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Induction of seed germination in *Orobanche* spp. by extracts of traditional Chinese medicinal herbs

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The co-evolution of *Orobanche* spp. and their hosts within the same environment has resulted in a high degree of adaptation and effective parasitism whereby the host releases parasite germination stimulants, which are likely to be unstable in the soil. Our objective was to investigate whether extracts from non-host plants, specifically, Chinese medicinal plants, could stimulate germination of *Orobanche* spp. Samples of 606 Chinese medicinal herb species were extracted with deionized water and methanol. The extracts were used to induce germination of three *Orobanche* species; *Orobanche minor*, *Orobanche cumana*, and *Orobanche aegyptiaca*. *O. minor* exhibited a wide range of germination responses to the various herbal extracts. *O. cumana* and *O. aegyptiaca* exhibited an intermediate germination response to the herbal extracts. *O. minor*, which has a narrow host spectrum, showed higher germination rates in response to different herbal extracts compared with those of *O. cumana* and *O. aegyptiaca*, which have a broader host spectrum. Methanolic extracts of many Chinese herbal species effectively stimulated seed germination among the *Orobanche* spp., even though they were not the typical hosts. The effective herbs represent interesting examples of potential trap crops. Different countries can also screen extracts from indigenous herbaceous plants for their ability to induce germination of *Orobanche* spp. seeds. The use of such species as trap plants could diminish the global soil seed bank of *Orobanche*.

***Orobanche* spp., methanolic extracts, deionized water extracts, germination, Chinese medicinal plants**

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Orobanche spp. are holoparasites of higher plants. Parasitic weeds of the *Orobanchaceae* family are widely distributed throughout the Mediterranean region, western Asia, and eastern Europe, and they cause substantial losses to global crop production. They are considered to be the most important parasitic weeds on a world-wide scale [1]. There are 11 species of the genus *Orobanche* in northern and southwestern China [2–4]. The genus is particularly prevalent in the Xinjiang Uygur Autonomous Region, where it has caused yield losses to several economically important plants such as sunflower (*Helianthus annuus* L.), tobacco (*Nicotiana tabacum* L.), and tomato (*Lycopersicon esculentum* Mill.).

Orobanche spp. are difficult weeds to control because of their complex life cycle. There are several discrete steps in the life cycle; the production of a large number of seeds that require a post-ripening period as well as warm and moist conditions, induction of germination by host-derived stimulants, haustorial initiation by host-plant haustorium inducers, attachment to the host root and penetration, establishment of contact with host vascular system, subterranean development, emergence, and flowering. The most serious damage

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to host crops occurs underground before emergence, as described by Parker and Riches [1]. Thus, the depletion of its soil seed bank by inducing "suicidal germination" would be an efficient way to control *Orobanche*.

Investigations into another parasitic weed, *Striga*, have advanced research on *Orobanche* seed germination stimulants. The first compound isolated as a *Striga* germination stimulant was strigol, later classified as one of a group of plant hormones known as strigolactones. Strigol was isolated from a non-host cotton (*Gossypium hirsutum* Linn.) plant in the 1960s and 1970s [5,6]. Strigolactones have been extensively characterized as seed germination stimulants of parasitic weeds. They are exuded from plant roots and released into the rhizosphere [7]. To date, three classes of plant secondary metabolites have been shown to induce seed germination in *Striga* and *Orobanche*; dihydrosorgoleones, sesquiterpene lactones, and strigolactones [8–10]. Among these, strigolactones show the strongest activity. Different strigolactones have been isolated from host and non-host plants of *Orobanche*, *Phelipanche*, and *Striga* [10,11]. To date, the structures of nine strigolactones have been elucidated. These include sorgomol [12], isolated from root exudates of sorghum (*Sorghum bicolor* L.), and fabacyl acetate [13], isolated from root exudates of pea (*Pisum sativum* L.). Didehydroorobanchol, orobanchol, orobanchyl acetate, and 5-deoxystrigol were also identified from the root exudates of *Fabaceae* plants [14]. Research on the isolation and identification of orobanchol [15,16], the first *Orobanche* germination stimulant isolated from root exudates of red clover (*Trifolium pratense* L.), demonstrated that strigolactones stimulate germination in both *Striga* and *Orobanche* spp. Recently, Evidente *et al*. [17] reported the isolation of two strigolactone-like compounds (peagol and peagoldione) from pea root exudates. Both compounds showed selective stimulation of *Orobanche* seed germination.

Much attention has been paid to the isolation and identification of germination stimulants from host plants of *Striga* and *Orobanche*. Although the first germination stimulant, strigol, was isolated from a non-host cotton plant, most attempts to isolate germination stimulants have been made using host plants. Fernández-Apariciol *et al*. [18] reported the induction of seed germination of nine *Orobanche* and *Pheliphanche* species by root exudates of 41 plants. However, most of these plants were cultivated field crops rather than wild plants.

We hypothesized that, if host plants could exude stable germination stimulants, many of the parasitic seeds would be induced to germinate during the growing season and this would greatly reduce the soil seed bank. In theory, this would reduce or eliminate the parasite problem, but it is unknown whether this strategy is successful in practice. Therefore, it is important to identify potent germination stimulants from non-host plants. This raises the question of where to find such plant materials, since not all plants are able to produce the stimulants. It is an established practice in Chinese medicine to use medicinal herbs to cure human and animal diseases. We speculated that because these plants contain substances with anti-fungal and/or antibacterial activity, and because they did not co-evolve with *Orobanche* plants, they may produce alternative chemicals to the known strigolactones that might be stable in soil.

Previously, we reported the screening of extracts from 383 traditional Chinese medicinal herbs and tested their ability to induce or inhibit the germination of *Striga hermonthica* seeds [19]. Deionized water and methanolic extracts of more than 26 herbs induced germination of *S. hermonthica* [19]. It was also reported that the Chinese medicinal herbs *Menispermum dauricum* (DC.) and *Houttuynia cordata* Thunb. produced germination stimulants for *S. hermonthica* [20–22]. Jin *et al*. [23] reported the extraction of active substances from 240 Chinese medicinal herbs and found that more than 40 of them were able to induce seed germination of *O. minor*. Therefore, we assumed that there would be other Chinese herbs that could produce germination stimulants for *Orobanche* spp. weeds. To test this hypothesis, we evaluated 606 economically important and commonly used Chinese medicinal herbal extracts for their efficacy to stimulate seed germination in *Orobanche minor*, *Orobanche cumana*, and *Orobanche aegyptiaca.*

1 Materials and methods

1.1 Seeds and chemicals

Seeds of *O. cumana* and *O. aegyptiaca* were collected in 2008/2009 from infested sunflower (*H. annuus*) and tomato (*L. esculentum*) fields in Xinjiang Uygur Autonomous Region, China. Seeds of *O. minor* were provided by Professor Koichi Yoneyama (Utsunomiya University, Japan). The germination stimulant, GR24, a synthetic analogue of strigol, was provided by Professor Binne Zwanenburg (University of Nijmegen, The Netherlands).

1.2 Herbal powders

We obtained 606 species of Chinese medicinal herbs from Chinese medicinal drug stores distributed in all the provinces of mainland China, excluding Tibet. The medicinally useful parts of the herbs were air-dried and then milled using a herbal medicine disintegrator (FW135-177, Tianjin Taisite Instrument Co., Ltd., Tianjin, China) and passed through a sieve (0.45 mm in diameter).

1.3 Surface sterilization and conditioning of *Orobanche* seeds

All seeds were surface-sterilized by immersion for 3 min in 1% sodium hypochlorite. Seeds of *O. minor* were then thoroughly washed with deionized water, pre-treated (con-

ditioned) for 6–8 d on 8 mm disks of glass fiber filter paper (GFFP, Whatman, GE Healthcare UK Ltd., Buckinghamshire, UK), and then incubated in the dark at 25°C. Seeds of *O. cumana* and *O. aegyptiaca* were already receptive before conditioning based on preliminary testing and the results of Plakhine *et al*. [24]; hence these seeds were used directly for bioassays.

1.4 Germination assay

A sample (100 mg) of each herb was sonicated for 30 min in 1.5 mL deionized water. The aqueous extracts were centrifuged in a microcentrifuge (Millipore Cat. No. XX42 CF0, 60 Lot No. N8JMB042A, Nihon Millipore Ltd. Yonezawa, Japan). We prepared three concentrations of the supernatants; undiluted, and 10- and 100-fold dilutions in deionized water. The two aqueous solutions and the undiluted extracts were assayed immediately by applying 15 μL of each solution to the conditioned *Orobanche* seeds on 8 mm disks of GFFP in Petri dishes. The disks with the conditioned seeds were briefly blotted on filter paper (Shuangquan GB/T1914-2007, Hangzhou Wohua Filter Paper Co., Ltd., Hangzhou, China) to remove excess water before treatments with the extracts. Three concentrations of methanolic extracts were obtained in the same way. Aliquots (15 μL) of the test solution were applied to 8-mm disks of GFFP and the disks were allowed to dry. A disk with conditioned *Orobanche* seeds was placed on top of the dried treated disk and moistened with 30 μL deionized water. The treated seeds were incubated in the dark at 25°C and examined for germination 8 days later. Controls comprised three GR24 treated disks with conditioned *Orobanche* seeds (positive control) and three deionized water treated disks (negative control).

Individual treatments were replicated four times. We used 20 herbs in each batch of bioassay experiments. The herbal extracts shown to affect *Orobanche* seed germination were included in the next round of germination testing. When the screening of the 606 herbs was complete, the ones that positively affected *Orobanche* seed germination were retested using the same group of conditioned seeds. Germinated and non-germinated seeds were counted under a binocular dissecting microscope at 20× magnification. Seeds were considered to have germinated on protrusion of the "germ-tube" from the seed coat.

1.5 Statistical analysis

Orobanche spp. germination data were subjected to analysis of variance. Tukey's multiple range tests were used to separate the means. Data were processed using Excel 2007 and DPS 6.55 software (DPS Soft Inc., Hangzhou, China).

2 Results

Seeds of all the three *Orobanche* spp. germinated at a high rate (>78%) when treated with GR24. None of them germinated when treated with deionized water. In this study, only those herb species whose extracts induced more than 30% of *Orobanche* seeds to germinate at one or more of the concentrations (undiluted, 10- , or 100-fold dilutions) were considered.

2.1 Induction of germination of *Orobanche minor* seeds

Deionized water extracts of 20 herbal species, at at least one concentration, resulted in high germination rates (>60%) of *O. minor*. The maximum germination rate of 90.3% was induced by the 10-fold dilution of the extract of *Juncus effusus* L. var. decipiens Buchen. Most of the undiluted extracts induced germination at lower rates. However, the undiluted *J. effusus* L. var. decipiens Buchen extract induced germination at the rate 72.7% (Table 1).

Based on our results, we classified the herbs into three

Table 1 Germination of *O. minor* seeds induced by deionized water extracts of traditional Chinese medicinal herbs (%)[a)]

Medicinal herbs	Undiluted	10-fold dilution	100-fold dilution
Aconitum carmichaeli Debx.	0.0 g[†]	24.7 cdefg	41.5 abcdefgh
Akebia quinata (Thunb.) Decne.	0.0 g	59.4 abcdef	65.8 abc
Aloe vera L.	21.9 cd	51.7 abcdefg	50.2 abcdefg
Alpinia katsumadai Hayata.	0.0 g	63.7 abcde	62.9 abcd
Angelica sinensis (Oliv.) Diels	0.0 g	25.2 cdefg	40.0 abcdefgh
Ardisia japonica (Hornsted) Blume.	0.0 g	14.7 efg	47.6 abcdefgh
Areca catecthu L.	0.0 g	42.2 abcdefg	30.0 abcdefgh
Arenaria kansuensis Maxim.	0.0 g	52.7 abcdefg	45.7 abcdefgh
Artemisia argyi Levl. et Vant.	7.7 efg	43.9 abcdefg	64.2 abcd
Artemisiae anomalae Herba.	0.0 g	46.0 abcdefg	27.7 abcdefgh
Benincasa hispida (Thunb.) Cogn.	0.0 g	36.8 bcdefg	20.9 cdefgh
Blumea balsamifera DC.	0.0 g	10.2 fg	45.5 abcdefgh
Buddleja officinalis Maxim.	0.0 g	31.5 bcdefg	30.1 abcdefgh
Cannablis sativa L.	8.0 efg	66.3 abcde	5.6 gh

(*To be continued on the next page*)

(*Continued*)

Medicinal herbs	Undiluted	10-fold dilution	100-fold dilution
Chrysanthemum indicum L.	0.0 g	25.8 cdefg	39.2 abcdefgh
Cibotium barometz (L.) J. Sm.	0.0 g	48.4 abcdefg	54.2 abcdef
Cinnamomum cassia Presl	0.0 g	40.4 abcdefg	32.9 abcdefgh
Citrus aurantium L.	0.0 g	0.0 g	43.3 abcdefgh
Citrus reticulata Blanco	0.0 g	15.4 efg	49.4 abcdefg
Citrus tangerina Hort. et Tanaka	0.0 g	0.0 g	34.6 abcdefgh
Cyathula officinalis Kuan.	0.0 g	40.1 abcdefg	72.2 ab
Cynanchum paniculatum (Bunge) Kitagawa	0.0 g	59.4 abcdef	65.8 abc
Dalbergia odorifera T. Chen.	21.7 cd	79.3 ab	61.7 abcd
Epimedium brevicornum Maxim.	0.0 g	33.0 bcdefg	39.1 abcdefgh
Eriocaulon buergerianum Koern.	14.0 def	41.7 abcdefg	24.3 bcdefgh
Eupatorium fortunei Turcz.	0.0 g	48.4 abcdefg	61.3 abcd
Farfugium japonicum (L.) Kitam	15.3 de	37.1 bcdefg	9.9 efgh
Glycine max (L.) Merr.	0.0 g	33.6 bcdefg	33.6 abcdefgh
Helianthus annuus L.	0.0 g	55.2 abcdef	51.7 abcdefg
Ilex rotunda Thunb.	2.5 fg	16.5 defg	66.9 abc
Illicium verum Hook. f.	0.0 g	8.6 fg	70.3 ab
Jasminum sambac (L.) Ait.	44.7 b	27.8 bcdefg	34.2 abcdefgh
Juncus effusus L. var. decipiens Buchen	72.7 a	90.3 a	29.0 abcdefgh
Kaempferia galanga L.	0.0 g	44.8 abcdefg	36.8 abcdefgh
Ligusticum chuanxiong Hort	0.0 g	38.1 abcdefg	62.9 abcd
Lophatherum gracile Bongn.	0.0 g	67.3 abcde	28.7 abcdefgh
Lycopodium clavatum L.	5.6 efg	44.9 abcdefg	9.0 efgh
Magnolia officinalis Rehd. et Wils.	0.0 g	9.8 fg	33.7 abcdefgh
Nardostachys chinensis Batal.	16.0 de	37.2 bcdefg	35.7 abcdefgh
Nelumbo nucifera Gaertn.	2.0 fg	69.0 abcd	72.0 ab
Ottelia alismoides (L.) Pers.	0.0 g	30.4 bcdefg	25.4 bcdefgh
Paeonia lactiflora Pall.	0.0 g	50.6 abcdefg	54.8 abcdef
Phyllanthus urinaria Linn.	0.0 g	15.3 efg	43.5 abcdefgh
Phyllostachys sulphurea (Carr.) A. et C. Riv.	0.0 g	41.8 abcdefg	74.5 a
Pinellia pedatisecta Schott.	48.4 b	59.1 abcdef	24.7 bcdefgh
Poeonia suffruticosa Andr.	0.0 g	29.6 bcdefg	55.3 abcde
Polygonum orientale L.	0.0 g	17.5 defg	33.9 abcdefgh
Polyporus umbellatus (Pers.) Fries	47.0 b	38.2 abcdefg	45.2 abcdefgh
Poria cocos (Schw.) Wolf.	29.3 c	40.4 abcdefg	16.5 defgh
Prunus mume Sieb. et. Zucc.	0.0 g	44.6 abcdefgl	8.9 efgh
Pteris multifida Poir.	0.0 g	35.5 bcdefg	0.0 h
Pueraria lobata (Willd.) Ohwi .	0.0 g	44.2 abcdefg	20.7 cdefgh
Pulsatilla chinensis (Bge.) Reg.	0.0 g	0.0 g	50.7 abcdefg
Pyrola calliantha H. Andres.	0.0 g	20.3 cdefg	31.9 abcdefgh
Pyrrosia lingua (Thunb.) Farw.	0.0 g	48.5 abcdefg	27.5 abcdefgh
Rhaponticum uniflorum (L.) DC	4.0 efg	54.0 abcdef	7.3 fgh
Rheum forrestii Diels.	0.0 g	33.0 bcdefg	62.2 abcd
Rhododendron molle (Bl.) G. Don	0.0 g	54.3 abcdef	44.7 abcdefgh
Rhus chinensis Mill.	0.0 g	39.0 abcdefg	0.0 h
Sargentodoxa cuneata (oliv.) Rehd. et Wils.	0.0 g	37.1 bcdefg	20.1 cdefgh
Saruma henryi Oliv.	0.0 g	42.1 abcdefg	38.0 abcdefgh
Saussurea costus (Falc.) Lipsch.	0.0 g	28.2 bcdefg	62.2 abcd
Scrophularia ningpoensis Hemsl.	0.0 g	41.5 abcdefg	53.3 abcdefg
Scutellaria barbata D. Don	0.0 g	25.6 cdefg	32.6 abcdefgh
Senecio scandens Buch.-Ham.	0.0 g	29.1 bcdefg	50.4 abcdefg
Setaria viridis (L.) Beauv.	0.0 g	50.7 abcdefg	61.9 abcd
Stemona japonica (BL.) Miq.	5.0 efg	44.5 abcdefg	47.4 abcdefgh
Sterculia scaphigera Wall.	0.0 g	38.7 abcdefg	0 h
Tinospora sinensis (Lour.) Merr.	0.0 g	72.4 abc	31.6 abcdefgh
Vaccaria segetalis (Neck.) Garcke	8.3 efg	65.0 abcde	40.5 abcdefgh
Viola yedoensis Mak.	0.0 g	0.0 g	54.6 abcdef

a) †, Means in the same column followed by different letters differ significantly according to Tukey's multiple range tests ($P<0.05$). *O. minor* germination rate in deionized water was 0.0%.

groups according to their ability to induce germination of *Orobanche* seeds. The first group consisted of those species whose undiluted extracts induced little or no germination, their 10-fold dilution induced a high germination rate (>60%), and their 100-fold dilution induced a lower germination rate. This group included *Cannablis sativa* L.; *Dalbergia odorifera* T. Chen., *Lophatherum gracile* Bongn., *Tinospora sinensis* (Lour.) Merr., and *Vaccaria segetalis* (Neck.) Garcke (Table 1).

The second group comprised herbs whose undiluted water extracts induced little or no germination of *O. minor* seeds, but their dilutions induced higher germination rates with greater dilution. This group included *Aconitum carmichaeli* Debx., *Akebia quinata* (Thunb.) Decne., *Angelica sinensis* (Oliv.) Diels, *Ardisia japonica* (Hornsted) Blume., *Artemisia argy*i Levl. et Vant., *Blumea balsamifera* DC., *Chrysanthemum indicum* L., *Cibotium barometz* (L.) J. Sm., *Citrus aurantium* L., *Citrus reticulata* Blanco, *Citrus tangerina* Hort. et Tanaka, *Cyathula officinalis* Kuan., *Cynanchum paniculatum* (Bunge) Kitagawa, *Epimedium brevicornum* Maxim., *Eupatorium fortunei* Turcz., *Ilex rotunda* Thunb., *Illicium verum* Hook. f., *Ligusticum chuanxiong* Hort, *Magnolia officinalis* Rehd. et Wils., *Phyllanthus urinaria* Linn., *Phyllostachys sulphurea* (Carr.) A. et C. Riv., *Poeonia suffruticosa* Andr., *Polygonum orientale* L., *Pulsatilla chinensis* (Bge.) Reg., *Pyrola calliantha* H. Andres., *Rheum forrestii* Diels., *Saussurea costus* (Falc.) Lipsch., *Scrophularia ningpoensis* Hemsl., *Scutellaria barbata* D. Don, *Senecio scandens* Buch.-Ham., *Setaria viridis* (L.) Beauv., and *Viola yedoensis* Mak. (Table 1). This group of herbs has the potential for further purification of germination stimulants or could be considered as trap plants. The results indicated that these extracts contained high concentrations of highly active substances.

The third group included herbs whose undiluted extracts resulted in little or no germination of *O. minor* seeds, and whose 10- and 100-fold-diluted extracts induced similar levels of germination. The species in this group included *Aloe vera* L., *Alpinia katsumadai* Hayata., *Arenaria kansuensis* Maxim., *Buddleja officinalis* Maxim., *Glycine max* (L.) Merr., *Helianthus annuus* L., *Nardostachys chinensis* Batal., *Nelumbo nucifera* Gaertn., *Paeonia lactiflora* Pall., *Saruma henryi* Oliv. and *Stemona japonica* (BL.) Miq. (Table 1).

The methanolic extracts of 100 herbs induced seed germination in *O. minor* (Table 2). Compared with deionized water extracts, there were more undiluted methanolic extracts that induced germination of *O. minor* seeds. Germination rates of more than 60% were observed for undiluted methanolic extracts from *Fritillaria cirrhosa* D. Don, *Ginkgo biloba* L., *Oryza sativa* L., *Pinellia ternate* (Thunb.) Breit and *V. segetalis* (Neck.) Garcke. However, when the extracts from these five herbs were diluted, all showed a sharp reduction in their ability to induce germination. Induction of germination by methanolic extracts of other herbs was similar to that of deionized water extracts, and could also be similarly divided into three groups. There were 47 herbs for which both the deionized water and methanolic extracts induced seed germination. Of these, five herbs induced a germination rate of more than 60% when supplied as dilutions of either deionized water or methanolic extracts. These five herbs were *A. katsumadai* Hayata., *A. argyi* Levl. et Vant., *E. fortunei* Turcz., *L. gracile* Bongn. and *V. segetalis* (Neck.) Garcke (Tables 1 and 2). Further research should be conducted on those herbs that showed germination induction ability only as a methanolic extract, with greater activity as the dilution increased. These included *Abrus fruticulosus* Wall. ex wight et Arn, *Albizzia julibrissin* Durazz, *Elsholtzia splendens* Nakai ex F. Maekawa, *Selaginella tamariscina* (Beauv.) Spring, and *Soroseris umbrella* (Franch.) Stebb. Further dilutions of their methanolic extracts should be tested for their ability to induce germination. This may reveal different compounds from those in the deionized water extracts.

2.2 Induction of germination of *Orobanche cumana* seeds

There were lower rates of germination of *O. cumana* seeds, compared with those of *O. minor*. The water extracts of only 18 species stimulated germination rates exceeding 30% (Table 3). Extracts from *Peucedanum praeruptorum* Dunn. and *S. viridis* (L.) Beauv. induced germination rates of 49.2% and 48.8%, respectively. The water extracts of six herbs, *Areca catechu* L., *A. kansuensis* Maxim., *C. indicum* L., *P. urinaria* Linn., *Polyporus umbellatus* (Pers.) Fries. and *S. viridis* (Linn.) Beauv., stimulated germination of both *O. cumana* and *O. minor* seeds. Similarly, methanolic extracts of only 16 herb species stimulated *O. cumana* seed germination at rates exceeding 30% (Table 4) although methanolic extracts of *Sterculia scaphigera* Wall. induced germination rates greater than 60%. Methanolic extracts of *C. paniculatum* (Bunge) Kitagawa, *H. annuus* L., *J. effusus* L. var. decipiens Buchen, *Prunus mume* Sieb. et Zucc., and *S. scaphigera* Wall. stimulated germination of both *O. minor* and *O. cumana* seeds (Tables 2 and 4), and their deionized water extracts also induced germination of *O. minor* seeds (Tables 1 and 4).

2.3 Induction of germination of *Orobanche aegyptiaca* seeds

Seed germination rates of *O. aegyptiaca* in response to herbal extracts were also lower than those of *O. minor*. Deionized water extracts of only 21 out of 606 herbs induced *O. aegyptiaca* seeds to germinate. The maximum germination rate (81.9%) was induced by the 10-fold dilution of *Hedyotis diffusa* Willd. (Table 5). Deionized water extracts of four herbs, *A. kansuensis* Maxim., *C. indicum* L., *P. urinaria* Linn., and *S. viridis* (L.) Beauv., induced germination of all three *Orobanche* spp. (Tables 1, 3, and 5). Deionized

Table 2 Germination of *O. minor* seeds induced by methanolic extracts of traditional Chinese medicinal herbs (%)[a)]

Medicinal herbs	Undiluted	10-fold dilution	100-fold dilution
Abrus fruticulosus Wall. ex wight et Arn	0.0 p	37.3 abcdefgh	54.0 abcde
Aconitum carmichaeli Debx.	35.0 bcdefghijklmn	22.0 defgh	5.6 lmn
Aconitum coreanum (Lévl.) Raipaics	41.9 bcdefghijk	38.7 abcdefgh	28.7 abcdefghijklmn
Acorus gramineus Soland.	2.9 nop	36.3 abcdefgh	9.8 ijklmn
Ailanthus altissima (Mill.) Swingle.	0.0 p	60.3 abcdef	0.0 n
Akebia quinata (Thunb.) Decne.	0.0 p	17.0 fgh	34.8 abcdefghijklmn
Albizzia julibrissin Durazz	0.0 p	41.7 abcdefgh	52.3 abcdefg
Aloe vera L.	0.0 p	27.4 cdefgh	35.8 abcdefghijklmn
Alpinia katsumadai Hayata.	30.7 cdefghijklmnop	64.7 abcde	50.1 abcdefghi
Amomum tsao-ko Crevost et lem.	53.0 abcd	29.3 abcdefgh	10.0 ijklmn
Arctium lappa L.	12.5 jklmnop	45.8 abcdefgh	6.1 lmn
Ardisia japonica (Hornsted) Blume.	0.0 p	40.3 abcdefgh	39.2 abcdefghijklmn
Arenaria kansuensis Maxim.	36.2 bcdefghijklm	41.7 abcdefgh	48.6 abcdefghij
Arisaema consanguineum Schott	6.3 mnop	60.0 abcdef	11.0 hijklmn
Artemisia apiacea Hance.	0.0 p	34.3 abcdefgh	34.9 abcdefghijklmn
Artemisia argyi Lévl. et Vant	25.3 defghijklmnop	62.0 abcdef	33.7 abcdefghijklmn
Artemisia capillaris Thunb.	0.0 p	55.7 abcdefg	18.5 abcdefghijklmn
Benincasa hispida (Thunb.) Cogn.	0.0 p	36.5 abcdefgh	52.9 abcdef
Blumea balsamifera DC.	0.0 p	24.8 defgh	42.9 abcdefghijklm
Cannabis sativa L.	16.4 ghijklmnop	41.8 abcdefgh	32.5 abcdefghijklmn
Cassia obtusifolia L.	18.6 efghijklmnop	51.7 abcdefgh	46.5 abcdefghijkl
Cibotium barometz (L.) J. Sm.	0.0 p	43.0 abcdefgh	11.6 ghijklmn
Cimicifuga foetida L.	0.0 p	37.2 abcdefgh	4.9 mn
Citrus aurantium L.	0.0 p	35.1 abcdefgh	41.6 abcdefghijklm
Citrus sinensis (L.) Osbeck	0.0 p	42.6 abcdefgh	35.5 abcdefghijklmn
Citrus tangerina Hort. et Tanaka	8.9 lmnop	42.3 abcdefgh	15.4 defghijklmn
Cremastra variabilis (Bl.) Nakai	45.2 abcdefghi	25.7 cdefgh	4.8 mn
Curcuma longa L.	21.0 defghijklmnop	66.7 abcd	8.0 jklmn
Cyathula officinalis Kuan.	0.0 p	37.9 abcdefgh	0.0 n
Cynanchum paniculatum (Bunge) Kitagawa	9.3 lmnop	50.5 abcdefgh	31.8 abcdefghijklmn
Cyperus rotundus L.	0.0 p	46.9 abcdefgh	3.9 mn
Dalbergia odorifera T. Chen.	0.0 p	27.1 cdefgh	38.0 abcdefghijklmn
Dichroa febrifuga Lour.	28.5 defghijklmnop	26.9 cdefgh	50.0 abcdefghi
Dioscorea hypoglauca Palib	13.7 hijklmnop	72.7 abc	13.0 efghijklmn
Elsholtzia splendens Nakai ex F. Maekawa	0.0 p	36.2 abcdefgh	52.9 abdef
Eupatorium fortunei Turcz.	0.0 p	42.9 abcdefgh	66.3 a
Euphoria longan (Lour.) Steud.	0.0 p	46.5 abcdefgh	2.7 mn
Farfugium japonicum (L.) Kitam	39.2 bcdefghijkl	20.5 defgh	4.1 mn
Fritillaria cirrhosa D. Don	61.3 abc	58.0 abcdefg	10.7 hijklmn
Gardenia jasminoides Ellis.	0.0 p	47.3 abcdefgh	28.4 abcdefghijklmn
Ginkgo biloba L.	63.9 ab	22.9 defgh	7.3 klmn
Glycine max (L.) Merr.	1.3 op	22.3 defgh	59.3 ab
Helianthus annuus L.	46.0 abcdefgh	44.5 abcdefgh	38.5 abcdefghijklmn
Herba Artemisiae Anomalae.	43.1 abcdefghij	60.4 abcdef	19.9 bcdefghijklmn
Homalomena occulta (Lour.) Schott	36.5 bcdefghijklm	41.1 abcdefgh	27.8 abcdefghijklmn
Ilex rotunda Thunb.	0.0 p	44.3 abcdefgh	31.0 abcdefghijklmn
Illicium verum Hook. f.	0.0 p	48.4 abcdefgh	58.8 ab
Indigofera tinctoria L.	47.2 abcdefg	12.9 gh	0.0 n
Jasminum sambac (L.) Ait.	34.2 bcdefghijklmn	38.9 abcdefgh	20.3 bcdefghijklmn
Juncus effusus L. var. decipiens Buchen	47.7 abcdefg	53.0 abcdefgh	52.7 abcdefg
Kaempferia galanga L.	0.0 p	51.1 abcdefgh	39.9 abcdefghijklmn
Lemna minor L.	23.8 defghijklmnop	41.5 abcdefgh	17.4 cdefghijklmn
Lepidium apetalum Willd.	0.0 p	44.0 abcdefgh	42.0 abcdefghijklmn
Ligusticum chuanxiong Hort	0.0 p	46.5 abcdefgh	55.1 abcd
Lonicera japonica Thunb.	0.0 p	41.0 abcdefgh	14.7 defghijklmn

(*To be continued on the next page*)

(*Continued*)

Medicinal herbs	Undiluted	10-fold dilution	100-fold dilution
Lophatherum gracile Bongn.	0.0 p	75.2 ab	66.0 a
Lycopodium clavatum L.	48.8 abcdef	39.8 abcdefgh	12.0 fghijklmn
Magnolia officinalis Rehd. et Wils.	0.0 p	29.7 abcdefgh	31.2 abcdefghijklmn
Morus alba L.	13.4 ijklmnop	44.4 abcdefgh	26.2 abcdefghijklmn
Nardostachys chinensis Batal.	0.0 p	32.7 abcdefgh	64.3 a
Nelumbo nucifera Gaertn.	6.4 mnop	39.2 abcdefgh	4.8 mn
Oryza sativa L	74.3 a	49.7 abcdefgh	27.0 abcdefghijklmn
Panax pseudo-ginseng Wall. var. notoginseng (Burkill) Hoo & Tseng	28.7 defghijklmnop	50.3 abcdefgh	51.7 abcdefgh
Phyllostachys sulphurea (Carr.) A. et C. Riv.	0.0 p	43.8 abcdefgh	30.6 abcdefghijklmn
Physalis alkekengi L. var. franchetii (Mast.) Makino	0.0 p	34.1 abcdefgh	29.3 abcdefghijklmn
Pinellia ternata (Thunb.) Breit	62.0 abc	7.0 h	4.7 mn
Polygonum cuspidatum Sieb. et Zucc.	11.9 jklmnop	33.8 abcdefgh	3.4 mn
Polygonum multiflorum Thunb.	32.9 bcdefghijklmno	40.2 abcdefgh	10.5 ijklmn
Portulaca oleracea L.	27.7 defghijklmnop	61.0 abcdef	52.7 abcdefg
Prunus mume Sieb. et Zucc.	0.0 p	26.3 cdefgh	48.2 abcdefghijk
Pueraria lobata (Willd.) Ohwi.	39.4 bcdefghijkl	25.4 cdefgh	6.1 lmn
Pulsatilla chinensis (Bge.) Reg.	0.0 p	17.7 efgh	31.8 abcdefghijklmn
Punica granatum L.	10.0 klmnop	29.3 abcdefgh	4.0 mn
Pyrola calliantha H. Andres.	6.4 mnop	32.5 abcdefgh	25.5 abcdefghijklmn
Pyrrosia lingua (Thunb.) Farw.	24.0 defghijklmnop	49.9 abcdefgh	28.5 abcdefghijklmn
Ranunculus japonicus Thunb.	0.0 p	32.4 abcdefgh	22.9 bcdefghijklmn
Ranunculus ternatus Thunb.	48.8 abcdef	51.4 abcdefgh	10.7 hijklmn
Rhaponticum uniflorum (L.) DC.	46.5 abcdefg	44.2 abcdefgh	14.9 defghijklmn
Rheum forrestii Diels.	42.0 bcdefghijk	6.8 h	6.5 lmn
Rhododendron molle (Bl.) G. Don	0.0 p	40.2 abcdefgh	8.9 jklm
Rubia cordifolia L.	4.9 mnop	44.2 abcdefgh	7.4 klmn
Sargassum siliguastrum (Turn.) C. Ag.	50.1 abcde	24.6 defgh	0.0 n
Sargentodoxa cuneata (oliv.) Rehd. et Wils.	6.3 mnop	50.0 abcdefgh	16.3 cdefghijklmn
Schisandra chinensis (Turcz.) Baill.	0.0 p	32.3 abcdefgh	22.2 bcdefghijklmn
Scrophularia ningpoensis Hemsl.	0.0 p	53.9 abcdefgh	54.3 abcd
Scutellaria barbata D. Don.	21.7 defghijklmnop	40.0 abcdefgh	29.5 abcdefghijklmn
Selaginella tamariscina (Beauv.) Spring.	0.0 p	28.4 bcdefgh	34.4 abcdefghijklmn
Semiaquilegia adoxoides (DC.) Mak.	21.0 defghijklmnop	57.7 abcdefgh	22.7 bcdefghijklmn
Sesamum indicum DC.	0.0 p	63.7 abcdef	9.3 ijklmn
Setaria viridis (L.) Beauv.	0.0 p	41.5 abcdefgh	34.0 abcdefghijklmn
Soroseris umbrella (Franch.) Stebb.	17.0 fghijklmnop	34.4 abcdefgh	48.3 abcdefghijk
Speranskia tuberculata (Bge.) Baill.	15.7 ghijklmnop	44.7 abcdefgh	8.2 jklmn
Stemona japonica (BL.) Miq.	0.0 p	76.3 a	4.3 mn
Stephania tetrandra S. Moore.	14.1 hijklmnop	42.5 abcdefgh	8.2 jklmn
Sterculia scaphigera Wall.	19.8 efghijklmnop	50.5 abcdefgh	56.8 abc
Tribulus terrestris L.	0.0 p	36.5 abcdefgh	39.5 abcdefghijklmn
Trichosanthes kirilowii Maxim.	35.0 bcdefghijklmn	22.9 defgh	20.0 bcdefghijklmn
Vaccaria segetalis (Neck.) Garcke	64.0 ab	54.3 abcdefgh	7.7 jklmn
Vatica mangachapoi Blauco.	0.0 p	46.2 abcdefgh	37.7 abcdefghijklmn
Viola yedoensis Mak.	0.0 p	35.2 abcdefgh	41.6 abcdefghijklm

a) *O. minor* germination rate in deionized water was 0.0%.

water extracts of *Benincasa hispida* (Thunb.) Cogn., *C. paniculatum* (Bunge) Kitagawa, *H. annuus* L. *Pueraria lobata* (Willd.) Ohwi and *S. viridis* (L.) Beauv. induced germination of both *O. aegyptiaca* and *O. minor* seeds (Tables 1 and 5). Deionized water extracts of *Artemisia capillaris* Thunb., *A. kansuensis* Maxim., *C. indicum* L., *Elsholtzia ciliata* (Thunb.) Hyland., *P. praeruptorum* Dunn., *P. urinaria* Linn., *S. viridis* (L.) Beauv. and *Tamarix chinensis* Lour. induced germination of both *O. aegyptiaca* and *O. cumana* seeds (Tables 3 and 5).

Among the methanolic extracts, the maximum germination rate (69.6%) was induced by the extract from *S. scaphigera* Wall. Similar to water extracts, methanolic extracts of only 20 species induced germination of *O. aegyptiaca* seeds. Only the methanol extract of *Lonicera japonica* Thunb. at 100-fold dilution induced a germination rate greater than

Table 3 Germination of *O. cumana* seeds induced by deionized water extracts of traditional Chinese medicinal herbs (%)[a)]

Medicinal herb	Undiluted	10-fold dilution	100-fold dilution
Achyranthes aspera Linn.	0.0 d	35.3 a	0.0 c
Alpinia officinarum Hance	0.0 d	39.3 a	0.0 c
Amomum cardamomum L.	0.0 d	34.2 a	0.0 c
Antenoron filiforme (Thunb.) Rob. et Vaut.	28.3 bc	39.4 a	0.0 c
Areca catechu L.	0.0 d	33.6 a	23.3 ab
Arenaria kansuensis Maxim.	20.1 c	36.7 a	0.0 c
Artemisia capillaris Thunb.	40.3 ab	21.9 ab	0.0 c
Atractylodes macrocephala Koidz.	0.0 d	0.0 b	30.9 ab
Chrysanthemum indicum L.	0.0 d	33.2 a	41.2 a
Elsholtzia ciliata (Thunb.) Hyland.	0.0 d	47.4 a	0.0 c
Gleditsia sinensis Lam.	53.6 a	39.1 a	22.4 b
Pelargonium hortorum Bailey	0.0 d	35.4 a	20.1 b
Peucedanum praeruptorum Dunn.	0.0 d	49.2 a	0.0 c
Pharbitis nil (L.) Choisy.	0.0 d	30.0 ab	0.0 c
Phyllanthus urinaria Linn.	0.0 d	40.6 a	24.5 ab
Polyporus umbellatus (Pers.) Fries.	25.8 c	32.5 a	0.0 c
Setaria viridis (L.) Beauv.	20.8 c	48.8 a	0.0 c
Tamarix chinensis Lour.	0.0 d	42.5 a	12.7 bc

a) *O. cumana* germination rate in deionized water was 0.0%.

Table 4 Germination of *O. cumana* seeds induced by methanolic extracts of traditional Chinese medicinal herbs (%)[a)]

Medicinal herbs	Undiluted	10-fold dilution	100-fold dilution
Antenoron filiforme (Thunb.) Rob. et Vaut.	58.9 a	51.5 ab	0.0 d
Crotalaria ferruginea Grah.	52.7 ab	45.0 ab	0.0 d
Cynanchum paniculatum (Bunge) Kitagawa	0.0 d	56.9 ab	10.5 cd
Diospyros kaki L. f.	50.9 abc	8.1 c	0.0 d
Emilia sonchifolia (Linn.) DC.	7.5 d	55.7 ab	36.1 abcd
Glehnia littoralis F. Schmidt ex Miq.	39.2 abcd	26.5 bc	17.8 bcd
Helianthus annuus L.	56.7 a	43.4 ab	0.0 d
Juncus effusus L. var. decipiens Buchen	38.3 abcd	52.8 ab	50.3 abc
Liquidambar formosana Hance	42.2 abcd	58.3 a	40.4 abcd
Murdannia divergens (C. B. Clarke.) Bruckn.	0.0 d	56.7 ab	0.0 d
Pelargonium hortorum Bailey	12.9 bcd	0.0 c	55.6 ab
Picrasma quassioides (D. Don) Benn	52.0 abc	43.9 ab	0.0 d
Prunus mume Sieb. et Zucc.	0.0 d	53.9 ab	37.6 abcd
Scutellaria baicalensis Georgi	0.0 d	58.4 a	52.8 ab
Selaginella tamariscina (Beauv.) Spring.	34.9 abcd	49.1 ab	53.1 ab
Sterculia scaphigera Wall.	9.5 cd	60.8 a	63.6 a

a) *O. cumana* germination rate in deionized water was 0.0%.

50%; others induced germination at rates lower than 50% (Table 6).

3 Discussion

It is widely believed that seeds of *Orobanche* spp. require a conditioning period of several days under suitable temperature and moisture conditions before germinating in response to germination stimulants [1,25–27]. We found that *O. minor* seeds indeed required a conditioning period before responding to germination stimulants (herbal extracts). This finding was consistent with other reports [24]. However, seeds of *O. cumana* and *O. aegyptiaca*, which infested sunflower and tomato fields in the Xinjiang Uygur Autonomous Region of China, did not require a preconditioning treatment. They germinated directly when treated with GR24 or medicinal herbal extracts, which is consistent with the findings of more recent studies [24,28].

Some undiluted herbal extracts did not induce germination. This may have been because the concentration of the stimulant was too high, or because germination inhibitors were present. In some cases, a 10-fold dilution was sufficient to overcome the inhibiting effects and allow the germination stimulant to induce seed germination. When the germination rate decreased with the 100-fold dilution, it is

Table 5 Germination of *O. aegyptiaca* seeds induced by deionized water extracts of traditional Chinese medicinal herbs (%)[a)]

Medicinal herbs	Undiluted	10-fold dilution	100-fold dilution
Arenaria kansuensis Maxim.	0.0 d	51.7 abc	49.6 ab
Artemisia capillaris Thunb.	0.0 d	24.1 cde	53.4 ab
Benincasa hispida (Thunb.) Cogn.	0.0 d	64.0 abc	39.5 ab
Centella asiatica (L.) Urban	0.0 d	64.1 abc	69.4 a
Chrysanthemum indicum L.	31.2 abcd	61.1 abc	70.7 a
Cissus pteroclada Hayata	36.8 abc	54.3 abc	38.7 ab
Curcuma longa L.	0.0 d	53.3 abc	45.3 ab
Cynanchum paniculatum (Bunge) Kitagawa	57.6 a	52.3 abc	36.6 ab
Elsholtzia ciliata (Thunb.) Hyland.	23.6 bcd	74.0 ab	59.6 ab
Ginkgo biloba L.	55.5 ab	63.4 abc	20.6 b
Hedyotis diffusa Willd.	0.0 d	81.9 a	39.5 ab
Helianthus annuus L.	0.0 d	75.8 ab	64.8 a
Ipomoea cairica (Linn.) Sweet	42.2 abc	74.9 ab	44.5 ab
Peucedanum praeruptorum Dunn.	61.3 a	49.9 abcd	43.5 ab
Phyllanthus urinaria Linn.	0.0 d	40.1 bcd	53.4 ab
Piper longum L.	0.0 d	37.2 bcde	51.4 ab
Pueraria lobata (Willd.) Ohwi	0.0 d	58.4 abc	44.6 ab
Setaria viridis (L.) Beauv.	20.2 cd	59.6 abc	55.6 ab
Tamarix chinensis Lour.	0.0 d	72.0 ab	45.1 ab
Trichosanthes kirilowii Maxim.	38.7 abc	0.0 e	61.1 ab
Vigna umbellata (Thunb.) Ohwi et Ohashi	30.6 abcd	11.7 de	55.7 ab

a) *O. aegyptiaca* germination rate in deionized water was 0.0%.

Table 6 Germination of *O. aegyptiaca* seeds induced by methanolic extracts of traditional Chinese medicinal herbs (%)[a)]

Medicinal herbs	Undiluted	10-fold dilution	100-fold dilution
Antenoron filiforme (Thunb.) Rob. et Vaut.	66.4 ab	49.2 a	19.7 abc
Blumea balsamifera DC.	0.0 e	29.4 a	41.4 ab
Buddleja officinalis Maxim.	29.8 bcde	40.4 a	28.2 abc
Citrus aurantium L.	19.3 cde	40.8 a	37.6 ab
Crotalaria ferruginea Grah.	55.6 abc	41.1 a	44.0 ab
Cynanchum paniculatum (Bunge) Kitagawa	39.7 abcd	44.7 a	18.4 bc
Dianthus superbus Linn.	22.0 cde	32.1 a	24.3 abc
Fructus tritici Levis	26.2 cde	31.2 a	15.0 bc
Ginkgo biloba L.	16.1 de	30.0 a	22.7 abc
Glycine max (L.) Merr.	0.0 e	43.8 a	43.1 ab
Helianthus annuus L.	36.3 abcde	38.4 a	22.2 abc
Ipomoea cairica (Linn.) Sweet	36.6 abcde	27.3 a	23.2 abc
Lobelia chinensis Lour.	49.0 abcd	33.5 a	4.4 c
Lonicera japonica Thunb.	20.4 cde	34.0 a	51.9 a
Momordica grosvenori Swingle	24.0 cde	31.2 a	27.5 abc
Murdannia divergens (C. B. Clarke.) Bruckn.	0.0 e	40.1 a	22.5 abc
Picrasma quassioides (D.Don) Benn	39.2 abcd	22.7 a	13.6 bc
Setaria italica (L.) Beauv	14.4 de	38.0 a	41.2 ab
Sterculia scaphigera Wall.	69.6 a	39.7 a	0.0 c
Taxillus chinensis (DC.) Danser	25.5 cde	36.5 a	18.5 bc

a) *O. aegyptiaca* germination rate in deionized water was 0.0%.

likely that the concentration of the stimulant was not high enough in these solutions. Further isolations of germination stimulants should exclude this group of herbs.

The present study on *Orobanche* germination stimulants has built upon the study of the parasite *Striga*. One reason for this is because germination stimulants that affected *Striga* also affected *Orobanche*. Isolation, identification, and structural elucidation of compounds that stimulate *Striga* germination from genuine host plants has been given utmost priority for almost 50 years [1]. The primary objective has been to synthesize structural mimics with high activities to induce suicidal germination of the parasite seeds,

thereby reducing or eliminating the seed bank in the soil. However, isolation of the active compounds has been difficult, as host plants produce very small amounts of these germination stimulants. Most of the germination stimulants isolated and identified so far possess the same basic molecular structure, and are collectively known as strigolactones [7–9,29–32]. To date, more than 14 strigolactones have been identified, mainly from plant root exudates [33].

In our experiment, approximately 20% of the medicinal herb extracts induced *O. minor* seeds to germinate. This percentage was much lower for germination of *O. cumana* (<3%) and *O. aegyptiaca* (<4%). These results were consistent with the findings of Fernández-Aparicio *et al*. [19], and suggested that not all plants produce sufficient amounts of strigolactones to induce germination of *Orobanche* spp., as also suggested by Akiyama and Hayashi [34] and Klee [35]. In addition, Chinese medicinal herb extracts may contain substances that inhibit the germination-promoting activities of strigolactones. In our previous study on *S. hermonthica*, water extracts of 27 of the 383 herbs tested (7%), inhibited germination of *S. hermonthica* seeds, even when GR24 was added before applying the extracts [20]. Only a very small number of medicinal herb extracts induced germination of *O. cumana* and *O. aegyptiaca* seeds (Tables 3–6), disproving the hypothesis that genes involved in strigolactone biosynthesis would be present in all higher plants. It is estimated that no more than 7% of herbaceous plants (3% for *O. cumana* and 4% for *O. aegyptiaca* stimulation) could induce seed germination of *O. cumana* and *O. aegyptiaca*. This leaves unanswered the question, why were most of the herbs unable to induce germination of *Orobanche* spp.?

To date, there is no definitive proof that the germination of parasitic weed seeds in the field is induced by one single signal compound or class of compounds [7]. In the present experiments, we used synthetic strigol as a positive control (data not shown), and found that it induced high germination rates among the three tested species of *Orobanche*. Large quantities of long-lived seeds allow the parasite to show a high degree of genetic adaptability to changes in host resistance and cultivation practices. As long as the seed bank is not controlled, the need to control the parasite will persist whenever a susceptible host is grown in an infested field [36].

On the basis of circumstantial evidence and experimental results, we concluded that germination stimulants from susceptible hosts and their synthetic mimics are likely to be unstable in soil [37–39]. As observed in the field, host plants are unable to exude sufficient stable germination stimulants during the growing season, and therefore, few parasitic seeds germinate. This increases the seed bank of the parasite in the soil, leading to a persistent parasite problem. To reduce the seed bank of the parasite in the soil, we must find potent germination stimulants from genuine non-host plants. The question is which plant materials are best suited to meet this objective. We suggest that similar research should now be conducted in *Orobanche*-infested areas by introducing herbaceous plants that have not co-evolved with the parasites. Introduction of species that can induce germination of *Orobanche* spp. into affected cropping systems may result in 'suicidal germination' of the weed. This practice would have the additional advantage of producing valuable herbal plants that can improve animal and human health at the same time. Furthermore, because these herbal plants did not co-evolve with the parasites, they may produce and store parasitic seed germination stimulants with completely different molecular structures from those that have already been isolated. From these different stimulants, synthetic structural analogs may be developed that have a high capability to induce suicidal germination of the parasites, decreasing or eliminating their seed reserves in soil.

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