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Broomrape (*Orobanche cernua*) control before attachment to host through chemically or biologically manipulating seed germination

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Abstract

The germination phase of *Orobanche cernua* Loefl. is a critical period in the life cycle of this parasitic weed. By stimulating the germination through chemicals in the absence of hosts or through natural stimulants by exposing seeds to trap crops the seed bank can be reduced. Seven series of laboratory and glasshouse experiments were conducted to investigate different methods of testing and to study the effect of several chemicals, root exudates of germinating crop seeds, and their interactions on *O. cernua*. Compared to experiments in the incubator better results were obtained when germination was tested in the presence of seedlings of host plants under glasshouse conditions. GR24 at 0.1 and 1.0 mg/kg was found most effective in stimulating the germination of the seeds followed by gibberellic acid at 10 and 20 mg/kg. Without addition of chemicals, trap crops strongly increased germination. Especially greengram (*Vigna radiata*) and sunhemp (*Crotalaria juncea*) were effective. The effects of (concentrations of) chemicals and trap crops interacted. The positive effect of trap crops was observed even when there was already a strong stimulation by GR24 at 1.0 mg/kg, but under these conditions trap crops were less effective at 0.1 mg/kg GR24. Within one host plant species, no differences between cultivars in their effect on *Orobanche* germination could be detected.

Keywords: broomrape, *Orobanche cernua*, trap crops, parasitic weed, germination stimulants, chemical control, biological control, suicidal germination

Introduction

Orobanche cernua Loefl. is a holoparasite and the most pernicious parasitic species of the genus *Orobanche* in tobacco in India; it is a prolific seed producer and seeds may remain viable in the soil for a long period (Puzzilli, 1983). In their seed stage

broomrapes are quite resistant to most of the weed control measures, except fumigation and solarization (Foy *et al.*, 1989), which are relatively expensive methods. Germination can only take place in the presence of root exudates containing a germination stimulant (Chabrolin, 1938; Brown, 1946). Once broomrape seeds have germinated, they must establish contact with the host roots rapidly in order to derive nutrients and water for further growth and development. This germination phase is a critical period in the life cycle of the parasite (Musselman & Press, 1995; Dhanapal *et al.*, 1996 and papers cited therein). If broomrape seeds are stimulated to germinate in the absence of host plants, the parasite seedlings die because of lack of nutrition. This phenomenon could be a basis for control, using chemicals that stimulate germination in the absence of host plants.

Strigol, a natural germination stimulant, which was isolated from cotton (Cook *et al.*, 1972), has long been the subject of many synthesis studies, and to date seven total syntheses and several partial syntheses have been reported (Zwanenburg *et al.*, 1994). The isolation and purification of strigol in large amounts from root exudates appears impractical; an artificial synthesis of strigol is necessary to obtain enough compounds for commercial use.

The strigol analogue GR24 is active at 0.1 to 1.0 mg/kg concentrations on *Orobanche* seed germination (Spelce & Musselman, 1981; Parker & Riches, 1993). Jacobsohn *et al.* (1988) observed over 90% germination of broomrape conditioned in water and then stimulated to germinate by GR24. GR7, GR24, GR28, and GR41 are most active at 0.1 to 1.0 mg/kg on *Orobanche* seed germination (Saghir, 1979; Spelce & Musselman, 1981; Saghir *et al.*, 1983; Saghir, 1986); these compounds tend to inhibit germination at higher concentrations (Saghir, 1979).

Gibberellic acid (GA_3) induced germination of *Orobanche ramosa* seeds (Al-Menoufi,1986). Possible stimulating effects on germination have also been reported for ethylene (Jain, 1987), cytokinins (Edwards *et al.*, 1976; Strelyaeva, 1978) and pyridoxine (Nash & Wilhelm, 1960). Similarly, numerous pesticides, chemically pure substances, nutrients and growth regulators have been tested for their efficacy on broomrape seed germination; only few of them could induce germination of broomrape seeds and the results are extremely variable and contradictory (Pieterse, 1981; Riches & Parker, 1995).

A suitable chemical that could induce a high per cent germination of broomrape seeds under field conditions is still lacking. Broomrape seedlings are most vulnerable to destruction immediately after germination but prior to their establishment on host roots. Therefore, a combination of such a stimulant with a control measure can help to reduce the problem. The present investigation aimed at developing a standard method to study germination of *Orobanche cernua*, finding suitable chemicals and their optimal concentrations. Moreover, the effects of chemicals were compared with those of natural stimulants by exposing seeds to trap crops. This also allows to study the residual effect of chemicals after stimulation of germination by trap crops, to study the effect of different trap crops and to compare their cultivars.

Materials and methods

Seven separate series of laboratory experiments were conducted at the Department of Crop Physiology, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bangalore, India. In these series of experiments, different methods of testing, the effects of several chemicals, of germinating crop seeds and their interactions on *Orobanche cernua* Loefl. were investigated.

The seeds of *Orobanche cernua* Loefl. which were used for the study, had been obtained from Mr. G.V.G. Krishnamurthy, Central Tobacco Research Institute, Rajahmundry, Andhra Pradesh, India during 1992, 1993 and 1994. The one-year old seeds were sieved, cleaned and stored in the dark at room temperature (20–25 °C) until use. GR24 (analogue of strigol) was obtained from Professor B. Zwanenburg (NSR-Center for Molecular Structure, Design and Synthesis, Department of Organic Chemistry, University of Nijmegen, Nijmegen, The Netherlands). Gibberellic acid (GA) came from LUPIN-Agrochemicals India Pvt. Ltd.; ethephon (2-chloroethylphosphonic acid) was obtained from Rhone Poulenc Agro B.V., The Netherlands; naphthalene acetic acid (NAA; 2-(1-napthyl) acetic acid) from SISCO Research Laboratory Pvt. Ltd., Bombay, India; pyridoxine (pyridoxine monohydrochloride) and benzyl adenine (BA; 6-benzyl amino purine N^6 benzyl adenine) from SIGMA, India, through commercial channels.

Series of experiments on broomrape germination were done by following various methods during August, 1992 to July, 1995 intermittently. The protocols in the various methods are as follows:

1. Direct method

a. wash Petri dishes thoroughly with tap water;
b. keep the washed Petri dishes in the oven for sterilization for 24 hours;
c. after sterilization, take out the Petri dishes from the oven and place 4.5 cm diameter filter paper (Whatman No. 42) on the bottom of the lower lid of the Petri dish and add distilled water on the filter paper just to moisten it, and then keep it in the oven for 24 hours for complete sterilization;
d. take out the Petri dishes from the oven and wet the 4.5 cm diameter filter paper and put one tip pin of *Orobanche cernua* seeds (approximately 500 seeds) on the wetted filter paper and add 6 ml of a solution of known concentration of one of the chemicals gently without disturbing the seeds placed on the filter paper;
e. keep the Petri dishes in an incubator at 25 °C and keep on adding 6 ml solution of various chemicals every day and observe for germination after 20 days of incubation under a stereo microscope;
f. germination in obligate parasites is usually cryptocotylar, meaning that the cotyledons remain within the seed coat (Musselman & Press, 1995). Therefore, protruberance of a whitish radicle through the micropylar end of the seed is the correct diagnosis of germination.

This method was carried out for four series, with three replicates each. These series are called Experiment 1 and the results (average over four series) are presented in Table 1. The incubators contained fluorescent lamps providing an illumination of 100 lux.

Table 1. Effect of chemicals on the germination of *Orobanche cernua* Loefl. seeds by direct and pre-soaking methods under incubator conditions.

Chemicals	Concentration (mg/kg)	Germination (%)	
		Direct method*	Pre-soaking method**
GR24	0.1	7.7[g]	8.4[g]
	1.0	8.9[g]	8.7[g]
Gibberellic acid	10	5.4[f]	5.5[ef]
	20	5.1[f]	6.4[f]
Ethephon	2	0.9[bc]	2.3[b]
	20	3.2[e]	4.4[de]
	50	3.1[e]	3.8[d]
Naphthalene acetic acid	1	1.0[bc]	2.5[b]
	20	2.4[de]	3.6[cd]
Pyridoxine	1	0.6[b]	2.3[b]
	10	1.6[cd]	4.0[d]
Benzyl adenine	0.2	0.5[b]	2.8[bc]
	2.0	2.0[de]	2.7[bc]
Control (distilled water)		0.0[a]	0.4[a]
	P	<0.01	<0.01
	CV (%)	12.7	11.6

[a] Different letters indicate significant differences between the treatments based on protected LSD values (0.05); CV (%) values for transformed data.

* Experiment 1: Average of four series with three replications each (approx. 6000 seeds per treatment in total).

** Experiment 2: Average of four series with three replications each (approx. 6000 seeds per treatment in total).

2. Pre-soaking method

In this method, the seeds of *O. cernua* were gently rubbed with zero sand paper and subsequently soaked in water. The pre-conditioning period was 10 days. Thereafter, the protocol already presented under 'Direct method' was followed.

This method was also carried out for four series, with three replicates each. These series are called Experiment 2 and the average results are presented in Table 1.

3. Coir method

a. follow steps a–c of the direct method but use blotting paper discs instead of filter paper discs;

b. place about 500 *Orobanche cernua* seeds on a wet blotting paper disc (4.5 cm diameter) and cover with a similar disc;

c. place the discs in a Petri dish and place 'coir', i.e. coconut (*Cocos nucifera*) fibre pieces (wetted with water) on discs to a thickness of one centimeter;

d. sow 10 crop seeds over the coir pad;

e-1. water the bioassay unit daily, keep it in daylight for 30 minutes and place it back

in the incubator at 25 °C; incubation should last 10 days; an alternative is to keep the bioassay unit under glasshouse conditions (see e-2);

e-2. water the bioassay unit daily and keep it under the glasshouse conditions, placed under partial shade, where the temperature ranges from 27–30 °C; the greenhouse period should last 14 days;

f. remove seedlings and fibre pieces pad and deblot the two wet blotting paper discs with dry blotting paper to remove the excessive water;

g. examine the discs under a stereo microscope for assessing *Orobanche cernua* seeds germination;

h. protruberance of a whitish radicle through the micropylar end of the seed is the correct diagnosis of the germinated seed.

This method was carried out for Experiments 3–7 (see below). It is similar to the in situ root absorption method described by Krishnamurthy *et al.* (1981) and the rapid bioassay test described by Krishnamurthy & Nagarajan (1991).

In Experiments 1 and 2, the treatments consisted of: GR24 at 0.1 and 1.0 mg/kg, GA at 10 and 20 mg/kg, ethephon at 2, 20 and 50 mg/kg, NAA at 1 and 20 mg/kg, pyridoxine (vitamin B-6) at 1 and 10 mg/kg, BA at 0.2 and 2 mg/kg and a control (distilled water). These chemicals were selected on the basis of their possible effects reported in the literature (see introduction). GR24, a strigol analogue, was added as a standard to assess potential germination.

In Experiment 3, various crop seeds viz. sunhemp (*Crotalaria juncea*), greengram (*Vigna radiata*), redgram (*Cajanus cajan*), blackgram (*Vigna mungo*), pea (*Pisum sativum*), sunflower (*Helianthus annuus*), soybean (*Glycine max*), sesamum (*Sesamum indicum*) and control (only distilled water) were the treatments to induce the germination of *O. cernua* seeds using the coir method both in the incubator and under glasshouse conditions. Similar trap crops were studied under field conditions (Dhanapal & Struik, 1996). This experiment was run for three series with three replications each for both incubator and glasshouse conditions and the results (average of three series) are presented in Table 2. GR24, a strigol analogue, was added as a standard to assess potential germination.

In Experiments 4 and 5, the coir method was used to test the effects of the same chemicals in the same concentrations for Experiments 1 and 2 with and without greengram seeds. Twenty nine treatment combinations were included: GR24 with and without greengram seeds at 0.1 and 1 mg/kg, GA with and without greengram seeds at 10 and 20 mg/kg, ethephon with and without greengram seeds at 2, 20 and 50 mg/kg, NAA with and without greengram seeds at 1 and 20 mg/kg, pyridoxine with and without greengram seeds at 1 and 10 mg/kg, BA with and without greengram seeds at 0.2 and 2 mg/kg, greengram seeds with distilled water, coir without greengram seeds and control (only distilled water). Experiment 4 was kept in the incubator, whereas Experiment 5 was put under glasshouse conditions. Experiment 4 included two series with three replications each and Experiment 5 included three series with three replications each (Table 3).

In Experiment 6, the combined effects of the root exudates of the same crop species as for Experiment 3 and GR24 at different concentrations on the germination

Table 2. Effect of various crops on the percentage germination of *Orobanche cernua* Loefl. seeds by the coir method under incubator and glasshouse conditions.

Trap crops		Germination (%)	
		Incubator*	Glasshouse*
Sunhemp (*Crotalaria juncea*)		19.2^{cd}	24.4^{d}
Greengram (*Vigna radiata*)		22.6^{d}	27.1^{d}
Redgram (*Cajanus cajan*)		4.0^{b}	4.9^{b}
Blackgram (*Vigna mungo*)		16.5^{c}	19.5^{c}
Pea (*Pisum sativum*)		3.8^{b}	5.2^{b}
Sunflower (*Helianthus annuus*)		14.8^{c}	16.6^{c}
Sesamum (*Sesamum indicum*)		17.8^{cd}	23.6^{d}
Soybean (*Glycine max*)		3.6^{b}	6.4^{b}
Control (distilled water)		0.2^{a}	0.8^{a}
	P	<0.01	<0.01
	CV (%)	13.3	7.4

* Different letters indicate significant differences between the treatments based on protected LSD values (0.05); CV (%) values for the transformed data.
* Experiment 3: Average of three series, each with three replications (approx. 4500 seeds per treatment in total), for both incubator and glasshouse conditions.

were studied under glasshouse conditions (Table 4). There were 27 treatments: sunhemp, greengram, redgram, blackgram, pea, sunflower, soybean and sesamum combined with 0, 0.1 and 1.0 mg/kg GR24, and GR24 at 0, 0.1 and 1.0 mg/kg without crop seeds.

In Experiment 7, the effects of different cultivars of five crops with and without GR24 on the germination of the parasitic seeds were studied (Table 5). There were 33 treatments with greengram, blackgram, redgram, sunflower and soybean with two cultivars of each crop in combination with and without GR24 at 0, 0.1 and 1.0 mg/kg, GR24 at 0, 0.1 and 1.0 mg/kg without crop seeds.

Statistical procedures

Results are expressed in percentage germination.

There were no statistically significant differences between series within one experiment. Both the trends and the magnitudes of the effects were very consistent. Data were therefore pooled.

Germination percentages were arcsine-root transformed. Transformed data were subjected to a one-way ANOVA but the results of the original values are presented in the tables. Fischer's method of 'Analysis of Variance' was applied for the analysis and interpretation of the data. The level of significance used in 'F' and protected 't' tests was $P<0.05$. The values of 'F' and 't' and critical differences (LSD) were calculated following the method outlined for Completely Randomised Block Design by Panse & Sukhatme (1967).

Table 3. Effect of chemicals on the percentage germination of *Orobanche cernua* Loefl. seeds by the coir method under incubator and glasshouse conditions.

Chemicals	Concentration (mg/kg)	Germination (%)			
		Incubator*		Glasshouse**	
		No GG#	+GG#	No GG#	+GG#
GR24	0.1	27.2^{e}	41.8^{g}	42.8^{kl}	53.9^{n}
	1.0	33.5^{f}	44.5^{g}	45.2^{lm}	58.0^{n}
Gibberellic acid	10	25.7^{de}	32.5^{f}	33.7^{ij}	47.8^{m}
	20	23.0^{bcde}	31.2^{ef}	35.7^{j}	40.5^{k}
Ethephon	2	21.8^{bcd}	27.2^{ef}	21.0^{cd}	28.9^{gh}
	20	25.5^{cde}	32.5^{f}	26.0^{efg}	32.4^{hij}
	50	21.0^{bcd}	23.2^{bcde}	22.0^{bcde}	23.4^{cde}
Naphthalene acetic acid	1	20.3^{bc}	28.3^{ef}	23.2^{bcde}	30.4^{hi}
	20	19.0^{b}	24.5^{bcde}	19.9^{bc}	28.6^{gh}
Pyridoxine	1	21.2^{bcd}	25.7^{bcde}	22.3^{bcde}	25.0^{def}
	10	21.8^{bcd}	24.7^{bcde}	19.9^{bc}	23.0^{bcde}
Benzyl adenine	0.2	19.3^{b}	24.0^{bcde}	19.5^{b}	25.3^{efg}
	2.0	19.2^{b}	23.8^{bcde}	19.6^{bc}	24.3^{def}
Greengram + distilled water		–	23.5^{bcde}	–	21.4^{bcd}
Coir + distilled water		0.3^{a}	–	0.0^{a}	–
Control (distilled water)		0.0^{a}	–	0.0^{a}	–
	P	<0.01		<0.01	
	CV (%)	7.4		5.3	

a Different letters indicate significant differences between the treatments based on protected LSD values (0.05); CV (%) values for the transformed data.
* Experiment 4: Average of two series with three replications each (approx. 3000 seeds per treatment in total).
** Experiment 5: Average of three series with three replications each (approx. 4500 seeds per treatment in total).
\# Greengram seeds.

Results

Table 1 reveals that the seed germination of the parasite was significantly affected by chemicals both when using the direct or the pre-soaking method of testing. Germination percentage of *O. cernua* seeds was highest for GR24 at 0.1 and 1.0 mg/kg in both experiments, followed by GA at 10 or 20 mg/kg, and ethephon at 20 or 50 mg/kg. However, NAA, pyridoxine and BA had only a small, but significant effect on the germination percentage and these effects were mostly affected by the concentration used. Virtually no germination of broomrape seeds was observed in the control treatment. Conditioning of *O. cernua* seeds with distilled water for a period of 10 days (pre-soaking method) generally enhanced germination (Table 1).

Germination was strongly stimulated by the root exudates of various crops (Table 2). Significant differences among the crops in their ability to induce germination of the parasite were observed both under incubator and glasshouse conditions. Maximum percentage of germination of the parasite was observed when the seeds were exposed to greengram, sunhemp and sesamum crops followed by blackgram and sunflower crops both in incubator and glasshouse conditions. Comparatively low percentages of germination of the parasite were found when induction had to be performed by redgram, soybean or pea. Germination in the control was negligible. Germination of the parasitic seeds was better in the glasshouse than in the incubator.

The effects of chemicals on the germination of *O. cernua* seeds in the coir method are illustrated in Table 3. GR24 at 0.1 and 1.0 mg/kg concentrations significantly induced the germination of the parasite both under incubator and glasshouse conditions (Table 3). GR24 at 1.0 mg/kg was capable of inducing 34–45% of the seeds to germinate in the absence of a host. This was followed by GA at 10 and 20 mg/kg and ethephon at 20 mg/kg concentration. Significantly lower percentages of germination of the parasite were observed for the other chemicals tested, although the effects were much larger than in Experiments 1 and 2. When a host was present the germination was even more enhanced, especially in those treatments in which germination was already considerable without greengram seeds. The positive effect was much lower for the other treatments. Therefore, we could see clearly the interaction between the effect of greengram and the effect of the chemicals in stimulating the germination of the parasitic seeds. There was still a significant effect of the chemical when there was a host present. Virtually no germination could be seen in the control and in the treatment with coir and distilled water.

Table 4. Effect of trap crops with and without GR24 on the percentage germination of *Orobanche cernua* Loefl. seeds by the coir method under glasshouse conditions. Experiment 6: One series with three replications (approx. 1500 seeds per treatment in total).

Trap crop	GR24 (mg/kg)		
	0	0.1	1.0
Sunhemp	20.3^{c}	47.7^{defg}	57.7^{i}
Greengram	22.0^{c}	48.0^{defg}	56.7^{i}
Redgram	5.3^{b}	46.7^{def}	54.3^{fghi}
Blackgram	17.7^{c}	46.7^{def}	58.7^{i}
Pea	6.7^{b}	45.3^{de}	51.3^{efghi}
Sunflower	18.3^{c}	46.7^{def}	51.7^{ghi}
Sesamum	19.3^{c}	47.3^{defg}	55.7^{hi}
Soybean	5.3^{b}	44.3^{de}	54.0^{fghi}
Range	5.3–22.0	44.3–48.0	51.3–58.7
No crop	0.0^{a}	41.3^{d}	48.3^{defgh}
P		<0.01	
CV (%)		7.68	

*Different letters indicate significant differences between the treatments based on protected LSD values (0.05); CV (%) values for the transformed data.

Table 5. Effect of cultivars of trap crops with and without GR24 on the percentage germination of *Orobanche cernua* Loefl. seeds by the coir method under glasshouse conditions. Experiment 7: One series with three replications (approx. 1500 seeds per treatment in total).

Trap crop	Cultivar	GR24 (mg/kg)		
		0	0.1	1.0
Greengram	PS-16	23.0^{de}	49.3^{ghij}	58.7^{k}
	KGG-1	23.3^{e}	48.7^{ghij}	59.3^{k}
Blackgram	T-9	18.0^{cde}	46.0^{fg}	59.3^{k}
	K-3	16.7^{cd}	47.0^{fgh}	58.7^{k}
Redgram	TTB-7	6.7^{b}	47.3^{fghi}	55.3^{ijk}
	Hyd-3C	5.7^{b}	47.3^{fghi}	55.0^{ijk}
Soybean	Hardee	6.7^{b}	44.0^{fg}	54.3^{hijk}
	KHSB	3.7^{b}	46.7^{fgh}	49.7^{ghij}
Sunflower	Morden	15.0^{c}	48.0^{fghj}	55.0^{ijk}
	KBSH-1	16.7^{cd}	46.0^{fg}	54.0^{hijk}
Range		3.7–23.3	44.0–49.3	49.7–59.3
No crop		0.0^{a}	40.7^{f}	49.3^{ghij}
	P		<0.01	
	CV (%)		7.69	

*Different letters indicate significant differences between the treatments based on protected LSD values (0.05); CV (%) values for the transformed data.

Table 4 shows the interaction between the best chemical stimulant and different trap crops. Significantly higher levels of germination of broomrape were obtained when greengram, sunhemp, blackgram, sesamum and sunflower were present as compared to treatments with redgram, pea or soybean in the absence of GR24. GR24 at 0.1 mg/kg strongly enhanced germination but did not allow the positive effect of the trap crop to express itself significantly. GR24 at 1.0 mg/kg stimulated the germination even more than GR24 at 0.1 mg/kg and with the highest concentration positive effects of the germinating trap crops were again observed. This suggests that natural stimulants may even have an effect on germination when there is already a strong induction by GR24.

The effect of cultivars of different trap crops with and without GR24 on the germination of *Orobanche cernua* seeds by the coir method under glasshouse conditions is presented in Table 5. The overall results are very similar to the ones of the previous experiment: GR24 enhanced germination even in the presence of a host, whereas there were significant differences in efficiency of hosts, especially at 0 and 1 mg/kg GR24. The host effect was still significant, even when GR24 was applied at high concentrations. The difference in stimulating effect on the germination of the parasite among cultivars of the same crop was never significant.

Discussion

The results of the present investigation showed very little variation regarding effects of chemicals or trap crops among different series but large variations from one method to another, both under incubator and glasshouse conditions. This is often found in research on the seed germination of broomrapes. Basler (1981) reported that the germination of *O. crenata* seed of the same origin can vary to a large extent and over a short period. He suggested that the variation is due to dormancy which is controlled by endogenous factors.

In the present study, the low germination percentage of *O. cernua* in Experiments 1 and 2 may be attributed to poor seed quality, lack of surface sterilization or pre-soaking of the seeds (except in the pre-soaking method) before treating the seeds with chemicals or natural stimulants. The 'coir method' proved an excellent bioassay method to induce the germination of *O. cernua*.

The germination percentage of the parasite was higher under glasshouse conditions than under incubator conditions in Experiments 3–7. This may be due to the fact that the seedlings of the crop plants grown under glasshouse conditions under partial shade were sturdy and had green leaves. On the other hand, crop seedlings grown in the incubator were lanky and etiolated, and could only be maintained for 10 days.

GR24 at 0.1 and 1.0 mg/kg, GA at 10 and 20 mg/kg and ethephon at 20 mg/kg induced the germination of *O. cernua* seeds better than the other chemicals, both in direct and pre-soaking methods. These effects may be related: germination stimulants may play a role in ethylene biosynthesis, thus enhancing germination (Boone *et al.*, 1995). Relatively more germination was observed in the pre-soaking method. This may be due to the rubbing of *O. cernua* seeds with zero sand paper. Moreover, the pre-soaking period might have broken the dormancy and may have provided better conditions for imbibition.

It is evident from Experiment 3 that the *in situ* production and supply of root exudates from the seedlings of various crops induced the germination of the parasitic seeds. The results confirm field studies (Dhanapal & Struik, 1996): sunhemp and greengram are promising trap crops, whereas redgram, sunflower, and pea trap crops were found to be less effective in inducing the germination of *O. cernua* seeds in a rotation of tobacco – (fallow) – trap crop – tobacco. High levels of germination were attained after 10 days under incubator conditions and 14 days in glasshouse conditions, suggesting that root production after such a period was sufficient to provide the root exudates required for inducing the germination of *O. cernua* seeds.

GR24, a strigol analogue, at 0.1 mg/kg and 1.0 mg/kg concentrations was extremely effective in inducing the germination of *Orobanche cernua* seeds, both in the incubator and glasshouse conditions in all the methods. GR24, GR28 and GR41 stimulated the germination of *O. minor* seeds in the laboratory (Spelce & Musselman, 1981; Parker & Riches, 1993). They also reported that maximum germination of broomrape seeds was obtained with these strigol analogues at 0.1 to 1.0 mg/kg when seeds were pre-conditioned with water and incubated for 15 days at 25°C. The isolation and purification of strigol in large amounts from root exudates

appears impractical (Vail *et al.*, 1985); an artificial synthesis of GR24 or related compounds is necessary to obtain enough compounds for extensive field testing or for commercial use (Zwanenburg *et al.*, 1994). The use of strigol or its analogues as seed germination stimulants in the field has not yet been possible due to the high cost involved in their synthesis and also their instability in soils with high pH, which are common in broomrape-infested areas. Strigol and its analogues remain of primary interest as research tools due to their activity not only on parasitic seeds but also on a few autotrophic weeds. An insight into the mechanism of action of strigol and its analogues may help to develop chemicals that have similar activity and can be synthesized economically. The isolation and identification of the factor in host root exudates may also open new avenues into the chemistry of germination stimulants. The GR24 compound gave maximum germination of *O. aegyptiaca* seeds.

The combination of chemicals and trap crops showed an interaction in stimulating the germination. The partly additional effect of natural stimulants is more pronounced in the presence of GR24 at 1.0 mg/kg than at 0.1 mg/kg concentration. The additional effect of natural stimulants is partly hidden by GR24 at 0.1 mg/kg.

Gibberellic acid at 10 mg/kg and 20 mg/kg stimulated the germination of the parasitic seeds both in the incubator and under glasshouse conditions, by direct as well as the coir methods either with greengram or without greengram as a trap crop. This is consistent with literature (Al-Menoufi, 1986; Hiron, 1973). In our study, GA performed better than the other chemicals but was considerably less effective than GR24 in stimulating the germination of *O. cernua* seeds.

Reports on the effect of ethephon on broomrape seed germination are scarce. In their review paper Parker & Riches (1993) claim that the response of *Orobanche* germination to ethylene (or ethephon) is still a matter of controversy: no effects were observed in *O. crenata* or *O. ramosa* and a significant response was shown in *O. ramosa*. In our experiments, ethephon at lower concentrations strongly stimulated germination of *O. cernua* seeds in the coir method but ethephon at 50 mg/kg reduced the germination when compared to 2 mg/kg and 20 mg/kg suggesting that higher concentrations show signs of inhibition of *O. cernua* seeds germination. Also Chun *et al.* (1979) observed that ethylene at high concentrations inhibited *O. ramosa* seed germination instead of stimulating it.

Auxins such as indole acetic acid and naphthalene acetic acid and cytokinins have been found to stimulate the germination of broomrape seeds to some extent (Nash & Wilhelm, 1960; Edwards *et al.*, 1976; Strelyaeva, 1978). NAA, pyridoxine and BA, however, were found less effective in stimulating the germination of *O. cernua* seeds.

We also observed interactions between natural host stimulant by growing greengram seedlings and different chemicals both in incubator and glasshouse conditions. An enhanced germination percentage of the parasite by greengram seedlings was observed in the chemical treatments when there was already a strong stimulation by the chemical themselves; especially greengram with GR24 at 0.1 and 1.0 mg/kg followed by GA at 10 and 20 mg/kg and ethephon at 2 and 20 mg/kg concentrations. This positive effect of greengram was not pronounced in the other chemicals tested because such chemicals induced low germination in the absence of greengram

seedlings. Ethephon at 50 mg/kg with greengram also showed less germination indicating its toxicity at higher concentrations. Similar observations were made by Chun *et al.* (1979).

It can be concluded that the stimulatory effect of the different chemicals, trap crops or combination of chemicals and crops on the germination of *O. cernua* seeds was different in various methods under incubator or glasshouse conditions. GR24 at 0.1 and 1.0 mg/kg was found extremely effective in stimulating the germination of the *O. cernua* seeds followed by GA at 10 mg/kg and 20 mg/kg concentrations, especially under glasshouse conditions using the coir method. Under such conditions there is an additional effect of trap crops.

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