A Critical Account on the Inception of *Striga* Seed Germination

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The seeds of the parasitic weed *Striga* germinate in response to stimulants exuded by the roots of host plants and some nonhost plants. Literature data are summarized that support the view that strigolactones induce germination of parasitic weed seeds via a receptor-mediated mechanism. The suggestion by Lynn et al. that the strigol D-ring is solely responsible for germinating *Striga* seeds via a redox reaction was based on hypothesized structural similarities between the natural compound dihydrosorgoleone (SXSg) and the strigol D-ring. Experiments have shown that the mechanistic connection between SXSg and the strigol D-ring is not valid, and therefore the proposed redox mechanism for the induction of *Striga* seed germination by strigolactones does not hold.

Keywords: *Bioorganic chemistry; germination; parasitic weeds; Striga; SXSg*

INTRODUCTION

Parasitic weeds belonging to the genera *Striga* and *Orobanche* severely reduce the yields of economically important crops in tropical and semitropical areas of the eastern hemisphere and in the Mediterranean region. Germination of the weeds is induced by stimulants present in the root exudates of host plants and some nonhost plants (Butler, 1995; Musselman, 1987; Parker and Riches, 1993). The first naturally occurring germination stimulant, (+)-strigol (**1**), was isolated in 1966 from the root exudate of cotton (Cook et al., 1966). The significance of strigol in the host−parasite interaction was uncertain for a long time, because cotton is not a host for *Striga* or *Orobanche*. A breakthrough was achieved when Hauck et al. (1992) identified sorgolactone (**2**), a structural analogue of strigol, as the major *Striga* germination stimulant produced by sorghum, a cereal crop which is severely affected by *Striga*. At about the same time, these authors also reported the isolation of another germination stimulant, viz., alectrol (**3**), from the root exudate of cowpea (Müller et al., 1992) (Figure 1). Soon thereafter, Siame et al. (1993) showed that strigol itself is the major germination stimulant produced by the *Striga* hosts maize and millet. The collective name "strigolactones" was proposed for this class of compounds (Butler, 1995). In addition, it has been demonstrated that root exudates of *Striga* hosts generally contain a mixture of these strigolactones, albeit in different ratios (Siame et al., 1993).

Structure activity studies of a series of synthetic strigol analogues (among others: Johnson et al., 1981; Mangnus and Zwanenburg, 1992a; Mangnus et al., 1992a; Bergmann et al., 1993; Zwanenburg et al., 1994; Kranz et al., 1996) revealed that the bioactiphore of the stimulants resides in the CD-part of the molecule, and a tentative molecular mechanism for the induction of germination has been proposed (Mangnus and Zwanenburg, 1992a). This molecular mechanism involves addition of a nucleophilic species, present at the receptor site, in a Michael fashion, followed by elimination of the D-ring. The ultimate result is that the ABC part of the stimulant is covalently bound to the receptor, a chemical change that may be responsible for triggering germination (Scheme 1).

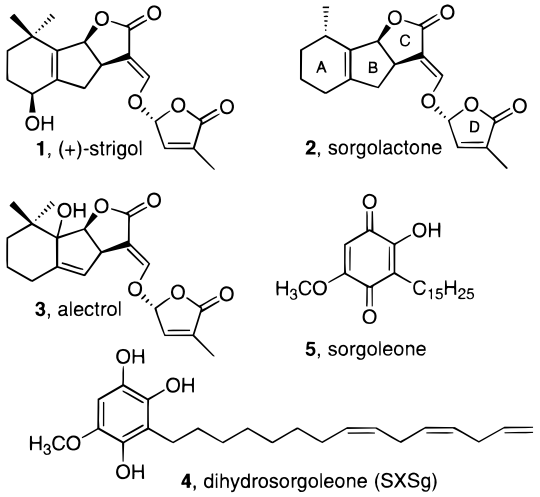

Figure 1. Structural formulas of (+)-strigol, sorgolactone, alectrol, SXSg, and sorgoleone.

Furthermore, the synthesis and biological evaluation of all possible diastereomers of sorgolactone (Sugimoto et al., 1997 and 1998; Mori et al., 1997) and its analogues DMSL (Thuring et al., 1997a), GR 7 (Mangnus and Zwanenburg, 1992b), and GR 24 (Thuring et al., 1997b) have clearly demonstrated the effect of the stereochemistry of the stimulant molecules on the biological activity. Only those isomers possessing the same absolute stereochemical configuration as the natural germinating agents exhibit high germination stimulatory activity. The concentration range in which strigolactones express half-maximal germination inducing activity is generally on the order of 10^{-9} M. This

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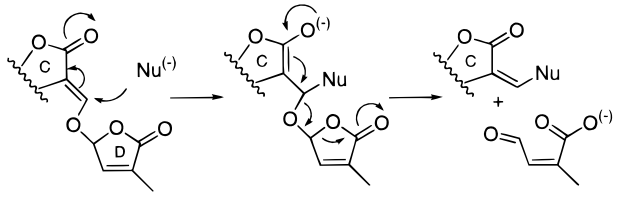
10.1021/jf980926e CCC: $18.00 © 1999 American Chemical Society
Published on Web 04/02/1999

Scheme 1. Tentative Molecular Mechanism for the Induction of *Striga* Germination



concentration range and the critical dependence of the bioactivity on the absolute stereochemistry are, in our view, strong indications that the inception of germination of parasitic weed seeds is mediated by a receptor.

In contrast, there is the explicit claim that the hydroquinone derivative, dihydrosorgoleone (**4**), also named SXSg, plays the key role during the inception of the seed germination process (Chang et al., 1986) (Figure 1). Moreover, Lynn and Boone (1993) make a connection between the functions of SXSg and strigolactones in the germination process. In the present paper, a critical account on the initial stages of *Striga* germination is given, with the aim to oppose the different views on this matter. The identity of the true stimulant is essential for the design of mimics with high potential to control these parasites selectively using the concept of "suicidal germination". This approach involves the application of a (synthetic) germination stimulant to the soil before planting the desired crop (Johnson et al., 1976; Eplee and Norris 1987). The *Striga* seeds will germinate, but in the absence of a suitable host plant they cannot survive.

EXPERIMENTAL PROCEDURES

General Remarks. ^{1}H NMR (100 MHz) and ^{1}H NMR (400 MHz) spectra were recorded on Bruker AC 100 and AM 400 spectrometers, respectively (Me_4Si as internal standard). All coupling constants (^3J) are given in Hz, unless indicated otherwise. GC was conducted with a Hewlett-Packard HP 5890 gas chromatograph, using a capillary column (25 m) of HP17 and nitrogen (2 mL/min) as the carrier gas. The temperature program was as follows: initial temperature, 100 °C during 5 min, then heating 5 °C/min for 10 min, followed by 5 min at the final temperature of 150 °C. Total run time was 20 min. Enantiomeric excesses were determined using a chiral Beta-dex 120 fused silica capillary column (60m × 0.25 mm ID, 0.25 μm film thickness) (Supelco) and nitrogen (1 mL/min) as the carrier gas. Solvents were of analytical grade and used as purchased.

5(R)-Ethoxy-3-methyl-2-(5H)-furanone (7). Optically active ethoxy butenolide **7** was prepared according to Van Oeveren (1996) and obtained as a colorless oil in 50−70% yield with ee's varying between 48 and 83%. Retention times of both enantiomers of **7** after separation on a Beta-dex 120 GC column at 130 °C isotherm: 34.4 and 35.6 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27 (t, 3H, J = 7.0 Hz, CH_3 ethyl), 1.94 (s, 3H, =CCH_3), 3.74 and 3.93 (AB, 2H, CH_2O), 5.79 (s, 1H, OC*H*O), 6.80 (s, 1H, H−C=).

3-Methyl-5-octyloxy-2-(5H)-furanone (8). Hydroxy furanone **6** (0.5 g; 4.4 mmol) was dissolved in a mixture of benzene (15 mL) and 1.1 equiv (0.63 g) of *n*-octanol. A catalytic amount of *p*-TsOH was added, and the mixture was refluxed under Dean−Stark conditions for 1 h. Subsequently, benzene was evaporated and the residue was dissolved in ethyl acetate and washed with water. Drying ($MgSO_4$) and concentration under reduced pressure followed by column chromatography over silica gel (hexane/ethyl acetate 9:1) afforded **8** (0.72 g, 72%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 7.0 Hz, CH_3 octyl), 1.27−1.35 (m, 10H, CH_2 octyl), 1.60 (m, 2H, C*H*$_2$CH$_2$O), 1.94 (s, 3H, =CCH_3), 3.65 and 3.85 (AB, 2H, CH_2O), 5.78 (s, 1H, OC*H*O), 6.80 (s, 1H, H−C=).

Equilibration Experiment. Hydroxy butenolide **6** (50 mg) was dissolved in a 5% aqueous solution of sulfuric acid (1 mL). The mixture was stirred at room temperature for 5 weeks. During this period, aliquots were assayed by gas chromatography for the formation of compound **10**. Retention time of **6**: 14.5 min. Retention time of **10**: 17.3 min.

Racemization Experiments. Optically active ethoxy furanone **7** (20 mg; ee 48%) was dissolved in 1 mL of acetone and added to 15 mL of water or phosphate buffer of pH 6, 7, and 8, respectively. The mixtures were stirred at room temperature. Aliquots were taken and extracted with ethyl acetate, and ee's were determined with chiral GC. In all cases, the mixture had become completely racemic within 24 h. Acetoxy butenolide **9** retained its ee when subjected to identical conditions. Retention time of both enantiomers of **9** after separation on a Beta-dex 120 GC column at 160 °C isotherm: 25.9 and 26.4 min.

Deuterium Exchange Experiments. Optically active ethoxy butenolide **7** (15 mg; ee 48%) was dissolved in 0.5 mL of acetone-D_6 and 4.5 mL of D_2O and stirred at room temperature. Aliquots were taken after 5 min, 1 h, 5 h, 8 h, and 5 days and assayed by ^{1}H NMR and chiral GC. Racemic octyloxy butenolide **8** was dissolved in a mixture of acetone-D_6 and D_2O (see above) in an NMR tube, and NMR spectra were recorded during 4 successive days.

RESULTS AND DISCUSSION

Prior to the identification of the strigolactones as the major stimulatory constituents produced by *Striga* hosts, Chang et al. (1986) suggested that dihydrosorgoleone (SXSg) (**4**) is the actual stimulant exuded from sorghum roots. The authors attributed significance to SXSg because it is readily oxidized to the corresponding inactive quinone, sorgoleone (**5**). This might explain the distance dependence observed in the germination of *Striga* seeds. Only those seeds less than 5 mm away from a host root germinate and can subsequently attach themselves to the host plant. On the basis of experiments in agar, Chang et al. (1986) claim that strigol is too stable to explain this distance dependence. It is relevant to mention, however, that it was established that strigol and its synthetic analogues are short-lived in soil, presumably because of hydrolytic degradation (Babiker et al., 1987 and 1988). Notably, neither SXSg nor sorgoleone was ever detected in the root exudate of maize, which is highly susceptible to *Striga* (Housley et al., 1987).

Evidence has been accumulated which demonstrates that strigolactones (vide supra) are the true stimulants for *Striga* germination. On the other hand, a series of articles continued to appear elaborating on the presumed prominent role of SXSg in the germination process (Fate et al., 1990; Lynn and Chang, 1990; Lynn and Boone, 1993; Boone et al., 1995; Fate and Lynn, 1996). The concentration at which SXSg induces half-maximal germination of *Striga asiatica* is reported to be 10^{-6} M. This is at least a 1000-fold higher than the concentration range in which the strigolactones exhibit half-maximal activity. Moreover, the variation in amounts of SXSg produced by different sorghum cultivars is at most 10-fold and shows little, if any, correlation between reported field resistance/susceptibility to *Striga* infection (Hess et al., 1992). The variation in the amount of strigolactones exuded by these sorghum species on the other hand, is a billionfold: high producers are almost always susceptible to *Striga* infection, whereas the lowest producers are invariably resistant (Hess et al., 1992; Olivier and Leroux, 1992). In other words, there is a good correlation between strigolactone

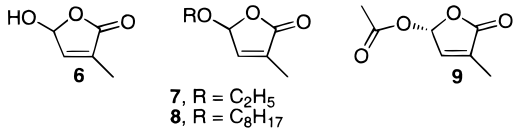


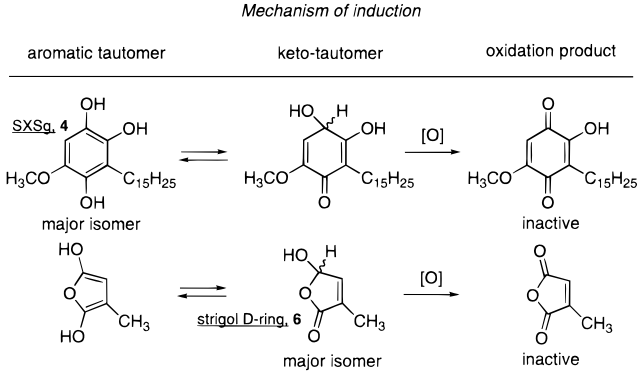
Figure 2. Structural formulas of hydroxy-, ethoxy-, octyloxy-, and acyloxy furanone.

exudation and field susceptibility for *Striga* attack. Hess et al. (1992) concluded that SXSg plays only a minor role, if any, in controlling germination of *Striga* seeds.

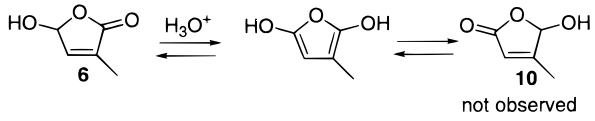
Despite these contradicting reports, Fate and Lynn (1996) stated that "SXSg is the only component exuded from sorghum roots capable of inducing *Striga* germination". It should be noted here that Hauck et al. (1992) isolated sorgolactone from the same sorghum root exudate. Inconsistent with their recurring view that strigolactones are not relevant for the induction of *Striga* germination, Boone et al. (1995) reported that "the active portion of the strigol molecule is restricted to the D-ring", i.e., 5-hydroxy-3-methyl-2-(5H)-furanone (**6**) (also named hydroxy butenolide). According to Lynn and Boone (1993), the stimulatory activity of strigolactones can be explained because their D-ring is structurally similar to SXSg and can be oxidized in the same manner. Therefore, these authors proposed a mechanistic connection between SXSg and strigolactones in the germination process. To confirm the suggestion that the butenolide is the active part of strigolactones, Lynn and Boone (1993) synthesized several furanone derivatives, such as hydroxy butenolide (**6**), 5-ethoxy-3-methyl-2-(5H)-furanone (**7**), and 5-octyloxy-3-methyl-2-(5H)-furanone (**8**) (Figure 2) and evaluated them for their capacity to induce germination of *S. asiatica* seeds. Their respective ED_{50} values were 10^{-5}, 10^{-6}, and 10^{-7} M. The difference in biological activity is explained by a difference in the ability of the inducer to permeate the seeds: the more hydrophobic alkyl acetals of the furanones have been shown to have activity comparable to that of strigol. The claim by Lynn et al. that only the strigol D-ring is responsible for the inception of *Striga* germination would imply that the ABC part of a strigolactone is just a lipophilic carrier, which is in sharp contrast with our results that clearly indicate the importance of the stereochemistry on the biological activity of strigol and its analogues (Sugimoto et al., 1998; Thuring et al., 1997a, Mangnus and Zwanenburg, 1992b; Thuring et al., 1997b). However, Lynn and coworkers do not offer any explanation about how the substituent group on the butenolide is removed inside the seeds to release the supposedly active species **6**. Interestingly, the views of these authors on the role of strigolactones are ambivalent as on one hand these compounds are too stable to explain the distance dependence observed in the germination of *Striga* seeds (Chang et al., 1986), whereas on the other hand their D-ring is readily split off to serve as a substrate in the redox reaction (Boone et al., 1995) (vide infra).

Lynn's claim that the D-ring is the only relevant part of the strigol molecule is also based on the results of Pepperman et al. (1982), who reported that ethoxy furanone **7** was active in inducing germination of *S. asiatica*. Notably, hydroxy butenolide **6** and methoxy-, propoxy-, and isopropoxy furanone were not active according to Pepperman. This in itself is inconsistent with Lynn's statement that the more lipophilic alkyl furanones show enhanced biological activity. In tests

Scheme 2. Comparison of the Structural Domains of SXSg and Strigol that Are Responsible for Germination Inducing Activity According to Boone et al. (1995). Two Different Tautomeric Forms of Each Are Shown, Together with Their Oxidation Products



Scheme 3. Presumed Tautomeric Equilibrium of the Strigolactone D-Ring (Lynn & Boone, 1993)



with *Striga hermontica* and *Orobanche crenata* seeds in our laboratory, neither hydroxy butenolide nor ethoxy furanone ever showed any stimulatory activity. Furthermore, Pepperman admits in the very same publication that the biological activity of ethoxy furanone was not reproducible and that also with other compounds tested there are "inconsistencies". He concludes that "the butenolide ring alone is not a structural feature capable of inducing germination of witchweed seed". In all of Pepperman's experiments, DMSO was used as a cosolvent. We know now that DMSO affects the permeability of the seed coats; it is therefore impossible to draw reliable conclusions about compounds tested for germination inducing activity in the presence of DMSO (Mangnus et al., 1992b). It is important to note that Lynn does not give any information about the reagents and conditions used in his bioassays with the various furanones.

In an attempt to provide a mechanistic explanation for the induction of germination, Boone and Lynn draw a parallel between SXSg and hydroxy butenolide (D-ring) (Lynn and Boone, 1993; Boone et al., 1995). It was hypothesized that the induction of the germination of *Striga* seeds proceeds via a redox reaction in which the somehow eliminated D-ring or SXSg is oxidized. This reaction is considered to trigger the germination process (Scheme 2).

Lynn's hypothesis is mainly based on the suggested structural similarity between SXSg and the D-ring (i.e., hydroxy butenolide **6**), especially because of the presumed existence of an equilibrium with its 2,5-dihydroxy furan tautomer. If such an equilibrium does indeed exist, stirring of furanone **6** under acidic conditions should result in the formation of its regioisomer **10** (Scheme 3).

The formation of this isomer **10** was not observed. Even after 5 weeks of stirring in a 5% H_2SO_4 solution, the only compound detected in the solution was the original hydroxy furanone **6**. This experiment clearly demonstrates that the actual occurrence of this tautomeric equilibrium is very unlikely.

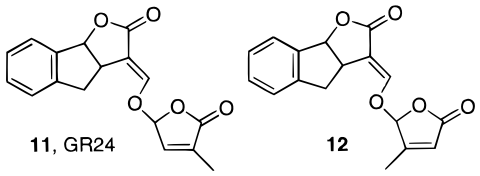


Figure 3. Structural formulas of the synthetic germination stimulant GR 24 **11** and its D-ring analogue **12**.

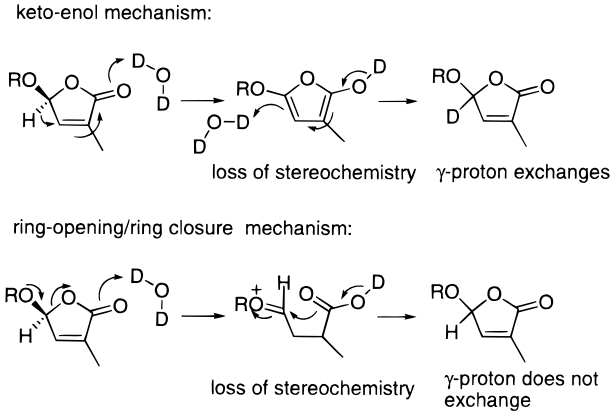
This conclusion is further supported by our results with GR 24 (**11**) (Mangnus et al., 1992c) and its D-ring isomer **12** (Mangnus et al., 1992b) (Figure 3). Upon liberation of the D-ring, which is a prerequisite for the oxidative mechanism, compounds **6** and **10** (see Scheme 3) should be obtained. According to the suggested tautomeric equilibrium, structures **6** and **10** are interchangeable and therefore they should both be active in stimulating *Striga* seed germination. Even when this structural interchange does not take place, oxidation of both **6** and **10** results in the same product, implying biological activity of both butenolides **6** and **10**. A substituent effect on the oxidation behavior of **6** and **10** is highly unlikely. However, in actual practice only compound **11** (GR 24) exhibited considerable germination stimulatory activity toward both *S. hermontica* and *O. crenata*, whereas its isomer **12** is virtually inactive. This observation raises serious doubts concerning the redox mechanism shown in Scheme 2.

In addition, the assumption of a tautomeric equilibrium between furanone and hydroxy furan, would imply that the stereogenic center present in alkoxy-substituted furanones is labile. According to this presumed intrinsic lability of the D-ring chiral center, the stereochemistry of the strigolactone D-ring would not have an effect on the biological activity. As mentioned before, the chirality of the stimulants does have a profound influence on their biological activity.

To study the stability of the furanone stereogenic center in more detail, ethoxy furanone **7** was prepared in optically active form and used as a model compound (Van Oeveren, 1996). When stirring optically active **7** under the conditions applied in our standardized bioassay (Mangnus et al., 1992b) (acetone in water), ethoxy furanone did, however, racemize completely within 20 h. Remarkably, when enantiopure acetoxy butenolide **9** (readily obtained by enzymatic resolution) (Thuring et al., 1996) was treated similarly, the optical activity was retained. These observations could be supportive for Lynn's views. However, racemization of alkoxy butenolides can be explained by two possible reaction mechanisms which are depicted in Scheme 4. If the racemization is explained by the keto−enol equilibrium, as is proposed by Lynn et al., then the γ-proton will be exchanged for a deuterium atom by performing the racemization experiment in D_2O. When the racemization proceeds via ring opening/ring closure, the γ-proton will not be exchangeable.

To investigate which mechanism is operative, racemic octyloxy butenolide **8** was stirred in a mixture of acetone-D_6 and D_2O and the signal of the γ-proton (δ 5.78 ppm) was monitored by ^{1}H NMR. Even after 3 days, no H/D exchange was detected. Optically active ethoxy butenolide **7** was also stirred in a mixture of acetone-D_6 and D_2O, and the racemization of the compound was monitored with chiral GC analysis. After 5 min, ee 48%; 1 h, ee 44%; 5 h, ee 25%; 8 h, ee 18%. After 5 days, the mixture had racemized completely. NMR analysis of the same aliquots showed that the signal of the γ-proton (δ 5.78 ppm) remained unchanged. This proton did not at all exchange for a deuterium atom during the racemization. The fact that in none of the cases any exchange of the γ-proton for deuterium was observed, while the optically active starting material had become completely racemic, is compelling evidence that the racemization proceeds via a ring opening/ring closure mechanism. The keto−enol equilibrium does not play a role. On the basis of these results, it is clear that Lynn's suggestion that the stimulatory activity of butenolides can be rationalized by the structural similarity of these compounds to SXSg does not hold. If R is an electron withdrawing group, as in **9**, the formation of an oxonium ion intermediate is much less likely, explaining the stability of acetoxy butenolide **9** toward racemization.

Scheme 4. Two Possible Mechanisms to Explain the Racemization of Alkoxy Butenolides. In the Keto−Enol Mechanism, the γ-proton Will Exchange for Deuterium in the Presence of D_2O, whereas in the Ring Opening/Ring Closure Mechanism This Proton Is Not Exchangeable



Finally, for the postulated redox mechanism to take place, the lipophilic part of the stimulant has to be detached from the furanone. In our proposed molecular mechanism for the induction of germination, the D-ring is split off in the key step of the process (Mangnus and Zwanenburg, 1992a) (see Scheme 1). Theoretically, Lynn's proposal cannot be excluded entirely. However, another very significant point is the concentration range in which Lynn et al. observed germination inducing activity of hydroxy butenolide **6**: 50% germination at a concentration of 10^{-5} mol L^{-1}. If we compare this with the concentrations of strigol and GR 24 that induce half-maximum germination ([stimulant] = $10^{-11} - 10^{-9}$ mol L^{-1}) there is a difference of at least 10^4 mol L^{-1}. In the concentration range in which strigol and GR 24 induce germination of *Striga* seeds, the D-ring (i.e., **6**) is not active. For this reason, Lynn's claim cannot be correct, especially if one assumes that the D-ring is originally derived from a strigolactone molecule in the root exudate.

The considerations given above are strong evidence that germination of *Striga* seeds is induced by strigolactones and proceeds via a receptor-mediated mechanism. The hypothesized mechanistic connection between the strigol D-ring (**6**) and SXSg (**4**) lacks credence. Therefore, the conclusion is justified that Lynn's redox mechanism for the induction of *Striga* germination by oxidation of the strigolactone D-ring cannot be valid. Whatever role SXSg may have in the germination

process, it is surely very different from that of the strigolactones.

ACKNOWLEDGMENT

J. W. J. F. Thuring is gratefully acknowledged for helpful discussions.

LITERATURE CITED

Babiker, A. G. T.; Hamdoun, A. M.; Rudwan, A.; Mansi, N. G.; Faki, H. H. Influence of soil moisture on activity and persistence of the strigol analogue GR 24. *Weed Res.* **1987**, *27*, 173−178.

Babiker, A. G. T.; Ibrahim, N. E.; Edwards, W. G. Persistence of GR 7 and *Striga* germination stimulants from *Euphorbia aegyptiaca* Boiss. in soils and in solutions. *Weed Res.* **1988**, *28*, 1−6.

Bergmann, C.; Wegmann, K.; Frischmuth, K.; Samson, E.; Kranz, A.; Weigelt, D.; Koll, P.; Welzel, P. Stimulation of *Orobanche crenata* seed germination by (+)-strigol and structural analogues. Dependence of constitution and configuration of the germination stimulants. *J. Plant Physiol.* **1993**, *142*, 338−342.

Boone, L. S.; Fate, G.; Chang, M.; Lynn, D. G. Seed germination. In *Parasitic Plants*; Press, M. C., Graves, J. D., Eds.; Chapman & Hall: London, 1995; pp 14−38.

Butler, L. G. Chemical communication between the parasitic weed *Striga* and its crop host. ACS Symposium Series 582; American Chemical Society: Washington, DC, 1995; 158−166.

Chang, M.; Netzly, D. H.; Butler, L. G.; Lynn, D. G. Chemical regulation of distance: characterization of the first natural host germination stimulant for *Striga asiatica. J. Am. Chem. Soc.* **1986**, *108*, 7858−7860.

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

Eplee, R. E.; Norris, R. S. Chemical control of *Striga.* In *Parasitic Weeds in Agriculture. Vol. I. Striga;* Musselman, L. J., Ed.; CRC Press: Boca Raton, FL, **1987**; pp 173−182.

Fate, G.; Chang, M.; Lynn, D. G. Control of germination in *Striga asiatica:* chemistry of spatial definition. *Plant Physiol.* **1990**, *93*, 201−207.

Fate, G.; Lynn, D. G. Xenognosin methylation is critical in defining the chemical potential gradient that regulates the spatial distribution in *Striga* pathogenesis. *J. Am. Chem. Soc.* **1996**, *118*, 11369−11376.

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.

Hess, D. E.; Ejeta, G.; Butler, L. G. Selecting sorghum genotypes expressing a quantitative biosynthetic trait that confers resistance to *Striga. Phytochemistry* **1992**, *31*, 493−497.

Housley, T. L.; Ejeta, G.; Cherif-Ari, O.; Netzly, D. H.; Butler, L. G. Progress towards an understanding of sorghum resistance to *Striga.* In *Proceedings of the 4th International Symposium on Parasitic Flowering Plants;* Weber, H. C., Forstreuter, W., Eds.; Philipps University: Marburg, Germany 1987; pp 411−419.

Johnson, A. W.; Roseberry, G.; Parker, C. A novel approach to *Striga* and *Orobanche* control using synthetic germination stimulants. *Weed Res.* **1976**, *16*, 223−227.

Johnson, A. W.; Gowda, G.; Hassanali, A.; Knox, J.; Monaco, S.; Razawi, Z.; Roseberry, G. The preparation of synthetic analogues of strigol. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1734−1743.

Kranz, A.; Samson-Schulz, E.; Hennig, L.; Welzel, P.; Müller, D.; Mayer-Figge, H.; Sheldrik, W. S. Synthesis of new strigol analogues. *Tetrahedron* **1996**, *52*, 14827−14840.

Lynn, D. G.; Chang, M. Phenolic signals in cohabitation: implications for plant development. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1990**, *41*, 497−526.

Lynn, D. G.; Boone, L. S. Signaling germination in *Striga asiatica.* In *Proceedings of the 8th Annual Penn State Symposium in Plant Physiology*; Schultz, J., Raskin, I., Eds.; ASPP Press: Pittsburg, 1993; pp 47−53.

Mangnus, E. M.; van Vliet, L. A.; Vandenput, D. A. L.; Zwanenburg, B. Structural modifications of strigol analogues. Influence of the B and C rings on the bioactivity of the germination stimulant GR 24. *J. Agric. Food Chem.* **1992a**, *40*, 1222−1229.

Mangnus, E. M.; Stommen, P. L. A.; Zwanenburg, B. A standardized bioassay for evaluation of potential germination stimulants for seeds of parasitic weeds. *J. Plant Growth Regul.* **1992b**, *11*, 91−98.

Mangnus, E. M.; Dommerholt, F. J.; De Jong, R. L. P.; Zwanenburg, B. Improved synthesis of strigol analogue GR 24 and evaluation of the biological activity of its diastereomers. *J. Agric. Food Chem.* **1992c**, *40*, 1230−1235.

Mangnus, E. M.; Zwanenburg, B. Tentative molecular mechanism for germination stimulation of *Striga* and *Orobanche* seeds by strigol and its synthetic analogues. *J. Agric. Food Chem.* **1992a**, *40*, 1066−1070.

Mangnus, E. M.; Zwanenburg, B. Synthesis, structural characterization and biological evaluation of all four enantiomers of strigol analogue GR7. *J. Agric. Food Chem.* **1992b**, *40*, 697−700.

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**, *14*, 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.

Musselman, L. J., Ed. *Parasitic Weeds in Agriculture. Vol. I. Striga*; CRC Press: Boca Raton, FL, 1987.

Olivier, A.; Leroux, G. D. Root development and production of a witchweed (*Striga* spp.) germination stimulant in sorghum *(Sorghum bicolor)* cultivars. *Weed Sci.* **1992**, *40*, 542−545.

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

Pepperman, A. B.; Connick, W. J.; Vail, S. L.; Worsham, A. D.; Pavlista, A. D.; Moreland, D. E. Evaluation of precursors and analogues of strigol as witchweed *(Striga asiatica)* seed germination stimulants. *Weed Sci.* **1982**, *30*, 561−566.

Siame, B. A.; 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.

Sugimoto, Y.; Wigchert, S. C. M.; Thuring, J. W. J. F.; Zwanenburg, B. The first total synthesis of the naturally occurring germination stimulant sorgolactone. *Tetrahedron Lett.* **1997**, *13*, 2321−2324.

Sugimoto, Y.; Wigchert, S. C. M.; Thuring, J. W. J. F.; Zwanenburg, B. Synthesis of all eight stereoisomers of the germination stimulant sorgolactone. *J. Org. Chem.* **1998**, *63*, 1259−1267.

Thuring, J. W. J. F.; Klunder, A. J. H.; Nefkens, G. H. L.; Wegman, M. A.; Zwanenburg, B. Lipase catalyzed dynamic kinetic resolution of some 5-hydroxy-2(5H)-furanones. *Tetrahedron Lett.* **1996**, *37*, 4759−4760.

Thuring, J. W. J. F.; Heinsman, N. W. J. T.; Jacobs, R. W. A. W. M.; Nefkens, G. H. L.; Zwanenburg, B. Asymmetric synthesis of all stereoisomers of demethylsorgolactone. Dependence of the stimulatory activity of *Striga hermontica* and *Orobanche crenata* seed germination on the absolute configuration. *J. Agric. Food Chem.* **1997a**, *45*, 507−513.

Thuring, J. W. J. F.; Nefkens, G. H. L.; Zwanenburg, B. Asymmetric synthesis of all stereoisomers of the strigol analogue GR 24. Dependence of absolute configuration on

stimulatory activity of *Striga hermontica* and *Orobanche crenata* seed germination. *J. Agric. Food Chem.* **1997b**, *45*, 2278−2283.

Van Oeveren, A. 5-Alkoxy-2-(5H)-furanones in asymmetric synthesis, Ph.D. thesis, University of Groningen, The Netherlands, 1996.

Zwanenburg, B.; Mangnus, E. M.; Thuring, J. W. J. F. Strigol analogues: design, synthesis and biological activity. In *Proceedings of the Third International Workshop on Orobanche and Related Striga Research*; Pieterse, A. H.; Verkley, J. A. C.; ter Borg, S. J., Eds.; Royal Tropical Institute: Amsterdam, 1994; pp 187−197.

Received for review August 19, 1998. Revised manuscript received February 8, 1999. Accepted February 19, 1999.

JF980926E