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RESEARCH ARTICLE

Switchgrass (*Panicum virgatum* L.) has ability to induce germination of *Orobanche cumana*

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Orobanche cumana Wallr. (sunflower broomrape) is a devastating root parasitic weed, causing enormous crop losses worldwide. The question was whether or not switchgrass has the potential to be a 'trap crop' for *O. cumana* control. To answer this question, the field experiments, pot experiments, and laboratory experiments were conducted in this study. The ability of nine switchgrass (*Panicum virgatum* L.) cultivars to induce *O. cumana* seeds germination was tested. Results indicated that root extracts shoot extracts, rhizosphere soil, and root exudates from switchgrass induced *O. cumana* germination. Ability to induce germination varied significantly among growing stages, with the earlier part of growing season (grown for 2 weeks) generally inducing the highest *O. cumana* germination rates. The methanol was a more suitable solvent than distilled water for extracting germination stimulants from switchgrass plants. Ten-fold dilutions of the extracts generally induced higher germination rates than either undiluted or 100-fold dilutions. The germination rates of *O. cumana* seeds in shoot extracts treatments were positively correlated with those in the root extracts ($R^2 = 0.6397$; $p < 0.01$) and negatively correlated with those in the rhizosphere soil treatments ($R^2 = 0.4433$; $p < 0.05$). In conclusion, it is believed that switchgrass is a potential trap crop for the control of root parasitic weed *O. cumana.*

Keywords: broomrape; germination; induction; switchgrass; trap crop

Introduction

Root parasitic weeds of the genus *Orobanche* (broomrapes) lack chlorophyll and depend totally on their host plants for water, assimilates, and inorganic nutrients. This also accounts for *O. cumana* Wallr. that represents a serious threat to sunflower (*Helianthus annuus* L.) (Labrousse et al. 2001). *O. cumana* has caused substantial crop losses in Southeastern Europe, the Middle East, and Asia (Parker 2013). The distribution of *O. cumana* coincides with that of sunflower fields in China, ranging from northeastern to northwestern China. For example, in Dingbian County, Shaanxi Province in the northern part of the Loess Plateau, at present sunflower plantation covers around 10,000 ha, and 64% of the field is infested by *O. cumana*, resulting in 40–50% yield losses (Chen 2010).

To germinate, *Orobanche* spp. seeds must be exposed to a chemical germination stimulant (Parker & Riches 1993). Sesquiterpene lactone dehydrocostus lactone is recently identified (Joel et al. 2011). Additional sesquiterpene lactones (costunolide, tomentosin, and 8-epixanthatin) released from sunflower root exudates are found to induce germination and trigger interaction of sunflower with *O. cumana* (Raupp & Spring 2013). In addition, a recent study shows that the strigolactone (SL) heliolactone may also stimulate *O. cumana* germination particularly in late developmental stages of sunflower (Ueno et al. 2014). *O. cumana* is extremely difficult to control because of its huge seed bank and species-specific stimulant of seeds germination. Several methods including crop rotation, soil fumigation, soil solarization, clean seeds, sowing dates, genetic resistance, and herbicides are used for broomrapes control and management (Goldwasser et al. 2003; Eizenberg et al. 2009; Hershenhorn et al. 2009). Due to the high requirements in manpower and material resources, most of those control measures are inadequate and difficult to apply in large-scale field (Aybeke et al. 2014).

Some plant species called 'trap crops' induce broomrape seed germination without being parasitized (Kasasian 1973). Rotation with trap crops is considered an attractive solution for controlling broomrapes. The broomrapes seedlings die for lack of nutritional support, a process termed 'suicidal germination', thus depleting the broomrapes seed bank (Parker & Riches 1993). Some crops, such as corn (*Zea mays* L.), rice (*Oryza sativa* L.), soybean (*Glycine max* L.), and rye (*Secale cereale* L.), have been proposed as trap crops for controlling *O. cumana* (Ma et al. 2013, 2014; Zhang et al. 2013; Cimmino et al. 2015). Additionally, some novel stimulants but no known ones are further characterized, such as ryecarbonitriline A which is isolated from root exudates of rye (Cimmino et al. 2015).

Switchgrass (*Panicum virgatum* L.) is a widely adapted, warm-season perennial grass with considerable potential as a bioenergy crop (Sanderson 1999). In recent years, switchgrass has been successfully cultivated in China's Loess Plateau as a favored plant for restoring vegetation and a forage for livestock production (Ma et al. 2011). In the arid and semiarid areas of the Loess Plateau, animal husbandry is the main source of livelihood and

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income of the local people. The artificial grassland construction is currently impeded by problems such as a lack of grass species and plant community formed by single species (Xu et al. 2011). Planting switchgrass could help solve these problems. Field trials show that some switchgrass cultivars grow well and achieve a higher yield (An et al 2013a; Ma et al. 2011).

For the *O. cumana* control and management, researchers have attempted to screen out some crops or their varieties that have potential on suicidal germination of *O. cumana* (Ma et al. 2013, 2014; Zhang et al. 2013). However, their practical applications are restricted by the limited adaptability of these crops in the arid and semiarid Loess Plateau. To date, there is no report about the ability of switchgrass to induce *O. cumana* germination. The objectives of this study are to (1) test if plant extracts, rhizosphere soil, and root exudates of switchgrass have the ability to induce *O. cumana* seed germination, and (2) determine if there are correlations of the induction ability between the shoot extracts and rhizosphere soil and root.

Materials and methods

Field experiment

Plant materials and chemicals

Seeds of nine switchgrass cultivars (Alamo, Blackwell, Cave-in-Rock, Forestberg, Illinois USA, Kanlow, Nebraska 28, Pathfinder, and Sunburst) were provided by Professor Nobumasa Ichizen, Utsunomiya University, Japan. Switchgrass was planted in the spring of 2006 at the Institute of Soil and Water Conservation, Northwest A&F University, Yangling, Shaanxi Province (34°12′–34°20′N, 108°– 108°7′E). Information about the cultivars is given in Table 1. The average annual precipitation at the research site is 600 mm, and the average annual temperature is 13.0°C. The nine cultivars were planted separately. The experiment design was a randomized complete block with three replications. Seeds of *O. cumana* were collected from sunflower fields in the Xinjiang Uygur Autonomous Region, China. A synthetic analog of strigol, GR24, was provided by Dr. Binne Zwanenburg, Radboud University, Nijmegen, Holland.

Table 1. Ecotypes, ploidy levels, and origins of the switchgrass cultivars in this study.

Switchgrass cultivars	Ecotype	Ploidy	Origin
Alamo	Lowland	Tetraploid (4n)	South Texas 28°
Blackwell	Upland	Octoploid (8n)	Northern Oklahoma 37°
Cave-in-Rock	Intermediate	Octoploid (8n)	Southern Illinois 38°
Forestberg	Upland	Tetraploid (4n)	South Dakota 44°
Illinois USA	Unknown	Unknown	Unknown
Kanlow	Lowland	Tetraploid (4n)	Central Oklahoma 35°
Nebraska 28	Upland	Unknown	Northern Nebraska 42°
Pathfinder	Upland	Octoploid (8n)	Nebraska/ Kansas 40°
Sunburst	Upland	Unknown	South Dakota 44°

Sample collection and extract preparation

In field experiment, shoot, root, and rhizosphere soil samples were collected from each switchgrass plot on 10 April (switchgrass grown for 2 weeks), 1 May (switchgrass grown for 5 weeks), and 21 May (switchgrass grown for 8 weeks) 2010. Switchgrass plants were removed from the plow layer soil (0–30 cm depth) with a trowel. The rhizosphere soil was collected according to the method described by Riley and Barber (1970). The roots were washed in running tap water. Shoots and roots were separated, air-dried, milled, and passed through a 0.35 mm sieve. Both aqueous and methanol extracts of the switchgrass shoots and roots were prepared. A 100-mg sample of switchgrass shoot or root powder was weighed into a 1.5 mL centrifuge tube with 1.0 mL of either distilled water or methanol and then sonicated for 30 min. The samples were centrifuged using a microcentrifuge (Millipore Cat. No. XX42 CF0, 60 Lot No. N8JMB042A, Nihon Millipore LTD., Yonezawa, Japan) at 6400 rpm for 2 min. The supernatants were hereafter referred to as the undiluted extracts. Both the rhizosphere soil and extracts were stored at 4°C until use.

Preparation of O. cumana *seeds*

The *O. cumana* seeds were surface-sterilized by immersion for 5 min in 1% (v/v) NaOCl (sodium hypochlorite), followed by soaking in 75% (v/v) ethanol for 3 min. The seeds were then rinsed with sterile distilled water and air-dried in clean bench. A Petri dish (9 cm diam.) was lined with two layers of filter paper. Glass fiber filter disks (5 mm Whatman GF/A, Whatman International Ltd., Maidstone, UK) were laid uniformly on the filter paper and sterile distilled water was added. The *O. cumana* seeds were put on the glass fiber filter disks (20–40 seeds per disk).

Germination assay

The aqueous extracts of switchgrass were diluted 10- and 100-fold with distilled water, and then 20 µL aliquots of each concentration were applied to condition *O. cumana* seeds on disks (5 mm diam.) of glass fiber filter paper in Petri dishes. Each extract was tested on three disks (i.e. three replications). A water-saturated and folded filter paper was placed in the center of each Petri dish to maintain a moist environment. The Petri dishes were then sealed with Parafilm (Pechiney Plastic Packaging Company, Menasha, WI 54952.) and incubated at 25°C for 10 d. Germination rates were examined microscopically. Each treatment was replicated three times. Three GR24-treated disks (20 µL of 0.1 mg GR24 L^{-1} per disk) were

used as positive controls to determine the normal germination rate of the *O. cumana* seeds. Three distilled water-treated disks were used as negative controls.

The methanolic extracts were diluted 10- and 100-fold with methanol, and then 20 μL aliquots of each concentration were applied to 5-mm-diam. disks (three disks). The disks were dried at room temperature, and then 5-mm glass fiber filters with *O. cumana* seeds were placed on top of each treated disk. Forty μL sterile water was added to each of the 5-mm-diam. disks. The other procedures were exactly the same as those used for the aqueous extracts.

Three grams of rhizosphere soil was added to Petri dishes (3.5 cm diam.) and then covered with filter paper (3.5 cm diam.) (Ma et al. 2005). Five 5-mm-diam. glass fiber filter disks, with 20–40 *Orobanche* seeds, were put on the upper surface of the filter paper. This position will be referred to as the bottom layer. The seeds were then covered with filter paper (3.5 cm diam.). Additional 3 grams of rhizosphere soil was spread across the filter paper and then moistened with 2 mL distilled water. Five glass fiber filter disks with *O. cumana* seeds were placed on the surface of rhizosphere soil. This position will be referred to as the top layer. Each treatment was replicated three times. Nonrhizosphere soil from a fallow plot at the study site was used as a control treatment. The Petri dishes were sealed and incubated at 25°C for 13 d. The *O. cumana* seeds were examined with a microscope to look for the emergence of a germ tube, which indicated that the seeds had germinated. The germination rates were then calculated.

Pot experiment

Plant materials

A pot experiment was conducted in 1 June 2011 at the Institute of Soil and Water Conservation, Northwest A&F University, Yangling, Shaanxi Province, to further verify the induction ability of switchgrass on *O. cumana* seed germination. Seeds of the nine switchgrass cultivar were sown in the pots (20 cm diam. × 30 cm deep) at the rate of 40 seeds per pot. Switchgrass seedlings were thinned to 10 plants per pot when grown for 20 d. The experimental soil was Lou soil (Typ-Eum-Orthic Anthrosols), collected from the test field of the Institute of Soil and Water Conservation, Northwest A&F University, with the pH of 7.98, organic matter content of 13.97 g kg^{-1}, available nitrogen of 71.3 mg kg^{-1}, available phosphorus of 24.2 mg kg^{-1}, available potassium of 166.1 mg kg^{-1}, respectively. The experiment was randomly designed. Each switchgrass cultivar had five replications. Pots without switchgrass were used as controls. Pots were watered daily and weeds removed manually until the sampling date. Shoot, intact root, and rhizosphere soil samples were collected at 1 August 2011 (seedlings grown for 8 weeks), and then prepared and assayed according to the filed experimental procedure.

Root exudates assay

Switchgrass root exudates were assayed to induce *O. cumana* germination on 1 June 2012. The 6-well tissue culture plate (Becton Dickinson and Company, Franklin Lakes, NJ, USA) with flat bottom was used. Five disks (5 mm diam.) of glass fiber filter paper carrying 20–40 *O. cumana* seeds were put at the bottom of lumen of the well, and then covered with a glass filter paper. Sixty seeds of the nine switchgrass cultivars with the germination rate of around 60% were immediately spread on the surface of the glass filter paper. The lumen of the well of the tissue culture plate was filled with sterilized vermiculite. Each switchgrass cultivar had five random replications. The tissue culture plates without switchgrass seeds were used as negative controls. Three GR24-treated disks (20 μL of 0.1 mg GR24 L^{-1} per disk) were used as positive controls to determine the normal germination rate of the *O. cumana* seeds. The tissue culture plates were then placed in an incubator at 25°C. Sufficient water was applied daily. After incubation for 30 d, germination rates of *O. cumana* seeds were determined microscopically.

Statistical analysis

Analysis of variance was calculated with the SPSS 10.0 software (SPSS, Chicago, IL, USA). Averages were separated by Least Significant Difference ($p < 0.05$). Linear regressions were performed to determine possible relationships between the shoot extracts, roots extracts, and rhizosphere soil on *O. cumana* germination. The regression coefficients (R^2) were considered significant at $p < 0.05$ and $p < 0.01$ level.

Results

The *O. cumana* germination rate induced by germination stimulant GR24 was around 60%. Distilled water was not able to induce *O. cumana* germination. Thus, the *O. cumana* seeds were viable in the study.

Field experiment

A summary of analysis of variance for the effects of switchgrass growth stage, cultivars, and their interaction on *O. cumana* seeds germination showed that the effect of growth stage was statistically significant ($F = 3.932$, $p < 0.05$). Among plant samples collected after 2 weeks of growth, aqueous or methanolic extracts of switchgrass shoots induced *O. cumana* seed germination, whereas root extracts generally had less effect (Table 2). Undiluted aqueous extracts of shoot could not induce *O. cumana* germination, whereas the undiluted methanolic extracts of shoot could induce germination. The 10-fold dilution of aqueous and methanolic extracts of shoot induced *O. cumana* germination to a higher level than the other concentrations did. Aqueous shoot extracts of four switchgrass cultivars, Alamo, Forestberg, Kanlow, and Nebraska 28, had greater ability to induce *O. cumana* seed

Table 2. Germination rate of *O. cumana* induced with extracts from switchgrass shoots and roots sampled after 2 weeks of growth in field experiment.

Switchgrass cultivars	Aqueous extracts				Methanolic extracts			
	Shoot		Root		Shoot		Root	
	Undiluted	10-fold dilution	100-fold dilution	Undiluted	10-fold dilution	100-fold dilution	Undiluted	10-fold dilution	100-fold dilution
	(%)								
Alamo	0.0 b	48.3 ab	40.5 b	0.0 b	5.1 c	0.0 c	20.2 e	46.8 ab	36.6 b	17.9 b	16.4 c	0.0 e
Blackwell	0.0 b	29.0 d	2.7 e	0.0 b	0.8 d	2.1 c	14.0 e	35.0bc	25.3 cd	6.0 c	0.0 d	28.1 c
Cave-in-Rock	0.0 b	43.4 b	23.4 cd	0.0 b	0.0 d	1.1 c	43.6 c	53.2 a	31.9 bc	0.0 d	1.3 d	4.5 e
Forestberg	0.0 b	58.8 a	57.6 a	0.0 b	0.0 d	0.0 c	32.0 d	38.9 bc	35.3 b	0.0 d	0.0 d	5.5 e
Illinois USA	0.0 b	40.4 bc	37.4 b	0.0 b	1.7 cd	20.1 b	53.2 ab	56.3 a	22.5 d	0.0 d	0.0 d	38.8 b
Kanlow	0.0 b	48.7 ab	32.2 bc	0.0 b	11.1 b	1.0 c	15.9 e	54.8 a	19.2 d	0.0 d	45.0 b	19.7 cd
Nebraska 28	0.0 b	49.9 ab	31.9 bc	0.0 b	0.0 d	1.7 c	45.8 bc	58.3 a	5.2 e	0.0 d	0.0 d	15.0 d
Pathfinder	0.0 b	30.5 cd	21.2 d	0.0 b	0.0 d	0.0 c	11.8 e	31.9 c	7.6 e	0.0 d	0.0 d	0.0 e
Sunburst	0.0 b	45.8 b	19.3 d	0.0 b	3.4 cd	3.1 c	3.0 f	18.0 d	1.1 e	0.0 d	0.0 d	43.7 b
Water	0.0 b	0.0 e	0.0 e	0.0 b	0.0 d	0.0 c	0.0 f	0.0 e	0.0 e	0.0 d	0.0 d	0.0 e
GR24	49.7 a	49.7 a	49.7 a	49.7 a	49.7 a	49.7 a	56.8 a	56.8 a	56.8 a	56.8 a	56.8 a	56.8 a

Note: Means in the same column followed by different letters differ significantly at $p < 0.05$ level.

germination. Forestberg maintained a greater induction effect on *O. cumana* seed germination when shoot extract was diluted to 100-fold. For the 10-fold dilution of methanolic extract from switchgrass shoot, five cultivars, Alamo, Cave-in-Rock, Illinois USA, Kanlow, and Nebraska 28, induced *O. cumana* seed germination to the similar level to that of GR24-treated seeds ($p > 0.05$).

The germination rates of *O. cumana* decreased substantially when treated with aqueous or methanolic shoot extracts from more mature switchgrass plants (grown for 5 weeks). The highest germination rate treated by aqueous extracts from switchgrass shoots was 35.1% (Table 3). Among the cultivars, the 10-fold dilution of methanolic extract from Alamo and Blackwell shoots induced germination rate to a similar level to that of GR24-treated seeds ($p > 0.05$). Ability of root extracts to induce *O. cumana* seed germination was generally weaker when switchgrass grown for 5 weeks.

The induction effects of aqueous extracts from shoots on *O. cumana* tended to disappear when switchgrass grown for 8 weeks (Table 4). *O. cumana* germination rates generally remained at a relative higher level when treated with methanolic extracts from shoots of more mature switchgrass. Among the cultivars, Cave-in-Rock had the greatest induction effect on *O. cumana* at 10-fold treatment (55.5%). The 10-fold methanolic extracts from switchgrass root of three cultivars, Alamo, Kanlow, Nebraska 28 and GR24, had similar induction activity on *O. cumana* seed germination.

The germination rates of *O. cumana* seeds treated with rhizosphere soil were much less than those of seeds treated with GR24 (Table 5). Moreover, the germination rates changed across time when induced with rhizosphere soil of switchgrass. The rhizosphere soil of switchgrass collected from 5-week switchgrass generally induced the highest level of germination. Rhizosphere soil of Nebraska 28 induced the highest *O. cumana* germination rate among the cultivars. *O. cumana* seed germination rate was less than 10% when switchgrass grown for 8 weeks.

Pot experiment

A summary of analysis of variance for the effects of extract solvent, concentration, and their interaction on *O. cumana* seed germination showed the effect of extract solvent and concentration to be statistically significant ($F = 10.953$, $p < 0.001$; $F = 4.253$, $p < 0.05$, respectively). Data in Table 6 showed that the germination rates of *O. cumana* treated with methanolic extracts were higher than that treated with aqueous extracts. For 10-fold dilution, germination rate of *O. cumana* treated with root methanolic extracts ranged between 39.2% and 59.8%, whereas that treated with aqueous extracts ranged between 10.9% and 35.9%. Moreover, the root extracts of some cultivars induced the *O. cumana* germination to a higher level compared with GR24 treatment. The 10- and 100-fold dilutions of methanolic extracts from roots of Forestberg, Nebraska 28, and Pathfinder induced

Table 3. Germination rate of *O. cumana* induced with extracts from switchgrass shoots and roots sampled after 5 weeks of growth in field experiment.

Switchgrass cultivars	Aqueous extracts						Methanolic extracts					
	Shoot			Root			Shoot			Root		
	Undiluted	10-fold dilution	100-fold dilution	Undiluted	10-fold dilution	100-fold dilution	Undiluted	10-fold dilution	100-fold dilution	Undiluted	10-fold dilution	100-fold dilution
	(%)											
Alamo	0.0 b	28.4 bc	21.9 b	0.0 b	0.0 b	0.0 b	45.4 b	48.4 ab	17.7 b	0.0 b	0.0 b	2.9 e
Blackwell	0.0 b	0.0 e	0.7 c	0.0 b	0.0 b	3.4 b	37.6 c	52.6 a	0.0 d	0.0 b	0.0 b	14.0 cd
Cave-in-Rock	0.0 b	35.1 b	15.3 b	0.0 b	0.0 b	0.0 b	31.0 cd	42.6 bc	18.4 b	0.0 b	0.0 b	12.5 d
Forestberg	0.0 b	14.6 d	6.0 c	0.0 b	0.0 b	0.0 b	22.0 e	38.9 c	6.7 c	0.0 b	0.0 b	28.8 b
Illinois USA	0.0 b	19.7 cd	0.0 c	0.0 b	0.0 b	0.0 b	29.7 d	34.5 c	1.5 d	0.0 b	0.0 b	10.1 d
Kanlow	0.0 b	30.4 b	18.5 b	0.0 b	0.0 b	1.7 b	20.7 e	33.1 c	0.0 d	0.0 b	0.0 b	28.6 b
Nebraska 28	0.0 b	4.0 e	4.3 c	0.0 b	0.0 b	0.0 b	19.4 e	34.5 c	0.0 d	0.0 b	0.0 b	17.9 c
Pathfinder	0.0 b	16.3 d	5.1 c	0.0 b	0.0 b	0.0 b	0.0 f	23.2 d	0.0 d	0.0 b	5.0 b	0.0 e
Sunburst	0.0 b	33.2 b	7.2 c	0.0 b	0.0 b	4.0 b	31.5 cd	34.5 c	0.0 d	0.0 b	0.0 b	12.7 d
Water	0.0 b	0.0 e	0.0 c	0.0 b	0.0 b	0.0 b	0.0 f	0.0 e	0.0 d	0.0 b	0.0 b	0.0 e
GR24	53.7 a	53.7 a	53.7 a	53.7 a	53.7 a	53.7 a	56.8 a	56.8 a	56.8 a	56.8 a	56.8 a	56.8 a

Note: Means in the same column followed by different letters differ significantly at $p < 0.05$ level.

Table 4. Germination rate of *O. cumana* induced with extracts from switchgrass shoots and roots sampled after 8 weeks of growth in field experiment.

Switchgrass cultivars	Aqueous extracts						Methanolic extracts					
	Shoot			Root			Shoot			Root		
	Undiluted	10-fold dilution	100-fold dilution	Undiluted	10-fold dilution	100-fold dilution	Undiluted	10-fold dilution	100-fold dilution	Undiluted	10-fold dilution	100-fold dilution
	(%)											
Alamo	0.0 b	7.1 c	0.0 c	0.0 b	0.0 b	0.0 b	47.0 b	34.6 de	0.0 d	0.0 b	52.4 a	16.3 e
Blackwell	0.0 b	2.8 cd	0.5 c	0.0 b	0.0 b	1.5 b	42.8 bc	25.7 ef	0.0 d	0.0 b	0.0 b	22.9 de
Cave-in-Rock	0.0 b	6.1 c	0.0 c	0.0 b	0.0 b	0.7 b	31.0 d	55.5 ab	7.0 b	0.0 b	5.0 b	1.1 f
Forestberg	0.0 b	0.0 d	2.5 c	0.0 b	0.0 b	1.2 b	43.8 bc	40.6 cd	0.0 d	0.0 b	0.0 b	35.0 bc
Illinois USA	0.0 b	0.0 d	1.3 c	0.0 b	0.0 b	0.0 b	43.9 bc	47.0 bc	3.0 c	0.0 b	0.0 b	0.0 f
Kanlow	0.0 b	0.0 d	0.0 c	0.0 b	0.0 b	0.0 b	41.8 bc	26.7 ef	0.0 d	0.0 b	51.1 a	41.3 b
Nebraska 28	0.0 b	6.1 c	8.6 b	0.0 b	0.0 b	0.0 b	40.6 bc	34.4 de	0.0 d	0.0 b	51.3 a	25.9 d
Pathfinder	0.0 b	0.0 d	0.0 c	0.0 b	0.0 b	0.0 b	46.6 b	44.2 c	0.0 d	0.0 b	0.0 b	25.1 d
Sunburst	0.0 b	12.1 b	0.0 c	0.0 b	3.0 b	0.0 b	36.4 cd	21.6 f	0.0 d	0.0 b	4.2 b	27.2 cd
Water	0.0 b	0.0 d	0.0 c	0.0 b	0.0 b	0.0 b	0.0 e	0.0 g	0.0 d	0.0 b	0.0 b	0.0 f
GR24	55.2 a	55.2 a	55.2 a	55.2 a	55.2 a	55.2 a	56.8 a	56.8 a	56.8 a	56.8 a	56.8 a	56.8 a

Note: Means in the same column followed by different letters differ significantly at $p < 0.05$ level.

Table 5. Germination rate of *O. cumana* seeds induced with rhizosphere soil from switchgrass in field experiment.

Switchgrass cultivars	Grown for 2 weeks		Grown for 5 weeks		Grown for 8 weeks	
	Top layer	Bottom layer	Top layer	Bottom layer	Top layer	Bottom layer
	(%)					
Alamo	4.8 c	4.1 cd	2.7 c	8.4 e	0.8 d	0.0 b
Blackwell	5.4 c	2.8 d	3.4 c	0.0 f	4.8 c	0.0 b
Cave-in-Rock	6.5 c	0.0 d	3.8 c	12.4 de	9.9 b	0.0 b
Forestberg	6.3 c	10.5 cd	2.3 c	25.9 c	9.3 b	0.0 b
Illinois USA	5.6 c	2.2 d	0.0 c	17.6 d	1.5 d	0.0 b
Kanlow	5.6 c	0.0 d	2.4 c	15.6 d	5.1 c	0.0 b
Nebraska 28	14.9 b	27.3 b	13.6 b	43.5 b	0.0 d	2.9 b
Pathfinder	3.0 c	15.5 c	4.7 c	12.6 de	0.0 d	0.0 b
Sunburst	6.8 c	0.0 d	0.0 c	0.0 f	0.0 d	2.4 b
Nonrhizosphere soil	0.0 c	0.0 d	0.0 c	0.0 f	0.0 d	0.0 b
GR24	56.3 a	56.3 a	57.2 a	57.2 a	58.7 a	58.7 a

Note: Means in the same column followed by different letters differ significantly at $p < 0.05$ level.

O. cumana germination rates at a similar level to those treated with GR24 ($p > 0.05$).

All the rhizosphere soil of switchgrass cultivars could induce *O. cumana* seed germination (Table 7). The germination rate of *O. cumana* treated with rhizosphere soil of Cave-in-Rock and Nebraska 28 was 45.3% and 51.0%, respectively, similar with those treated with GR24 ($p > 0.05$).

Root exudates assay

The laboratory experiment indicated that root exudates of switchgrass directly induced *O. cumana* seeds germination (Figure 1), with the germination rates ranging from 14.1% to 48.6%. Among all the tested switchgrass cultivars, root exudates of Nebraska 28 induced a relative higher level of *O. cumana* seed germination, whereas root exudates of Cave-in-Rock and Pathfinder had less ability to induce germination.

Discussion

Effects of switchgrass on *O. cumana* germination generally declined as the plants matured, suggesting that *O. cumana* seeds germination induced by extracts from switchgrass was depending on the growing stages of switchgrass. The result was supported by the previous study, which reported the induction ability of soybean on *O. cumana* germination reached highest at the beginning stages (Zhang et al. 2013). Similarly, *Orobanche minor* germination rate was the greatest in response to root exudates produced by the one-leaf and the beginning of tillering stages of wheat (*Triticum aestivum* L.), indicating that wheat produced germination stimulant only at early stages of development (Al-thahabi et al. 2014). Moreover, the result was partially proved by the previous study which demonstrated that the parasitic plant striga (*Striga hermonthica* [del.]) seed germination treated with the stimulants from 1-week-old Asiatic moonseed (*Menispermum dauricum* DC.) root cultures was higher than that treated with the stimulants from 2- to 3-week-old root cultures (Ma et al. 1996). In this study, the reduction in the *O. cumana* germination induced by plant extracts and rhizosphere soil of switchgrass at later growth stages might be explained partially by the presence of an inhibitory compound in the plant extract, or due to an increase in the exudates concentrations with increase in the plant size. Therefore, the changes in induction ability of switchgrass observed in the field experiment might be due to a change in either the quantity (composition) or the quality (concentration) of stimulant produced as a result of plant growth stages.

Among plant samples collected in the field experiment, switchgrass shoot extracts induced greater *O. cumana* germination rates than root extracts. The inducing trend was similar to switchgrass allelopathy (Shui et al. 2010), with switchgrass shoot having greater effect on the target plants than root. Unlike field experiment, pot experiment showed that root extracts induced higher *O. cumana* germination rates than shoot extracts. It was confirmed that allelopathic effect is strongly related to plant–environment interactions (Blanco 2007). Differences in *O. cumana* germination observed in the field and pot experiments might be attributed to several environmental factors (nutrient level, light intensity, temperature, etc.) and their heterogenicity. Further trials on exploring the change mechanism of the stimulants in order to provide greater control of *O. cumana* should be carried out. In addition, previous studies revealed that the *Orobanche* germination stimulants were mainly produced in the hairy roots (Bouwmeester et al. 2003; Besserer et al. 2006). These findings probably supported the results of the pot experiment, which indicated that the root extracts of switchgrass had greater stimulatory activity on *O. cumana* germination than the shoot extracts. Also, some studies indicated that germination stimulants of *Orobanche* were mainly synthesized in host plant roots and transported to shoots (Matúšová & Bouwmeester 2006; Kohlen et al. 2011). Furthermore, there was a positive correlation between the germination rates of *O. cumana* seeds treated with switchgrass shoot extracts and those treated with switchgrass roots extracts ($R^2 = 0.6397$; $p < 0.01$, Figure 2a). Likewise, there was a negative relationship between germination rate treated

Table 6. Germination rate of *O. cumana* induced with extracts from switchgrass shoots and roots sampled in pot experiment.

Switchgrass cultivars	Aqueous extracts						Methanol extracts					
	Shoot			Root			Shoot			Root		
	Undiluted	10-fold dilution	100-fold dilution	Undiluted	10-fold dilution	100-fold dilution	Undiluted	10-fold dilution	100-fold dilution	Undiluted	10-fold dilution	100-fold dilution
	(%)											
Alamo	0.0 b	0.0 b	0.0 c	0.0 b	35.9 b	0.0 b	0.0 b	12.7 c	0.0 c	31.0 c	56.2 ab	27.5 ef
Blackwell	0.0 b	0.0 b	0.0 c	0.0 b	18.9 de	0.0 b	0.0 b	6.5 d	0.0 c	19.5 d	42.0 cd	22.8 fg
Cave-in-Rock	0.0 b	0.0 b	1.1 c	0.0 b	16.7 ef	0.0 b	0.0 b	4.5 de	2.3 bc	26.2 c	48.9 bc	47.5 bc
Forestberg	0.0 b	0.0 b	0.0 c	0.0 b	10.9 f	0.0 b	0.0 b	24.3 b	0.0 c	44.1 b	59.8 a	54.9 ab
Illinois USA	0.0 b	0.0 b	0.7 c	0.0 b	19.5 de	0.0 b	0.0 b	0.0 ef	0.0 c	30.0 c	41.2 cd	36.2 de
Kanlow	0.0 b	0.0 b	0.0 c	0.0 b	25.4 cd	0.0 b	0.0 b	0.0 f	4.5 b	0.0 e	40.9 cd	13.6 g
Nebraska 28	0.0 b	0.0 b	0.0 c	0.0 b	28.0 c	0.0 b	0.0 b	12.0 c	0.0 c	38.4 b	58.9 a	51.3 ab
Pathfinder	0.0 b	0.0 b	11.7 b	0.0 b	17.0 ef	0.0 b	0.0 b	7.5 d	5.0 b	0.0 e	57.2 ab	55.2 ab
Sunburst	0.0 b	0.0 b	0.0 c	0.0 b	19.5 de	0.0 b	0.0 b	4.0 def	0.0 c	14.8 d	39.2 d	38.9 cd
Water	0.0 b	0.0 b	0.0 c	0.0 b	0.0 g	0.0 b	0.0 b	0.0 f	0.0 c	0.0 e	0.0 e	0.0 h
GR24	58.4 a	58.4 a	58.4 a	58.4 a	58.4 a	58.4 a	58.4 a	58.4 a	58.4 a	58.4 a	58.4 a	58.4 a

Note: Means in the same column followed by different letters differ significantly at $p < 0.05$ level.

Table 7. Germination rate of *O. cumana* induced with rhizosphere soil from switchgrass in pot experiment.

Switchgrass cultivars	Top layer	Bottom layer
	(%)	
Alamo	17.7 fg	23.5 d
Blackwell	27.0 ef	24.3 cd
Cave-in-Rock	45.3 abc	20.3 d
Forestberg	5.8 gh	23.0 d
Illinois USA	29.7 def	20.5 d
Kanlow	41.0 bcd	36.3 b
Nebraska 28	51.0 ab	31.6 bc
Pathfinder	32.5 cde	37.3 b
Sunburst	23.3 ef	25.3 cd
Nonrhizosphere soil	0.0 h	0.0 e
GR24	58.3 a	58.5 a

Note: Means in the same column followed by different letters differ significantly at $p < 0.05$ level.

with switchgrass rhizosphere soil and that treated with root extracts ($R^2 = 0.4433$; $p < 0.05$, Figure 2b). These findings were in partial agreement with the results obtained by Zhang et al. (2013), who reported the germination rates of *O. cumana* induced by 10-fold dilutions of stem extracts of soybean were positively correlated with those induced by 10-fold dilutions of root extracts. Similar relationships were also observed by Ma et al. (2012), who demonstrated that stem extracts could be used to assay the allelopathic potential of cotton toward *O. minor*. Thus, the relationships among the induction ability of switchgrass organs will provide important basis for the studies on the stimulants transportation, although the type and concentration of allelopathic substances might differ among plant organs (Khanh et al. 2005). And, use of shoot to screen and assay the induction ability of switchgrass cultivars is feasible when switchgrass is growing.

Results of the study showed that methanolic extracts had greater ability to induce *O. cumana* germination than distilled water extracts. One explanation is that stimulants such as SLs are not water soluble chemicals when purified (Siame et al. 1993), or have limited water solubility (de Luque et al. 2000). Another explanation is that the chemical composition of the aqueous and methanolic extracts might not be the same (Zhang et al. 2013). Among three concentrations of switchgrass root extracts, the 10-fold dilutions induced the germination to the highest level than other concentration treatments. The explanation may be that the undiluted extracts contain some compounds that hinder the effect of germination stimulation (Ma et al. 2012; Al-thahabi et al. 2014). The concentration of these compounds was consequently diluted in the 10- and 100-fold dilutions. Likewise, the concentration of the stimulants was too low to induce germination in the 100-fold dilutions. This finding was consistent with previous work of Dong et al. (2012), who found that aqueous extracts of wheat at 10% and 1% concentration induced *O. minor* seed germination effectively, whereas undiluted and 0.1% extracts could not induce seed germination. The dose-dependent

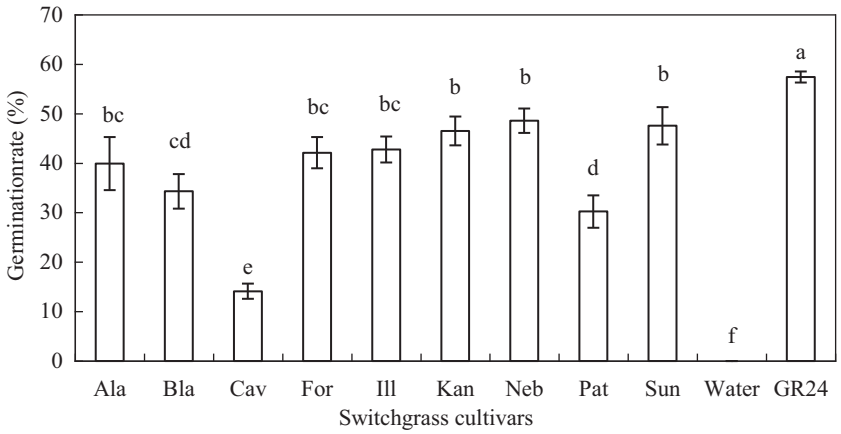

Figure 1. Effect of switchgrass root exudates on *O. cumana* germination. Mean values followed by the same letter are not significantly different ($p < 0.05$). Abbreviations are: Ala, Alamo; Bla, Blackwell; Cav, Cave-in-Rock; For, Forestberg; Ill, Illinois USA; Kan, Kanlow; Neb, Nebraska 28; Pat, Pathfinder; Sun, Sunburst.

response was generally illustrated in the studies on plant allelopathy (Alam et al. 2002; Zahed et al. 2010; An et al. 2013b).

Root exudates assay indicated that the root exudates of all the switchgrass cultivars could directly induce *O. cumana* seed germination. Similarly, root exudates of some gramineous plants, such as wheat, black oat (*Avena strigosa* Schreb.), and rye, could induce the genus *Orobanche* germination (Dong et al. 2012; Kim et al. 2014; Cimmino et al. 2015). Some new stimulants but not known SLs were consequently identified in root exudates (Kim et al. 2014; Cimmino et al. 2015), which were considered as secondary metabolites with allelopathic activity (Yoneyama et al. 2010). Thus, switchgrass

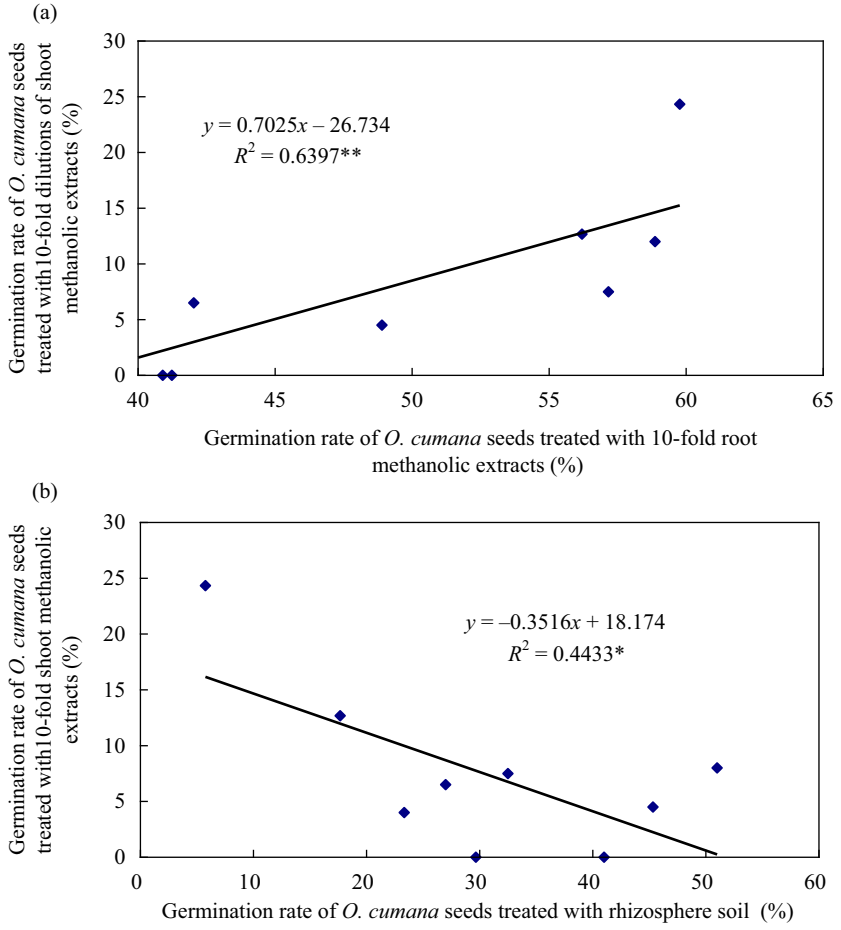

Figure 2. Linear regressions between the germination rates of *O. cumana* seeds treated with shoot methanolic extracts and those treated with switchgrass root extracts (Fig. a), and those treated with rhizosphere soil (Fig. b). ** and * indicate the regressions are significant at $p < 0.01$ and $p < 0.05$ level, respectively.

root exudates also provide an important medium to isolate and identify the stimulants. Although we did not try to chemically identify the germination stimulant, it is suffice to prove that switchgrass has potential to act as a trap crop for *O. cumana*.

Ecologically, all the switchgrass cultivars tested in this study produced *O. cumana* germination stimulants, although there were varietal differences among the field, pot, and laboratory experiments. Intercropping switchgrass with sunflower that induces 'suicidal germination' of *O. cumana* is a promising approach that might be possible not only to control *O. cumana* infestation but also to harvest forage for livestock use. Therefore, selecting cultivars of switchgrass that are adaptable in the regions infested by *O. cumana* will ultimately result in the determination of the ideal trap crop.

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References

Alam SM, Khan MA, Mujtaba SM, Shereen A. 2002. Influence of aqueous leaf extract of common lambsquarters and NaCl salinity on the germination, growth, and nutrient content of wheat. Acta Physiol Plant. 24:359–364.

Al-thahabi SA, Colquhoun JB, Mallory-Smith CA. 2014. Influence of temperature and plant growth stages of red clover and wheat on small broomrape (*Orobanche minor*) germination. Weed Technol. 28:266–271.

An Y, Ma YQ, Shui JF. 2013a. Switchgrass (*Panicum virgatum* L.) plants and switchgrass residue reduce the biomass and density of associated weeds. Acta Agr Scand B-S P. 63:107–113.

An Y, Ma YQ, Shui JF. 2013b. Switchgrass root exudates have allelopathic potential on lettuce germination and seedling growth. Acta Agr Scand B-S P. 63:497–505.

Aybeke M, Şen B, Ökten S. 2014. *Aspergillus alliaceus*, a new potential biological control of the root parasitic weed *Orobanche*. J. Basic Microbiol. 54:S93–S101.

Besserer A, Puech-Pagès V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais JC, Roux C, Bécard G, Séjalon-Delmas N. 2006. Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. PLoS Biol. 4:e226.

Blanco JA. 2007. The representation of allelopathy in ecosystem-level forest models. Ecol Model. 209:65–77.

Bouwmeester HJ, Matusova R, Zhongkui S, Beale MH. 2003. Secondary metabolite signalling in host–parasitic plant interactions. Curr Opin Plant Biol. 6:358–364.

Chen XF. 2010. A comprehensive treatment and the reason of sunflower broomrape occurring in Dingbian County. Modern Agric Sci. 15:215–217. Chinese.

Cimmino A, Fernández-Aparicio M, Avolio F, Yoneyama K, Rubiales D, Evidente A. 2015. Ryecyanatines A and B and ryecarbonitrilines A and B, substituted cyanatophenol, cyanatobenzo[1,3]dioxole, and benzo[1,3]dioxolecarbonitriles from rye (*Secale cereale* L.) root exudates: novel metabolites with allelopathic activity on *Orobanche* seed germination and radicle growth. Phytochemistry. 109:57–65.

de Luque AP, Galindo JCG, Macías FA, Jorrín J. 2000. Sunflower sesquiterpene lactone models induce *Orobanche cumana* seed germination. Phytochemistry. 53:45–50.

Dong S, Ma YQ, Wu H, Shui JF, Hao ZQ. 2012. Stimulatory effects of wheat (*Triticum aestivum* L.) on seed germination of *Orobanche minor*. Allelopathy J. 30:247–258.

Eizenberg, H, Hershenhorn, J, Ephrath, JE. 2009. Factors affecting the efficacy of *Orobanche cumana* chemical control in sunflower. Weed Res. 49:308–315.

Goldwasser Y, Eizenberg H, Golan S, Kleifeld Y. 2003. Control of *Orobanche crenata* and *Orobanche aegyptiaca* in parsley. Crop Prot. 22:295–305.

Hershenhorn J, Eizenberg H, Dor E, Kapulnik Y. Goldwasser Y. 2009. *Phelipanche aegyptiaca* management in tomato. Weed Res. 49:34–47.

Joel DM, Chaudhuri SK, Plakhine D, Ziadna H, Steffens JC. 2011. Dehydrocostus lactone is exuded from sunflower roots and stimulates germination of the root parasite *Orobanche cumana*. Phytochemistry. 72:624–634.

Kasasian L. 1973. Miscellaneous observations on the biology of *Orobanche crenata* and *O. aegyptiaca*. Proceedings of the European Weed Research Council, Symposium on Parasitic Weeds; Apr 11–13, Wageningen, The Netherlands: Malta University Press; p, 68–75.

Khanh TD, Hong NH, Xuan TD, Chung IM. 2005. Paddy weed control by medicinal and leguminous plants from Southeast Asia. Crop Prot. 24:421–431.

Kim HI, Kisugi T, Khetkam P, Xie X, Yoneyama K, Uchida K, Yokota T, Nomura T, McErlean CSP, Yoneyama K. 2014. Avenaol, a germination stimulant for root parasitic plants from *Avena strigosa*. Phytochemistry. 103:85–88.

Kohlen W, Charnikhova T, Liu Q, Bours R, Domagalska MA, Beguerie S, Verstappen F, Leyser O, Bouwmeester H, Ruyter-Spira C. 2011. Strigolactones are transported through the xylem and play a key role in shoot architectural response to phosphate deficiency in nonarbuscular mycorrhizal host Arabidopsis. Plant Physiol. 155:974–987.

Labrousse P, Arnaud MC, Serieys H, Berville A, Thalouarn P. 2001. Several Mechanisms are involved in resistance of helianthus to *Orobanche cumana* Wallr. Ann Bot. 88:859–868.

Ma YQ, An Y, Shui JF, Sun ZJ. 2011. Adaptability evaluation of switchgrass (*Panicum virgatum* L.) cultivars on the loess plateau of China. Plant Sci. 181:638–643.

Ma YQ, Babiker AGT, Ali IA, Sugimoto Y, Inanaga S. 1996. *Striga hermonthica* (Del.) Benth. germination stimulant(s) from *Menispermum dauricum* (DC.) root culture. J Agric Food Chem. 44:3355–3359.

Ma YQ, Jia JN, An Y, Wang Z, Mao JC. 2013. Potential of some hybrid maize lines to induce germination of sunflower broomrape. Crop Sci. 53:260–270.

Ma YQ, Lang M, Dong SQ, Shui JF, Zhao JX. 2012. Screening of some cotton varieties for allelopathic potential on clover broomrape germination. Agron J. 104:569–574.

Ma YQ, Zhang M, Li YL, Shui JF, Zhou YJ. 2014. Allelopathy of rice (*Oryza sativa* L.) root exudates and its relations with *Orobanche cumana* Wallr. and *Orobanche minor* Sm. Germination. J Plant Interact. 9:722–730.

Ma YQ, Shui JF, Inanaga S, Cheng JM. 2005. Stimulatory effects of *Houttuynia cordata* Thunb. on seed germination of *Striga hermonthica* (Del.) Benth. Allelopathy J. 15:49–56.

Matúšová R, Bouwmeester HJ. 2006. The effect of host-root-derived chemical signals on the germination of parasitic plants. In Dicke M, Takken W, editors. Chemical ecology: from gene to ecosystem. Berlin: Springer; p. 39–54.

Parker C, Riches CR. 1993. Parasitic weeds of the world biology and control. In Gutteridge RC, Skelton HM, editors. *Orobanche* species. Cambridge: Cambridge University Press; p. 111–164.

Parker C. 2013. The parasitic weeds of the Orobanchaceae. In: Joel DM, Gressel J, Musselman LJ, editors. Parasitic Orobanchaceae. Berlin: Springer-Verlag; p. 313–344.

Raupp FM. Spring O. 2013. New Sesquiterpene Lactones from sunflower root exudate as germination stimulants for *Orobanche cumana*. J Agric Food Chem. 61:10481–10487.

Riley D, Barber SA. 1970. Salt accumulation at the soybean (*Glycine max* (L.) Merr.) root-soil interface. Soil Sci Soc Am Proc. 34:154–155.

Sanderson MA. 1999. Switchgrass cultivars and germplasm forbiomass feedstock production in Texas. Bio Technol. 67:209–219.

Shui JF, An Y, Ma YQ, Ichizen N. 2010. Allelopathic potential of switchgrass (*Panicum virgatum* L.) on perennial ryegrass (*Lolium perenne* L.) and alfalfa (*Medicago sativa* L.). Environ Manage. 46:590–598.

Siame BA, Weerasuriya Y, Wood K, Ejeta G, Butler LG. 1993. Isolation of strigol, a germination stimulant for Striga asiatica, from host plants. J Agric Food Chem. 41:1486–1491.

Ueno K, Furumoto T, Umeda S, Mizutani M, Takikawa H, Batchvarova R, Sugimoto Y. 2014. Heliolactone, a non-sesquiterpene lactone germination stimulant for root parasitic weeds from sunflower. Phytochemistry. 108:122–128.

Xu BC, Xu WZ, Huang J, Shan L, Li FM. 2011. Biomass production and relative competitiveness of a C3 legume and a C4 grass co-dominant in the semiarid Loess Plateau of China. Plant Soil. 347:25–39.

Yoneyama K, Awad AA, Xie X, Yoneyama K, Takeuchi Y. 2010. Strigolactones as germination stimulants for root parasitic plants. Plant Cell Physiol. 51:1095–1103.

Zahed N, Hosni K, Brahim NB, Kallel M, Sebei H. 2010. Allelopathic effect of Schinus molle essential oils on wheat germination. Acta Physiol Plant. 32:1221–1227.

Zhang W, Ma Y, Wang Z, Ye X, Shui J. 2013. Some soybean cultivars have ability to induce germination of sunflower broomrape. PLoS ONE. 8:e59715.