *2*, 60−66.

Egley, G. H. Influence of the seed envelope and growth regulators upon seed dormancy in witchweed (*Striga lutea* Lour.). *Ann. Bot.* **1972**, *36*, 755−770.

Egley, G. H.; Dale, J. E. Ethylene, 2-chlorophosphonic acid, and witchweed germination. *Weed Sci.* **1970**, *18*, 586−589.

Foy, C. L.; Jain, R.; Jacobsohn, R. Recent approaches for chemical control of broomrape (*Orobanche* spp.). *Rev. Weed Sci.* **1989**, *4*, 123−152.

Hauck, C.; Müller, S.; Schildknecht, H. A germination stimulant for parasitic flowering plants from *Sorghum bicolor*, a genuine host plant. *J. Plant Physiol.* **1992**, *139*, 474−478.

Jackson, M. B.; Parker, C. Induction of germination by a strigol analogue requires ethylene action in *Striga hermonthica* but not in *S. forbesii*. *J. Plant Physiol.* **1991**, *138*, 383−386.

Joel, D. M.; Steffens, J. C.; Matthews, D. E. Germination of weedy root parasites. In *Seed Development and Germination*; Kigel, J., Galili, G., Eds.; Dekker: New York, 1995; pp 567−597.

Logan, D. C.; Stewart, G. R. Role of ethylene in the germination of the hemiparasite *Striga hermonthica*. *Plant Physiol.* **1991**, *97*, 1435−1438.

Lynn, D. G.; Steffens, J. C.; Kamut, V. S.; Graden, D. W.; Shabanowitz, J.; Riopel, J. L. Isolation and characterization of the first host recognition substance for parasitic angiosperms. *J. Am. Chem. Soc.* **1981**, *103*, 1868−1870.

Ma, Y.; Babiker, A. G. T.; Ali, I. A.; Sugimoto, Y.; Inanaga, S. *Striga hermonthica* (Del.) Benth germination stimulant(s) from *Menispermum dauricum* (DC.) root culture. *J. Agric. Food Chem.* **1996**, *44*, 3355−3359.

Marrè, E. Fusicoccin: a tool in plant physiology. *Annu. Rev. Plant Physiol.* **1979**, *30*, 273−288.

Mori, K.; Matsui, J.; Bando, M.; Kido, M.; Takeuchi, Y. Synthesis and biological evaluation of the four racemic stereoisomers of the structure proposed for sorgolactone, the germination stimulant from *Sorghum bicolor*. *Tetrahedron Lett.* **1997**, *38*, 2507−2510.

Müller, S.; Hauck, C.; Schildknecht, H. Germination stimulants produced by *Vigna unguiculata* Walp cv Saunders Upright. *J. Plant Growth Regul.* **1992**, *11*, 77−84.

Muromtsev, G. S.; Voblikova, V. D.; Kobrina, N. S.; Koreneva, V. M.; Krasnpoplskaya, L. M.; Sadovskaya, V. L. Occurrence of fusicoccanes in plants and fungi. *J. Plant Growth Regul.* **1994**, *13*, 39−49.

Netzly, D. H.; Riopel, J. L.; Ejeta, G.; Butler, L. G. Germination stimulants of witchweed (*Striga asiatica*) from hydrophobic root exudate of sorghum (*Sorghum bicolor*). *Weed Sci.* **1988**, *36*, 441−446.

Parker, C.; Riches, C. R. *Parasitic Weeds of the World: Biology and Control*; CAB International Press: Wallingford, Oxon, U.K., 1993; 332 pp.

Sassa, T. Promotion of the enlargement of cotyledons by cotylenins A and B. *Agric. Biol. Chem.* **1970**, *34*, 1588−1589.

Sassa, T. Cotylenins, leaf growth substances produced by fungus. Part I. Isolation and characterization of cotylenins. *Agric. Biol. Chem.* **1971**, *35*, 1415−1418.

Sassa, T.; Aoki, H.; Namiki, M.; Munakata, K. Plant growth promoting metabolites of *Sclerotinia sclerotiorum* Part I. Isolation and structure of sclerotinin A and B. *Agric. Biol. Chem.* **1968**, *32*, 1432−1439.

Sassa, T.; Tojyo, T.; Munakata, K. Isolation of new plant growth substances with cytokinin-like activity. *Nature* **1970**, *227*, 379.

Sassa, T.; Neguro, T.; Ueki, H. Production and characterization of a new fungal metabolite, cotylenol. *Agric. Biol. Chem.* **1972**, *36*, 2281−2285.

Sassa, T.; Togashi, M.; Kitaguchi, T. The structures of cotylenins A, B, C, D and E. *Agric. Biol. Chem.* **1975**, *39*, 1735−1744.

Sassa, T.; Yoshida, N.; Haruki, E. Isolation and structural elucidation of acremoauxin A. A novel auxin derivative produced by *Acremonium roseum* I 4267. *Agric. Biol. Chem.* **1989**, *53*, 3105−3108.

Siame, B. P.; Weerasuriya, Y.; Wood, K.; Ejeta, G.; Butler, L. G. Isolation of strigol, a germination stimulant for *Striga asiatica*, from host plants. *J. Agric. Food Chem.* **1993**, *41*, 1486−1491.

Stewart, G. R.; Press, M. C. The physiology and biochemistry of parasitic angiosperms. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1990**, *41*, 127−151.

Takeuchi, Y.; Sassa, T.; Kawaguchi, S.; Ogasawara, M.; Yoneyama, K.; Konnai, M. Stimulation of germination of *Monochoria vaginalis* seeds by seed coat puncture and cotylenins. *Zasso Kenkyu* **1995**, *40*, 221−224.

Visser, J. H. Germination requirements of some root-parasitic flowering plants. *Naturwissenschaften* **1989**, *76*, 253−261.

Worsham, A. D. Germination of witchweed seeds. In *Parasitic Weeds in Agriculture. Vol. I. Striga*; Musselman, L. J., Ed.; CRC Press: Boca Raton, FL, 1987; pp 45−61.

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