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Changes in the sensitivity of parasitic weed seeds to germination stimulants

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Abstract

The effects of preconditioning temperature and preconditioning period on the sensitivity of parasitic weed seeds to the synthetic germination stimulant GR24 were studied under laboratory and field conditions. The temperature during preconditioning of *Orobanche cumana* and *Striga hermonthica* seeds strongly affected the responsiveness of the seeds to the applied germination stimulant. Preconditioning at an optimal temperature (21°C for *O. cumana* and 30°C for *S. hermonthica*) rapidly released dormancy and increased the sensitivity to GR24 by several orders of magnitude. After reaching maximum sensitivity, prolonged preconditioning rapidly induced secondary dormancy, i.e. decreased sensitivity of *O. cumana* and *S. hermonthica* to GR24. The rapid change in sensitivity of preconditioned seeds to germination stimulants during prolonged preconditioning was particularly visible at low concentrations of GR24. GR24 at higher concentrations (0.1 and 1 mg l^{-1}) usually induced high germination of both species, regardless of the preconditioning period. The striking similarities between the response of parasitic weed seeds to GR24, described here, and results in the literature on non-parasitic wild plant seeds are discussed. Our results show that parasitic weed seeds are highly sensitive to the germination stimulant for a short period of time only, and then enter into secondary dormancy relatively quickly. The similar germination pattern of *S. hermonthica* seeds preconditioned for prolonged periods of time under laboratory and field conditions suggests that the mechanism observed is of ecological significance.

Keywords: GR24, *Orobanche*, parasitic weeds, preconditioning, seed dormancy, *Striga*

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Introduction

Parasitic weeds cause enormous yield losses in agriculture. Broomrapes (*Orobanche* spp., *Orobanchaceae*) and witchweeds (*Striga* spp., *Scrophulariaceae*) are serious pests in many countries. Infected crops can be heavily damaged even before *Orobanche* or *Striga* emerge from the soil. *Orobanche* spp. are holoparasites that lack chlorophyll, and for their development they obtain water and nutrients through the roots of their host. *O. cumana* Wallr. parasitizes sunflower in Spain, as well as in eastern Europe around the Black Sea (Akhtouch *et al*., 2002). *O. ramosa* is distributed widely in southern Europe and the Mediterranean region, and the pest has been introduced to regions of South Africa, USA and Central America (Musselman, 1994). It parasitizes mainly tomato, potato and tobacco. *Striga* spp. belong to the hemiparasites, with lower photosynthetic activity, and behave basically as holoparasites (Parker and Riches, 1993). Hosts of *S. hermonthica* include grain cereals, such as maize, sorghum, millet and upland rice (Press *et al*., 2001).

The first critical step in the life cycle of these parasites – germination of their seeds – is regulated by specific chemical signals exuded by the roots of host plants. For *Striga* spp. several germination stimulants have been identified from host and non-host plants. Most of them are known as strigolactones (Fig. 1). Germination stimulants in maize and sorghum were identified as strigol (Siame *et al*., 1993) and sorgolactone (Hauck *et al*., 1992). Alectrol and orobanchol, the germination stimulants for *O. minor*, were isolated and identified from the root exudate of red clover (Yokota *et al*., 1998). Knowledge of the biosynthetic pathways and genes involved in the biosynthesis of germination stimulants will help in the development of new methods (strategies) and crop varieties to control broomrape and striga parasitism (Bouwmeester *et al*., 2003).

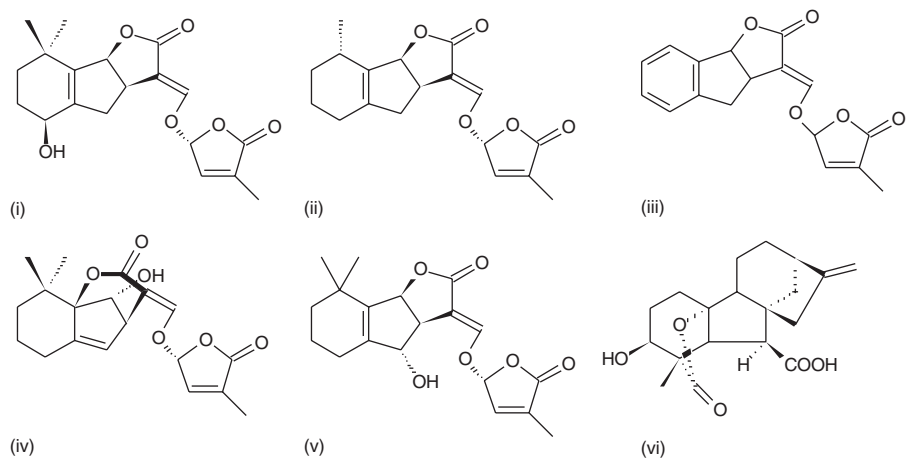

Figure 1. Structures of the germination stimulants of parasitic weed seeds, and gibberellin (GA). (i) (+)-Strigol, (ii) sorgolactone, (iii) the synthetic germination stimulant GR24, (iv) alectrol, (v) orobanchol, (vi) GA4.

The germination stimulants are active in extremely low concentrations, from 10^{-7} to 10^{-15} M (Joel, 2000), and thousands of plants were required to isolate and identify the germination stimulants mentioned above. To study the biosynthesis of germination stimulants and the effects of environmental factors or inhibitors, parasitic weed seeds with high sensitivity to the germination stimulants are required. It is well known that the seeds of *Orobanche* and *Striga* spp. require a moist environment for a certain period of time at a suitable temperature (Joel *et al*., 1995) before the seeds become responsive to the germination stimulants. This period is described as 'preconditioning' or 'conditioning'. During the preconditioning period seeds start to be metabolically active. Several peaks of respiratory activity and protein synthesis were observed during preconditioning of *O. aegyptiaca* seeds. Respiratory activity and protein biosynthesis decreased at the stage at which the seeds became responsive to the germination stimulant (Mayer and Bar Nun, 1997). Conditioning also removes a restriction to the ethylene biosynthetic pathway in *S. hermonthica* seeds (Babiker *et al*., 2000). Endogenous ethylene is required for *S. hermonthica* seed germination (Sugimoto *et al*., 2003). However, the exact role of ethylene in seed germination has not yet been elucidated (Brady and McCourt, 2003).

In this paper we describe the changes in sensitivity of *O. cumana* and *S. hermonthica* seeds to the synthetic germination stimulant GR24 during prolonged preconditioning. The effect of prolonged preconditioning on *S. hermonthica* seed dormancy was tested under laboratory and field conditions. The results are discussed and compared with the safety mechanisms of non-parasitic weed seeds that prevent germination under unfavourable conditions.

Materials and methods

Plant material

Seeds of *O. cumana* were collected in 1999 in Afek, Israel and were the kind gift of Dr D.M. Joel, Newe-Ya'ar Research Center, Agricultural Research Organization, Israel. *S. hermonthica* seeds were collected from a sorghum field in Cinzana, Mali, in 1998, and were the kind gift of Ch. Diarra. For the field experiment, seeds were collected from a *S. hermonthica* population growing on *Sorghum bicolor* at the experimental field station of ICRISAT, Samanko, Mali in 2001. The synthetic strigolactone analogue GR24 was kindly provided by Professor B. Zwanenburg, University of Nijmegen, The Netherlands.

Preconditioning

Preconditioning and germination assays were performed under sterile conditions. The seeds were surface sterilized in 2% sodium hypochlorite containing 0.02% (v/v) Tween 20 for 5 min, and rinsed thoroughly with sterile demineralized water. Subsequently, the seeds were dried for 30 min in a laminar air flow cabinet. Approximately 100–150 seeds were spread on a glass-fibre filter paper (GFFP) disc (9 mm diameter) and put into sterile Petri dishes (9 cm diameter) lined with Whatman filter paper wetted with 2.7 ml of demineralized water. Petri dishes were sealed with Parafilm and incubated for preconditioning. The seeds of *Orobanche* spp. were preconditioned at 21 or 25°C, and those of *S. hermonthica* at 21, 25 and 30°C, all in darkness. Petri dishes were checked regularly and water was added as needed.

The field experiment was carried out in the rainy season from 26 June until October 2002, at the ICRISAT experimental station in Samanko, Mali, on a sandy loam agricultural field. The experimental design consisted of 2 soil treatments (sterilized and non-sterilized), 8 sampling times (seed-bag exhumation dates) and 3 replicates per exhumation date. Soil columns (10 cm diameter × 25 cm high) were exhumed, and the soil kept separate for different soil depths (0–5, 5–10 and 10–25 cm). Then half of each batch of soil was sterilized by heating air-dry soil to 120°C for 48 h. The non-sterile soil treatment consisted of air drying the soil at ambient temperatures (between 20 and 35°C). Soil was returned to the columns according to soil depth (0–5, 5–10 and 10–25 cm) after insertion of plastic pipes (10 cm diameter × 30 cm high) into the field. A sample of 4 mg (about 1000) *Striga* seeds was placed in a polyamide gauze bag. The seeds in the bags were surface sterilized in 1% sodium hypochlorite for 5 min and then placed into the columns at a depth of 5 cm. To prevent fungal infection, 1 mg of the fungicide APRON+ was added. Soil moisture was analysed every 14 d, and ranged from 8.6 to 12.2% (dwt) during the first 80 d. Then regular rains stopped, and the soil dried to 5.9% at the end of the experiment. The soil temperature was not recorded. However, in 2003 at the same location, soil temperature was recorded at a depth of 5 cm at 2-hourly intervals.

Germination bioassay

In the laboratory experiments after the preconditioning period, the GFFP discs with seeds were removed from the Petri dish and dried for 20 min to remove surplus moisture. The discs were transferred into another Petri dish within a filter paper ring (outer diameter 9 cm, inner diameter 8 cm) wetted with 0.9 ml of water, which maintained a moist environment during the germination bioassay. Aliquots of 40 µl of the test solutions were added to each of two replicate discs. A negative control (demineralized water) was included in each bioassay. Seeds were incubated at 25°C (*Orobanche* spp.) and at 30°C (*S. hermonthica*) in darkness for 8 (*Orobanche* spp.) or 2 d (*S. hermonthica*). The germinated and non-germinated seeds were counted using a binocular microscope. Seeds were considered germinated when the radicle protruded through the seed coat.

In the field experiment, exhumed seeds of *S. hermonthica* were surface sterilized for 3 min in 4 ml 1% sodium hypochlorite containing 0.01% Tween 20. Two samples of 50–80 seeds each were placed between two GFFP discs in a Petri dish, between two layers of filter paper (9 cm diameter; Schleicher and Schuell, Dassel, Germany) wetted with 4 ml of distilled water. The Petri dishes were closed with Parafilm, and seeds were preconditioned for 12 d at 28°C in darkness. After preconditioning, 200 µl of 2 mg l^{-1} GR24 were added to the discs, which were then incubated at 28°C for 5 d in darkness. After incubation, the discs were placed on paper towels to dry for 30 min. Then 200 µl of 1% sodium hypochlorite were added, and removed again after 5 min. Four categories of seeds were scored under a binocular microscope: black seeds, germinated seeds, non-germinated intact and empty seeds. Black and empty seeds were considered dead. The germination percentage was calculated by dividing the number of germinated seeds by the number of germinated plus intact, non-germinated seeds.

Calculation of logistic dose–response curves

A quantitative description of the sensitivity of the seeds to the germination stimulant was made by calculating the logistic dose–response curves with non-linear regression, using Slide Write Plus 5.01 (Advanced Graphics Software Inc., Encinitas, California, USA). The equation proposed by Weyers *et al.* (1987), analogous to the Michaelis–Menten model of enzyme kinetics but incorporating a Hill coefficient, was used to analyse the data:

$$R = R_{\min} + \frac{R_{\max} - R_{\min}}{1 + \left(\frac{[GS]_{50}}{[GS]} \right)^{p}}$$

where: $R_{\min}$ is the germination in the absence of a germination stimulant; $R_{\max}$ is the maximum germination; [GS] is the applied dose concentration; $[GS]_{50,}$ the dose required to induce 50% of maximum germination; and p is the Hill, or interaction, coefficient.

Results

The sensitivity of preconditioned seeds to the applied GR24 first increased and then decreased with prolonged preconditioning period, regardless of the species studied or the temperature treatment used. *O. cumana* reached maximum sensitivity after preconditioning for 9–14 d at 21°C (Fig. 2). The change in sensitivity was best visible at the lower GR24 concentrations. Higher GR24 concentrations (0.1, 1 mg l^{-1}) induced high germination of *O. cumana* regardless of the preconditioning period tested.

The temperature during preconditioning of *O. cumana* affected the maximum attainable sensitivity of seeds to the applied germination stimulant (Fig. 3, Table 1). The sensitivity of the seeds to the germination stimulant increased with preconditioning time until the seeds reached the

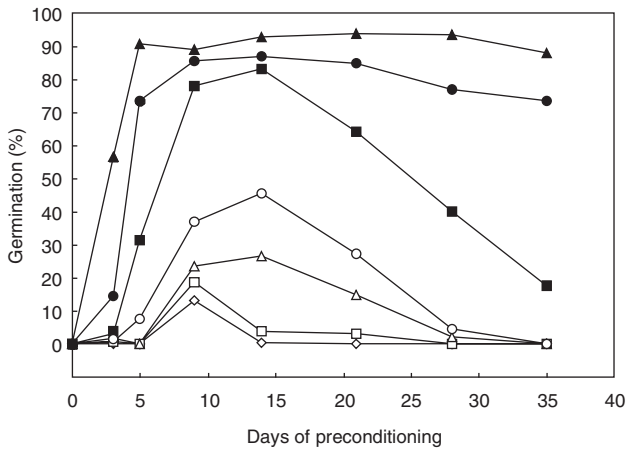

Figure 2. Changes in germination of *Orobanche cumana* seeds in a range of concentrations of GR24. Seeds were preconditioned at 21°C for the time indicated by the *x*-axis, and then germinated in duplicate for 8 d at 25°C in darkness. GR24 concentrations: 0 mg l^{-1} (◇), 0.00001 mg l^{-1} (□), 0.0001 mg l^{-1} (△), 0.001 mg l^{-1} (○), 0.01 mg l^{-1} (■), 0.1 mg l^{-1} (●), 1 mg l^{-1} (▲).

maximal possible sensitivity after 14 d of preconditioning and then decreased again. With an optimal preconditioning temperature, the sensitivity of the seeds to the germination stimulant increased fastest and the maximal sensitivity was highest (0.001 for 21°C versus 0.003 for 25°C) (Table 1, values are represented by [GS_{50}]; Fig. 3A, B). Preconditioning of *O. cumana* seeds at 21°C (Fig. 3A) resulted in higher germination percentages at lower germination stimulant concentrations in comparison to germination of seeds preconditioned at 25°C (Fig. 3B).

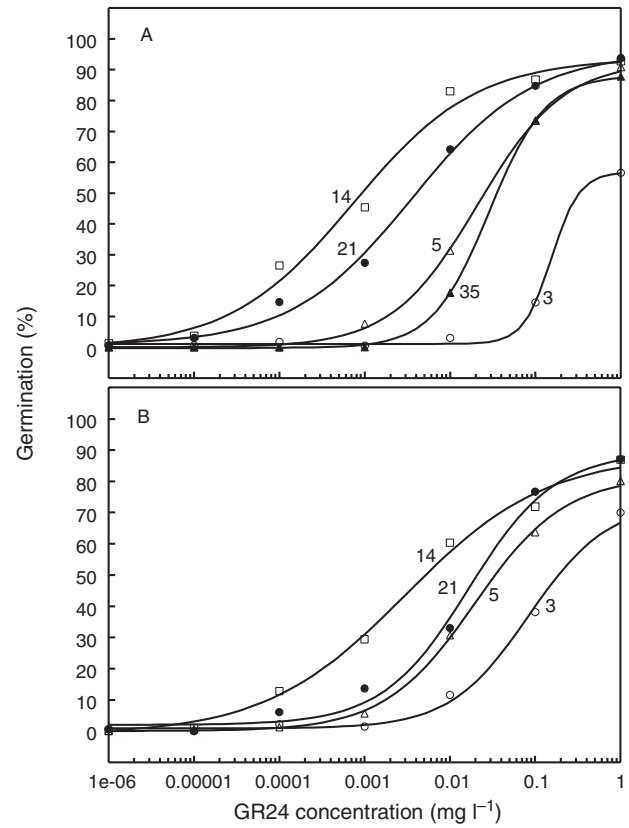

Figure 3. Effect of the preconditioning temperature and duration on the sensitivity of *Orobanche cumana* seeds to GR24 during prolonged preconditioning. Seeds were preconditioned at 21°C (A) and 25°C (B) for 3 d (○), 5 d (△), 14 d (□), 21 d (●) and 35 d (▲). Germination assays were performed in duplicate at 25°C in darkness at the range of GR24 concentrations. GR24 logistic dose–response curve parameters are shown in Table 1.

Table 1. Parameters of GR24 dose–response curves of *Orobanche cumana* seed germination (data from Fig. 3A, B). Dose–response curves were calculated with non-linear regression using Slide Write Plus 5.01. R_{min}, minimum germination, negative values are the result of the curve fitting; R_{max}, maximum germination induced by GR24; $[GS]_{50}$, dose required to induce 50% of maximum germination; *p*, Hill, or interaction, coefficient (Weyers *et al.*, 1987); SE, standard error

Days of preconditioning	R_{min} ± SE (%)	R_{max} ± SE (%)	$[GS]_{50}$ ± SE (mg l^{-1})	*p* ± SE
Preconditioning at 21°C				
3 d	1.1 ± 0.4	56 ± 1.2	0.154 ± 0.044	2.6 ±1.7
5 d	0.0 ± 0.6	93 ± 1.3	0.021 ± 0.001	0.9 ± 0.0
14 d	–0.3 ± 3.4	94 ± 5.2	0.001 ± 0.000	0.6 ± 0.1
21 d	0.8 ± 1.7	95 ± 3.1	0.004 ± 0.001	0.6 ± 0.1
35 d	–0.2 ± 0.2	88 ± 0.5	0.029 ± 0.001	1.3 ± 0.0
Preconditioning at 25°C				
3 d	0.9 ± 1.1	72 ± 2.8	0.084 ± 0.014	0.9 ± 0.1
5 d	0.0 ± 0.5	82 ± 1.2	0.019 ± 0.001	0.8 ± 0.0
14 d	–1.1 ± 2.2	90 ± 4.1	0.003 ± 0.001	0.5 ± 0.1
21 d	2.0 ± 1.8	87 ± 3.7	0.017 ± 0.003	0.9 ± 0.1

The release of dormancy in *S. hermonthica* seeds during preconditioning was also temperature dependent. The rate and percentage of germinated seeds increased with increased preconditioning temperature (up to 30°C). The seeds preconditioned at lower temperatures (21 and 25°C, data not shown) started to be responsive to GR24 later and at higher concentrations of germination stimulant only. The seeds preconditioned at 21 or 25°C did not reach the same maximum germination percentage as seeds preconditioned at 30°C, even after longer preconditioning (data not shown). Therefore, we chose preconditioning at 30°C for a long-term experiment. Seeds of *S. hermonthica* were conditioned at 30°C for up to 95 d (Figs 4 and 5, Table 2). The sensitivity of seeds to the germination stimulant increased with prolonged preconditioning for up to 21–28 d and then decreased again (Fig. 4). As for *O. cumana*, the changes in sensitivity of *S. hermonthica* seeds were best visible in treatments with lower concentrations of germination stimulant.

Figure 6 shows the results of experiments with *S. hermonthica* seeds preconditioned for prolonged periods of time under laboratory (data from Fig. 4) and field (sterile and non-sterile) conditions. The average soil temperature at 5 cm during a similar 140-day period in 2003 was almost the same as in the laboratory (29.3 versus 30°C), with a minimum temperature of 22.0 and a maximum of 47.7°C (Fig. 6). Remarkably, the germination trends in response to

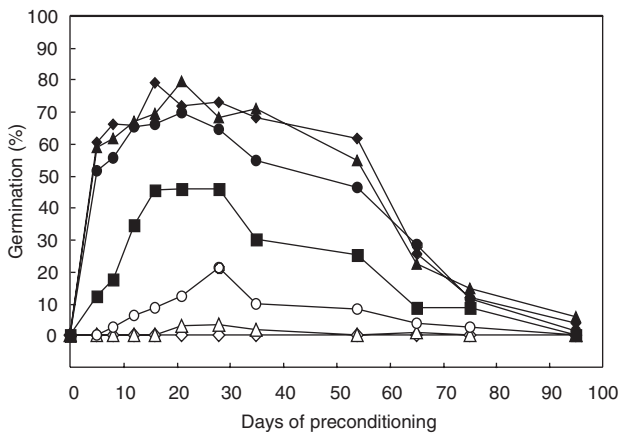

Figure 4. Changes in germination of *Striga hermonthica* seeds in a range of concentrations of GR24. Seeds were preconditioned at 30°C for the time indicated by the *x*-axis and then germinated for 4 d at 30°C. Germination assays were performed in duplicate at 30°C in darkness at the range of GR24 concentrations: 0 mg l^{-1} (◇), 0.00001 mg l^{-1} (□), 0.0001 mg l^{-1} (△), 0.001 mg l^{-1} (○), 0.01 mg l^{-1} (■), 0.1 mg l^{-1} (●), 1 mg l^{-1} (▲) and 10 mg l^{-1} (◆).

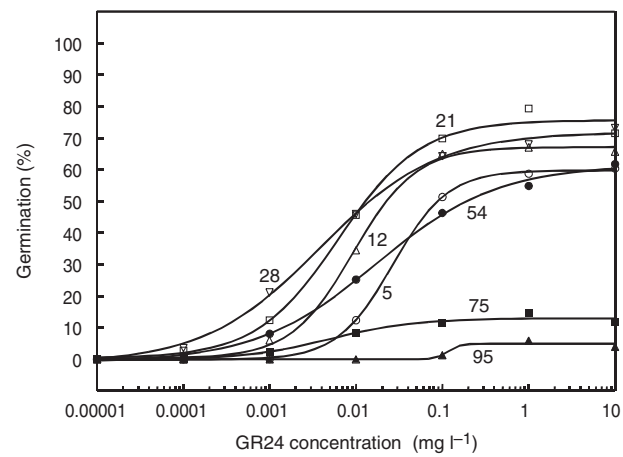

Figure 5. Effect of preconditioning on the sensitivity of *Striga hermonthica* seeds during prolonged preconditioning. Seeds were preconditioned at 30°C for 5 d (○), 12 d (△), 21 d (□), 28 d (▽), 54 d (●), 75 d (■) and 95 d (▲). Germination assays were performed in duplicate at 30°C in darkness at a range of GR24 concentrations. GR24 logistic dose–response curve parameters are shown in Table 2.

Table 2. Parameters of GR24 dose–response curves of germination of *Striga hermonthica* seeds (data from Fig. 5). Dose–response curves were calculated with non-linear regression using Slide Write Plus 5.01. R_{min}, minimum germination, negative values are the result of the curve fitting; R_{max}, maximum germination induced by GR24; $[GS]_{50}$, dose required to induce 50% of maximum germination; *p*, Hill, or interaction, coefficient (Weyers et al., 1987), SE, standard error

Days of preconditioning	R_{min} ± SE (%)	R_{max} ± SE (%)	$[GS]_{50}$ ± SE (mg l^{-1})	*p* ± SE
5 d	–0.2 ± 0.3	66 ± 0.5	0.027 ± 0.001	1.4 ± 0.0
12 d	0.3 ± 0.8	67 ± 1.3	0.009 ± 0.001	1.2 ± 0.1
21 d	0.3 ± 1.9	75 ± 3.1	0.006 ± 0.001	0.9 ± 0.1
28 d	–1.3 ± 1.3	73 ± 2.1	0.004 ± 0.001	0.6 ± 0.1
54 d	–1.0 ± 1.1	62 ± 2.2	0.017 ± 0.003	0.6 ± 0.1
75 d	–0.1 ± 0.8	13 ± 1.2	0.005 ± 0.002	0.9 ± 0.3
95 d	0.0 ± 0.3	5 ± 0.8	0.12 ± 204.040	6.3 ± 66729

GR24 of laboratory- and field-preconditioned seeds were similar. Under field soil conditions, germination was relatively stable from 12 to 40 d of preconditioning. After that, seeds seemed to enter into secondary dormancy. Results from the laboratory conditions showed the same pattern, albeit at a different level, which may be caused by the higher GR24 concentration used in the field experiment.

Discussion

Changes in sensitivity to germination stimulants

Our results show that preconditioning of parasitic weed seeds has a substantial effect on their sensitivity to germination stimulants. Preconditioning at a suitable temperature increases the sensitivity with several orders of magnitude, and prolonged preconditioning can decrease sensitivity again (Figs 2–5; Tables 1 and 2). This rapid change in the sensitivity of *Orobanche* and *Striga* seeds to the germination stimulant during prolonged preconditioning has not been reported before, although many authors have studied the effects of preconditioning and temperature on germination of parasitic weed seeds (e.g. Reid and Parker, 1979; Hsiao *et al*., 1988; Van Hezewijk *et al*., 1993). In most of these studies, relatively high concentrations of germination stimulants were used. These high concentrations do not allow us to appreciate the actual dormancy status of preconditioned seeds. In our experiments, the changes in sensitivity of *O. cumana* and *S. hermonthica* were also less visible at the higher concentrations of GR24, and particularly at the lower concentrations, the strong change in the response of preconditioned seeds to the germination stimulant could be observed (Figs 2 and 4). This is simply caused by the fact that the high concentrations of GR24 are saturating and, hence, induce maximal germination for a prolonged period of time, even when the sensitivity to the germination stimulant does change.

Although it has been reported before that the optimal preconditioning temperature and period vary between different species or different seed populations of the same species (Logan and Stewart, 1992), we feel that the strong influence of preconditioning has been underestimated in parasitic weed research. If seeds with a high sensitivity to germination stimulants are required for experiments, the optimal preconditioning temperature and preconditioning period should be carefully determined for each individual batch of parasitic weed seeds. From our results, it proved to be helpful to apply lower (suboptimal) concentrations of germination stimulants to find this optimal preconditioning period and temperature. Therefore, these lower concentrations of germination stimulants should be included routinely in germination experiments with parasitic weed seeds.

Comparison with non-parasitic plant seeds

The shape of the logistic dose–response curves and the changes due to the preconditioning period (Figs 3

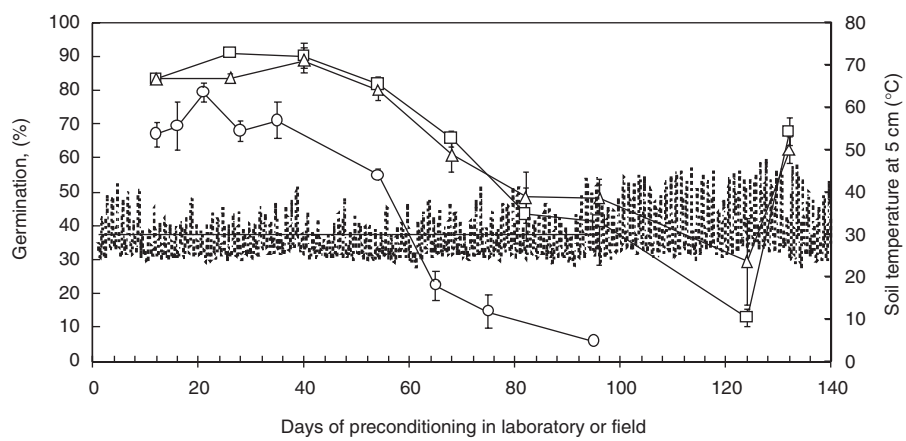

Figure 6. Effect of preconditioning time of *Striga hermonthica* seeds under laboratory and field soil conditions on germination of intact seeds. Seeds were preconditioned in darkness at 30°C under laboratory conditions (○) (in duplicate) and buried 5 cm deep in soil under sterile (□) and non-sterile (△) field conditions (in triplicate). Concentrations of GR24 used in the germination assays were 1 mg l^{-1} for the laboratory experiment and 2 mg l^{-1} for the field experiment. The broken line represents the soil temperature measured at 2-hourly intervals at 5 cm at the same location as the seeds were buried, but 1 year later (2003). The straight solid line represents temperature during laboratory preconditioning (30°C). Error bars represent SEM.

and 5) are similar to dormancy and sensitivity changes of seeds of non-parasitic wild plants to, for example, light, nitrate and gibberellin (Derkx and Karssen, 1993a, b; Derkx and Karssen, 1994). From their results, Karssen and co-workers concluded that changes in the sensitivity to light and nitrate are responsible for changes in dormancy in *Arabidopsis* and *Sisymbrium officinale*. Clearly, both parasitic, as well as non-parasitic, plants have evolved an important safety mechanism to prevent germination under adverse conditions. The latter have adapted to respond to a signal(s) indicative of a suitable position (light) or growth-conditions (nitrate), the former to a signal indicative of the presence of a host. In *Arabidopsis* and *S. officinale* the sensitivity to gibberellins increased during the release of primary dormancy, but it did not decrease again during induction of secondary dormancy (Derkx and Karssen 1993a, 1994). Therefore, the authors concluded that sensitivity to gibberellins is not the mechanism responsible for changes in dormancy in the seeds of these two species. Nevertheless, the similarity of the shift in the gibberellin-response curves during dormancy relief in *Arabidopsis*, as reported by Derkx and Karssen (1994), and the shift in the GR24-response curves we report here are striking. Also, gibberellins and a putative gibberellin receptor do play a crucial role in the germination of non-parasitic wild plant seeds. According to a model proposed by Hilhorst and Karssen, gibberellin biosynthesis and sensitivity to gibberellin in these seeds are controlled by a receptor that is activated by nitrate and red light (Hilhorst and Karssen, 1988; Hilhorst, 1993).

Considering the fair degree of similarity between the structures of the known parasitic weed seed germination stimulants and gibberellins (Fig. 1), it is tempting to speculate about a possible joint evolutionary origin of the receptors for these stimuli. Gibberellins seem to be important for effective preconditioning of *O. minor* and *O. cumana*, but they do not induce nor affect germination-stimulant-induced germination (Takeuchi *et al.*, 1995). The involvement of a receptor for the germination stimulants has been postulated by Zwanenburg and co-workers (Wigchert and Zwanenburg, 1999), and a first indication of a protein specifically binding GR24 obtained (Reizelman Lucassen, 2003; J. Beekwilder, A. Reizelman-Lucassen, P. Bakker, H.J. Bouwmeester and B. Zwanenburg, unpublished results). A gibberellin receptor in non-parasitic weed seeds was postulated (Hilhorst *et al.*, 1986, 1996). The cloning of both receptors, which is being attempted by several groups (Lovegrove *et al.*, 1998; B. Zwanenburg, H.J. Bouwmeester *et al.*, unpublished results), should shed light on this possible evolutionary relationship.

The involvement of a receptor in germination-stimulant recognition is also suggested by the dose–response curves in Figs 3 and 5. Overall, these curves are very similar to other dose–response systems, i.e. the effect of light and nitrate on germination of non-parasitic weed seeds (Derkx and Karssen, 1993a, 1994) and the response of dwarf mutants of barley to gibberellin (Chandler and Robertson, 1999), suggesting that the stimulant-induced germination of parasitic weed seeds is mediated by a receptor–ligand interaction. The changes in the dose–response curves with preconditioning may be used to interpret the mechanism of these changes. For example, R_{max} increases in the early stages of preconditioning and decreases again after prolonged preconditioning (Figs 3 and 5, Tables 1 and 2). According to Weyers *et al.* (1987), changes in this parameter could arise from changes in the number or availability of the receptors or from a change in the response reaction (signalling cascade downstream of the germination stimulant–receptor complex). Preconditioning results in virtually parallel shifts in the dose–response curves along the *x*-axis, which is apparent from the changes in the $[GS]_{50}$ (Figs 3 and 5; Tables 1 and 2). If the Hill coefficient equals 1, $[GS]_{50}$ is the dissociation constant (K_D) for the receptor–ligand complex and, as such, a measure for the affinity of the receptor or binding characteristics for the germination stimulant (Weyers *et al.*, 1987). Shifts along the *x*-axis to the left could indicate increased affinity; shifts to the right, decreased affinity (Firn, 1986). For example, during preconditioning of *O. cumana* seeds, the affinity increased 150-fold between 3 and 14 d of preconditioning at 21°C ($[GS]_{50}$ of 0.154 versus 0.001 mg l^{-1}).

Ecological consequences

All this shows that the dormancy of parasitic weed seeds is released during preconditioning and that seeds, therefore, begin to be sensitive to increasingly lower concentrations of the germination stimulant. With prolonged preconditioning, seeds enter secondary dormancy and become less sensitive to the germination stimulant. These substantial changes in sensitivity suggest that this is a safety mechanism that restricts the period in which the seeds can respond to the germination stimulants produced by host plants. Indeed, there are several publications showing that a later crop-sowing date strongly reduces infection by parasitic weeds; for example, of sunflower by *O. cumana* (Ish-Shalom-Gordon *et al.*, 1994; Sukno *et al.*, 2001; Eizenberg *et al.*, 2003), and a delay of 10 d in sowing of sorghum considerably reduced emergence of *S. hermonthica* plants in Mali (A. van Ast, personal communication). Although there is no direct proof that this is due to the induction of dormancy (= a

decrease in sensitivity to germination stimulants), it would be interesting to see whether this plays a role in the positive effect of delayed sowing, and whether it could be developed into a control strategy.

Although we do not know whether the same processes that we observed under laboratory conditions also occur in the field, the similar germination pattern of *S. hermonthica* seeds preconditioned for a prolonged period of time under laboratory (sterile) and field (sterile and non-sterile) conditions suggest that they do. This assumption is supported by the work of van Hezewijk *et al.* (1994) and López-Granados and Garcia-Torres (1999), who studied seasonal changes in the germination response of buried seeds of *O. crenata*. The results from field experiments were highly consistent with the results of *in vitro* experiments by van Hezewijk *et al.* (1993) on development of secondary dormancy during prolonged preconditioning of *O. crenata* at different temperatures. However, more detailed studies will be necessary to answer this question.

Redefining dormancy in parasitic plant seeds

Considering the wealth of literature, it is clear that release and re-induction of dormancy, which we have defined as the sensitivity to germination stimulants, depend on the preconditioning temperature and period. We feel that the term 'wet dormancy', introduced by Vallance (1950), does not characterize the factor that induces dormancy of parasitic weeds. Moisture is a prerequisite for preconditioning, but it is not the cause of dormancy. Considering the similarities between parasitic and non-parasitic wild plants in the changes in sensitivity to external stimuli, we suggest the adoption of the terminology and concepts used for dormancy and dormancy changes of the non-parasitic wild plants (for example, see Vleeshouwers *et al.*, 1995; Kebreab and Murdoch, 1999). This is further supported by the conclusions of seed ecophysiologists (Baskin and Baskin, 1998), who state that the germination ecology of the *Orobanchaceae* is similar to that of non-parasitic winter annuals. That is, with an increase in the dormancy-breaking temperature, the rate of dormancy loss and the maximum temperature for germination increase. Kebreab and Murdoch (1999) showed that the changes in dormancy of seeds of two O*robanche* spp. could be predicted using temperature as a driving force and a model describing temperature effects on dormancy of the non-parasitic *Rumex* spp., as proposed by Totterdell and Roberts (1979).

If we adopt the dormancy-terminology of non-parasitic wild plant seeds, freshly produced parasitic weed seeds are in a state of primary dormancy, i.e. they will not, or hardly, respond to an exogenous stimulus – the germination stimulant in the case of parasitic weed seeds. Primary dormancy is released upon pretreatment under warm, moist conditions. Generally, in parasitic plant research this pretreatment is called preconditioning, whereas in seed dormancy terms this would be called (warm dark-) stratification (Steadman, 2004). During release of primary dormancy, the sensitivity to the germination stimulant increases, until the seeds are non-dormant (maximally responsive to the germination stimulant). Upon longer preconditioning (warm stratification), secondary dormancy develops, and the seeds gradually lose their responsiveness to the germination stimulant until they will not germinate at all, even at high germination stimulant concentrations. The field results (Fig. 6) suggest that release of secondary dormancy also occurs (as for non-parasitic wild plant seeds), but we can not conclude whether this just requires prolonged preconditioning (warm stratification) under moist conditions (as is the case for non-parasitic weed seeds) or a dry period with increased temperatures (as occurs under field conditions in West Africa). Proof of this would require prolonged laboratory experiments and more detailed data on soil temperature and humidity under field conditions. In principle, these cyclic changes in dormancy could go on for many years until the seed germinates or dies (Vleeshouwers *et al.*, 1995), and for *O. crenata* this was indeed demonstrated by López-Granados and Garcia-Torres (1999). Finally, it is important to realize that release and induction of dormancy are dependent on time and temperature and occur gradually. Even if a seed batch has developed 'secondary dormancy' due to prolonged preconditioning (warm stratification; the seeds do not germinate, even at a high concentration of germination stimulant), the physiological status of the seeds may continue to change. For example, dormancy may further develop or be released, even if we can not (yet) see that from the response to the germination stimulant (because there is no germination). Hence, for the optimal characterization of the dormancy status of parasitic weed seeds, it is necessary to test germination over a range of germination stimulant concentrations.

Conclusion

It is clear that dormancy is a crucial safety mechanism in the parasitic weed life cycle, as it is in non-parasitic wild plant seeds. Parasitic *Orobanche* and *Striga* spp. germinate in response to germination stimulants, which are exuded by roots of host plants. The release of dormancy in parasitic weed seeds and re-induction of dormancy are highly dependent on the preconditioning temperature and preconditioning period. The sensitivity of the seeds to the germination stimulant increases during the preconditioning

period, and the seeds are highly sensitive to the germination stimulant for a short period of time only, and then enter into secondary dormancy. Assuming that the production of germination stimulants by host roots continues through host plant development, this relatively short period at which seeds are responsive to the germination stimulants ensures that the seeds will not germinate too late in the season, when – despite the presence of a host, moisture and a suitable temperature – the conditions will not allow completion of the life cycle of the parasite.

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