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RESEARCH ARTICLE

Extracts from cotton over the whole growing season induce *Orobanche cumana* (sunflower broomrape) germination with significant cultivar interactions

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Abstract Five cotton cultivars and their parents were tested for induction of germination of *Orobanche cumana* Wallr. (sunflower broomrape) seeds in pot and field experiments. Germination rates induced by cotton root extracts were the highest followed by stem extracts then leaf extracts. Cotton seedlings at the six-leaf stage induced higher germination than seedling at the two- and four-leaf stage, in all five cotton cultivars and their parents. In the field, the highest concentration of cotton root extracts gave the highest germination rate of *O. cumana*, and the lowest concentration of cotton root extracts gave the lowest germination rate. Methanol extracts of cotton rhizosphere soil gave the highest germination of *O. cumana*. In general, the root, stem and leaf extracts were more active at the six-leaf stage than other seedling stages. In conclusion, extracts of cotton rhizosphere soil and tissues have high activity in the seedling stage. Extracts of cotton across the whole growing season were able to induce *O. cumana* germination but displayed significant cultivar interactions.

Keywords agricultural systems, cotton, crop ecology, crop rotation systems, weed management

1 Introduction

Broomrapes (*Orobanche* spp.) are holoparasitic weeds that completely depend on their hosts for water and nutrients[1]. They heavily infest many important crops and have negative impacts on crop yield and quality, thus causing economic losses worldwide[2,3]. Of the 20 broomrape species in China, *Phelipanche aegyptiaca* Pers. (Egyptian broomrape), *O. cumana* Wallr. (Sunflower broomrape) and *O. ramosa* L. are the most common and have the widest host range[4].

Suitable temperature and moisture conditions are required for host plants (such as sunflower) to grow, but these conditions alone are not sufficient to cause *O. cumana* seeds germination. This parasite has evolved to require a chemical signal from a host or non-host plant to induce germination. In the absence of these germination stimulants, the seeds will revert back to secondary dormancy[2].

Cotton is a non-host plant that can induce germination of clover broomrape[5]. Botanga et al. showed that in planta production of the germination stimulant of *Striga hermonthica* (Del.) Benth in cotton was a qualitatively inherited trait, and the genes encoding this stimulant are monogenic and simply inherited[6]. It might therefore be possible to select and breed certain cotton genotypes that produce high concentrations of highly active germination stimulants, while maintaining or improving other agronomic attributes.

Efficient and economical control of *Orobanche* is extremely difficult because of the infested soil usually contains high seed reservoir densities. At present, mechanical and manual removal, pesticide spraying and other measures are used to control *Orobanche*, but these methods are costly and labor intensive. They also pose unwanted side effects of crop phytotoxicity and environmental pollution caused by chemical pesticide residues. Therefore, there is currently no consistent and sustainable method for controlling *Orobanche* anywhere in the world[2].

Trap crops are non-host crops that induce germination of *Orobanche*, and consequently lead to seed reservoir

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attrition. Research has shown that many crops including carrot, cucumber, maize, onion, soybean and wheat can be used as trap crops for clover broomrape[7,8]. Wheat can be used as a trap crop with germination rates as high as 25% to 40%, and even up to 70%[9]. Control of parasitic weeds with trap crops is by far the most economical and practical method for small-scale commercial farming[10]. Our research group has conducted investigations on trap crops for *Orobanche* spp. including wheat[9,11], maize[12], cotton[13], soybean[14], rice[15] and switchgrass (*Panicum virgatum*)[16]. In this study, pot and field experiments were conducted to study the effects of rhizosphere soil, root, stem and leaf extracts of different cotton cultivars on the germination of *O. cumana*. The objective was to find a new method for using cotton as a trap crop for the biocontrol of *O. cumana*.

2 Materials and methods

2.1 Source of seeds and chemicals

Seeds of the cotton cultivars were provided by the Cotton Institute Henan Academy of Agricultural Science. Strigol was provided by Prof. K. Yoneyama, Utsunomiya University, Japan and synthetic strigolactones GR24 by Prof. B. Zwanenburg, Radboud University, The Netherlands.

2.2 Seed surface sterilization

Cotton and *O. cumana* seeds were surface sterilized in 1% sodium hypochlorite for 3 min and then soaked in 75% (V/V) ethanol for 3 min. After thoroughly rinsing with sterile distilled water, seeds were air-dried.

2.3 Preconditioning of *O. cumana* seed

Five milliliter gibberellin (10^{-4} mol·L^{-1}) was added into a Petri dish (9 cm in diameter) containing two filter papers. Glass fiber filter disks (8 mm Whatman GF/A) were laid uniformly on the double filter paper and 20–50 *O. cumana* seeds placed on each disk. The Petri dishes were then sealed with Parafilm and incubated at 25°C for 6 d as described in Parker et al.[17].

2.4 *O. cumana* germination assay

Aqueous solutions were assayed directly, by applying 20 μL aliquots of the respective test solution to conditioned *O. cumana* seeds on glass fiber filter disks in the Petri dishes. For solutions and extracts containing organic solvents, aliquots (20 μL each) of the test solution were applied to an 8 mm disk of glass fiber filter paper without seeds and allowing it to dry. Then a disk of conditioned *O. cumana* seed was placed on top of each and moistened with 40 μL of distilled water. After 10 d incubation, the germination rates were examined under a binocular dissecting microscope. An *O. cumana* seed was considered as germinated when the germ-tube protruded from the seed coat. GR24 was also used at 10 mg·L^{-1} on seed stock in separate assays to establish a standard proportion of *O. cumana* seeds that were responsive to germination stimulants[18]. A distilled water control was also included. Individual treatments were replicated three times unless otherwise mentioned.

2.5 Field and pot experiments

Five cotton cultivars and their parents (Table 1), were sown in both pots and field at the Institute of Soil and Water Conservation, Northwest A&F University, Yangling, Shaanxi Province, China in May 2009. The previous crop in the field had been wheat. Five cotton hybrid cultivars and their parents were cultivated in each block, with 30 cotton seeds in a row. The plants were harvested at the two-leaf stage (May 25, 2010), four-leaf stage (June 20, 2010), six-leaf stage (July 8, 2010), squaring stage (September 10, 2010) and flowering-boll stage (October 12, 2010). The loosely held soil was gently shaken off the roots, which was referred to as rhizosphere soil[19,20] and the soil as well as plant samples were sampled at each stage.

Table 1 The cotton cultivars and the corresponding cotton number used in pot and field experiment

Cotton number	Cotton cultivar
Male 1	Zhongmiansuo 48 male parent
Female 1	Zhongmiansuo 48 female parent
Hybrid 1	Zhongmiansuo 48F1
Male 2	Zhongmiansuo 51 male parent
Female 2	Zhongmiansuo 51 female parent
Hybrid 2	Zhongmiansuo 51F1
Male 3	Zhongmiansuo 101 male parent
Female 3	Zhongmiansuo 101 female parent
Hybrid 3	Zhongmiansuo 101F1
Male 4	Batiao-083001
Female 4	Batiao-083001
Hybrid 4	Batiao-083001
Male 5	Qitiao-083001
Female 5	Qitiao-083001
Hybrid 5	Qitiao-083001

Pot experiments were conducted at the of Institute of Soil and Water Conservation, Northwest A&F University, Yangling, Shaanxi Province, China in May 2009. Eight kilogram of soil was put in each pot. Lou soil (Eum-orthic Anthrosols) was collected from the field at Institute of Soil

and Water Conservation, Northwest A&F University, with the pH of 7.98, organic matter content of 14.0 g·kg^{-1}, available nitrogen of 71.3 mg·kg^{-1}, available phosphorus of 24.2 mg·kg^{-1} and available potassium of 166.0 mg·kg^{-1}, respectively. Twenty cotton seeds were planted into each pot (15 cm× 10 cm). Each cultivar had three replications. The cotton plants were collected after 40 d at the six-leaf stage. Soils were sampled at the same time.

2.6 Rhizosphere soil on *O. cumana* germination

Five grams of the rhizosphere soil[19,20] and 1.5 mL distilled water were added to Petri dishes (3.5 cm in diameter). Five disks of glass fiber filter paper (8 mm Whatman GF/A) with conditioned *O. cumana* seeds were put on the surface of the soil and the Petri dishes were sealed and incubated at 25°C for 10 d and subsequently examined for germination using a binocular dissecting microscope.

2.7 Methanol and distilled water extracts of cotton roots, stems and leaves on *O. cumana* germination

Cotton root, stem, and leaf were freeze-dried, milled and sieved (0.35 mm). Samples (100 mg) were weighed into 1.5 mL centrifuge tubes and 1 mL of methanol or distilled water added. The tubes were sonicated for 30 min then centrifuged at 6400 rpm for 2 min. The supernatant was collected, and used undiluted and at 10- and 100-fold dilutions in *O. cumana* germination tests.

2.8 Data analysis

The germination rates among different cultivars at each growth stage were subjected to an analysis of variance. Data processing was done with Excel 2007 and DPS 9.5. Tukey's honest significant difference (HSD) test was used to compare the means.

3 Results and discussion

GR24 (1 mg·L^{-1}) gave the high germination rates (over 60%) in all experiments and distilled water did not induce germination.

In pot experiments, undiluted extracts did not induce any germination, but the 100-fold dilution gave the highest germination rate, higher than that induced by the 10-fold dilution (Table 2). Root extracts from the three cotton seedling stages (two-, four- and six-leaf stages) all induced *O. cumana* germination. There were significant interactions with cultivar and their parents. Generally, extracts from cotton plants at the six-leaf stage induced the highest germination, followed by the four-leaf stage and the two-leaf stage. The highest germination rates reached at each stage were 76.5% for Male 5 at the six-leaf stage, 68.8% for Female 4 at the four-leaf stage, and 37.7% for Female 4 at the two-leaf stage. At least five cotton cultivars and/or parents induced over 60% germination at the four- and six-leaf stages (Table 2).

There were similar trends for the methanol extracts of cotton stem and leaf. Also there were significant

Table 2 Germination rates of sunflower broomrape induced by 10- and 100-fold methanol extracts of cotton roots at two-, four- and six-leaf stages in pot experiment

unit: %

Cotton cultivar	Two-leaf		Four-leaf		Six-leaf	
	10-fold	100-fold	10-fold	100-fold	10-fold	100-fold
Male 1	26.9 abcd	38.2 ab	15.7 f	1.0 cd	32.7 de	0.0 b
Female 1	5.0 g	19.6 ef	60.3 abc	16.3 bcd	54.9 abcd	20.0 a
Hybrid 1	32.8 ab	30.9 abcde	27.6 def	12.5 bcd	57.5 abc	24.8 a
Male 2	34.9 a	33.1 abcd	54.2 abc	10.8 bcd	39 cde	0.0 b
Female 2	26.2 abcde	40.9 a	61.3 abc	16.0 bcd	70.7 a	0.0 b
Hybrid 2	31.0 abc	36.2 abc	38.8 cdef	8.9 bcd	62.2 ab	0.8 b
Male 3	14.7 defg	16.2 f	20.1 ef	10.1 bcd	4.2 f	0.0 b
Female 3	21.5 bcde	25.8 bcdef	60.6 abc	41.9 a	59.9 abc	0.0 b
Hybrid 3	34.6 a	35.8 abc	49.3 abcd	25.6 ab	27.2 ef	0.0 b
Male 4	26.3 abcde	39.7 a	18.7 ef	0.5 d	61.8 abc	2.1 b
Female 4	37.7 a	30.0 abcde	68.8 a	21.4 b	44.8 bcde	11.8 ab
Hybrid 4	13.9 efg	39.1 a	42.3 bcde	12.4 bcd	71.3 a	0.6 b
Male 5	36.9 a	38.1 ab	52.7 abc	1.7 cd	76.5 a	0.4 b
Female 5	18.9 cdef	23.9 cdef	62.9 ab	4.1 cd	41.6 bcde	22.6 a
Hybrid 5	7.7 fg	22.4 def	38.5 cdef	17.7 bc	40.9 bcde	0.5 b

Note: Only the means in the same column were compared. Different letters indicate significant differences between means at $P < 0.05$ (Tukey's HSD). The same bellow.

interaction with cultivar and their parents. Basically, extracts of the six-leaf stage induced the highest germination, followed by the four-leaf stage and the two-leaf stage (Table 3; Table 4).

The field experiment showed that extracts from the whole growth period of cotton, rhizosphere and tissues could induce *O. cumana* germination, but there were significant interactions with cotton cultivar (Tables 5–8).

The undiluted methanol extracts of rhizosphere soil induced the highest *O. cumana* germination compared to the 10- and 100-fold dilutions. The average germination rates were over 30% at squaring stage, with Male 1, Male 2 and Female 3 inducing over 35% germination at this stage. In contrast to the pot experiments, the germination induced by extracts collected at squaring stage were higher than germination for the four-leaf stage, followed by the two-leaf stage and the six-leaf stage (Table 6).

The 100-fold diluted methanol extracts of rhizosphere soil gave the highest *O. cumana* germination rates compared to the undiluted and 10-fold dilutions. As for the pot experiments, the germination induced by the soils collected at the six-leaf stage were highest for the whole growth period, while the extract from the flowering-boll stage showed the lowest germination rates. The Male 2 cotton parent induced 81.0% germination at the six-leaf stage (Table 6).

The 100-fold diluted methanol extracts of stem tissues gave the highest *O. cumana* germination rates compared to the undiluted and 10-fold dilutions. Among the five stages, the average germination rates induced by the two-leaf stage samples were the highest, while those from the flowering-boll stage were the lowest. The Hybrid 5 cotton induced 67.7% germination at the two-leaf stage (Table 7).

Again, the 100-fold diluted methanol leaf extract gave the highest *O. cumana* germination rates compared to the undiluted and 10-fold dilutions. Among the five stages, the germination rates induced by the extracts from the two-leaf stage were highest, while the squaring stage gave the lowest germination rates. The Female 5 cotton parent induced 53.1% germination at the two-leaf stage (Table 7).

The plants collected at flowering-boll cotton stage were divided into eight parts: root, upper stem xylem, lower part of stem xylem, upper stem phloem, and lower part of stem phloem, upper leaves, lower leaves, and flowering-boll. The methanol extracts of the tissues induced *O. cumana* germination. For nine cotton cultivars, extracts of the different organs gave the lowest germination rates. This demonstrated that the germination activity was weak at the flowering-boll stage. (Table 8)

Our observations are consistent with those of Botanga et al.[6] that showed cotton genotypes significantly affect the induction of *O. cumana* germination. Integrated management of parasitic weeds like *Orobanche* using trap crops is needed to reduce the use of herbicides in agriculture. Trap cropping is done to induce the germination of parasitic weed seeds, while preventing the parasite from producing its own seeds. *Orobanche* produces a large number of seeds that have prolonged dormancy in the soil. A chemical stimulant is required to break the seed dormancy and induce germination. This chemical is

Table 3 Germination rates of sunflower broomrape induced by 10- and 100-fold methanol extracts of cotton stem at two-, four- and six-leaf stages in pot experiments unit: %

Cotton cultivar	Two-leaf		Four-leaf		Six-leaf	
	10-fold	100-fold	10-fold	100-fold	10-fold	100-fold
Male 1	38.2 ab	26.9 abcd	1.0 cd	15.7 f	0.0 b	32.7 de
Female 1	19.6 ef	5.0 g	16.3 bcd	60.3 abc	20.0 a	54.9 abcd
Hybrid 1	30.9 abcde	32.8 ab	12.5 bcd	27.6 def	24.8 a	57.5 abc
Male 2	33.1 abcd	34.9 a	10.8 bcd	54.2 abc	0.0 b	39 cde
Female 2	40.9 a	26.2 abcde	16.0 bcd	61.3 abc	0.0 b	70.7 a
Hybrid 2	36.2 abc	31.0 abc	8.9 bcd	38.8 cdef	0.8 b	62.2 ab
Male 3	16.2 f	14.7 defg	10.1 bcd	20.1 ef	0.0 b	4.2 f
Female 3	25.8 bcdef	21.5 bcde	41.9 a	60.6 abc	0.0 b	59.9 abc
Hybrid 3	35.8 abc	34.6 a	25.6 ab	49.3 abcd	0.0 b	27.2 ef
Male 4	39.7 a	26.3 abcde	0.5 d	18.7 ef	2.1 b	61.8 abc
Female 4	30.0 abcde	37.7 a	21.4 b	68.8 a	11.8 ab	44.8 bcde
Hybrid 4	39.1a	13.9 efg	12.4 bcd	42.3 bcde	0.6 b	71.3 a
Male 5	38.1 ab	36.9 a	1.7 cd	52.7 abc	0.4 b	76.5 a
Female 5	23.9 cdef	18.9 cdef	4.1 cd	62.9 ab	22.6 a	41.6 bcde
Hybrid 5	22.4 def	7.7 fg	17.7 bc	38.5 cdef	0.5 b	40.9 bcde

Table 4 Germination rates of sunflower broomrape induced by 10-fold methanol extracts of cotton leaf at two-, four- and six-leaf stages in pot experiments

unit: %

Cotton cultivar	Two-leaf	Four-leaf	Six-leaf
Male 1	38.2 ab	1.0 cd	0.0 b
Female 1	19.6 ef	16.3 bcd	20.0 a
Hybrid 1	30.9 abcde	12.5 bcd	24.8 a
Male 2	33.1 abcd	10.8 bcd	0.0 b
Female 2	40.9 a	16.0 bcd	0.0 b
Hybrid 2	36.2 abc	8.9 bcd	0.8 b
Male 3	16.2 f	10.1 bcd	0.0 b
Female 3	25.8 bcdef	41.9 a	0.0 b
Hybrid 3	35.8 abc	25.6 ab	0.0 b
Male 4	39.7 a	0.5 d	2.1 b
Female 4	30.0 abcde	21.4 b	11.8 ab
Hybrid 4	39.1 a	12.4 bcd	0.6 b
Male 5	38.1 ab	1.7 cd	0.4 b
Female 5	23.9 cdef	4.1 cd	22.6 a
Hybrid 5	22.4 def	17.7 bc	0.5 b

Table 5 Germination rates of sunflower broomrape induced by methanol extracts of cotton rhizosphere soils at five stages in field experiments

unit: %

Cotton cultivars	Two-leaf	Four-leaf	Six-leaf	Squaring	Flowering-boll
Male 1	36.0 a	0.0 h	26.9 bcd	39.3 ab	0.0 b
Female 1	32.1 ab	10.1 fgh	4.8 e	31.8 abc	4.6 b
Hybrid 1	4.7 de	16.1 efg	0.0 e	28.1 abc	16.9 a
Male 2	13.6 cde	38.8 ab	15.9 de	36.1 abc	15.6 a
Female 2	26.9 abc	8.7 fgh	46.7 a	24.7 bc	9.5 ab
Hybrid 2	5.9 de	20.4 cdef	0.0 e	27.3 abc	8.3 ab
Male 3	4.3 de	31.3 abc	33.9 abc	34.1 abc	0.0 b
Female 3	6.1 de	10.7 fgh	9.3 e	35.6 abc	0.0 b
Hybrid 3	34.2 ab	6.3 gh	16.6 cde	24.3 bc	0.0 b
Male 4	21.8 abcd	29.5 bcd	1.9 e	28.7 abc	14.5 a
Female 4	1.9 e	18.2 defg	0.0 e	22.1 bc	0.0 b
Hybrid 4	17.6 bcde	37.3 ab	0.0 e	27.7 abc	0.0 b
Male 5	28.9 abc	33.9 ab	37.7 ab	34.9 abc	0.0 b
Female 5	13.2 cde	42.7 a	4.2 e	16.8 c	0.0 b
Hybrid 5	0.0 e	27.1 bcde	2.3 e	46.0 a	0.0 b

synthesized in planta and released as root exudates by hosts of the parasitic weed and other plants, and the latter can serve as trap crops. The primary consideration in parasitic weed management is the reduction of the parasitic weed seed reservoir in the soil[21].

The high heritability and simple inheritance of suicidal germination in *S. hermonthica* under cotton suggests that this cotton trait should be easily incorporated into cultivars with good agronomic attributes for use in *Striga*-infested areas[6]. It has been reported that the production of strigol by proso millet gradually increased, reaching a maximum of 25 pg per plant per day on days 5–7, and then decreased

Table 6 Germination rates of sunflower broomrape induced by 100-fold dilution methanol extracts of cotton stems at five stages in field experiments

unit: %

Cotton cultivars	Two-leaf	Four-leaf	Six-leaf	Squaring	Flowering-boll
Male 1	3.1 d	0.0 f	31.6 abcde	6.7 gh	0.0 e
Female 1	38.7 abc	61.1 a	26.7 bcde	27.1 def	21.1 c
Hybrid 1	22.2 bcd	7.6 ef	47.8 ab	26.8 def	36.5 b
Male 2	38.3 abc	0.0 f	43.9 abc	42.7 bcd	81.0 a
Female 2	42.9 abc	0.0 f	21.6 de	9.0 gh	7.4 de
Hybrid 2	42.4 abc	43.1 bc	41.9 abcd	0.0 h	22.5 c
Male 3	31.4 bc	4.5 f	19.2 e	0.0 h	0.0 e
Female 3	30.7 bc	12.1 ef	50.0 a	68.3 a	0.0 e
Hybrid 3	19.4 cd	31.3 bcd	52.6 a	20.4 efg	0.0 e
Male 4	32.5 bc	38.5 bcd	41.2 abcd	45.9 bc	2.2 e
Female 4	29.8 bc	45.8 ab	11.0 e	15.1 fgh	12.8 cde
Hybrid 4	44.3 ab	23.3 de	46.5 ab	26.1 ef	45.2 b
Male 5	46.2 ab	0.0 f	50.2 a	31.3 cde	12.3 cde
Female 5	38.9 abc	33.7 bcd	10.2 e	45.4 bc	0.0 e
Hybrid 5	60.8 a	28.5 cd	22.9 cde	49.6 b	19.4 cd

Table 7 Germination rates of sunflower broomrape induced by 100-fold dilution methanol extracts of cotton leaves at five stages in field experiments

unit: %

Cotton cultivars	Two-leaf	Four-leaf	Six-leaf	Squaring	Flowering-boll
Male 1	31.1 cdef	52.8 a	35.1 bcde	30.2 ab	0.0 e
Female 1	8.2 hi	0.0 d	49.7 abcd	22.4 bc	21.1 c
Hybrid 1	27.5 efg	13.3 bc	20.5 de	0.9 e	36.5 b
Male 2	22.6 fgh	0.0 d	62.5 ab	0.0 e	81.0 a
Female 2	0.0 i	0.0 d	55.9 abc	38.6 a	7.4 de
Hybrid 2	68.4 a	0.0 d	32.3 bcde	1.7 e	22.5 c
Male 3	6.4 i	22.1 b	19.2 de	2.1 e	0.0 e
Female 3	28.7 defg	0.0 d	28.9 cde	14.1 cde	0.0 e
Hybrid 3	22.5 fgh	0.0 d	27.1 cde	5.8 de	0.0 e
Male 4	26.5 fg	4.4 cd	56.7 abc	19.2 bcd	2.2 e
Female 4	14.6 ghi	10.5 bcd	32.2 bcde	32.3 ab	12.8 cde
Hybrid 4	44.1 bcd	0.0 d	40.1 abcd	11.7 cde	45.2 b
Male 5	42.8 bcde	0.0 d	22.3 de	0.0 e	12.3 cde
Female 5	53.1 ab	9.0 bcd	7.7 e	0.0 e	0.0 e
Hybrid 5	46.9 bc	21.7 b	67.7 a	0.0 e	19.4 cd

to a constant level of 14 pg per plant per day[22]. Strigol is produced by cotton, and is a germination stimulant for the root parasitic plants, *Striga* and *Orobanche*, which was first isolated by Cook et al.[23,24]. Strigol was later identified in the root exudates of sorghum, maize and proso millet [*Pennisetum glaucum* (L.) R. Br.][25]. Recently, the quantification of strigolactones produced by cotton was reported, which confirmed that strigol was the main stimulant for the germination of *Striga* and *Orobanche* seeds[22].

Since the *Striga* germination stimulant strigol was isolated from cotton, and in our experiment we observed

Table 8 Germination rates of sunflower broomrape induced by 100-fold dilution methanol extracts of various cotton tissues at boll period in field experiments unit: %

Cotton cultivars	Root	Up stem	Low stem	Up phloem	Low phloem	Up leaves	Low leaves	Flowering-boll
Male 1	0 d	44.2 a	0 e	18.8 b	0 e	0 e	0 d	0 c
Female 1	56.2 b	53.5 a	44.1 ab	7.9 cd	21.1 c	21.1 c	6.0 c	18.8 a
Hybrid 1	32.4 c	0 b	41.4 abc	0 d	36.5 b	36.5 b	19.1 b	18.9 a
Male 2	77.2 a	53.7 a	23.7 cd	31.4 a	81.0 a	81.0 a	81.0 a	3.7 bc
Female 2	0 d	6.4 b	60.6 a	10.2 c	7.4 de	7.4 de	6.5 c	9.2 b
Hybrid 2	56.2 b	55.2 a	50.8 a	21.5 b	22.5 c	22.5 c	0 d	0.8 c
Male 3	0 d	0 b	0 e	0 d	0 e	0 e	0 d	0 c
Female 3	0 d	0 b	0 e	0 d	0 e	0 e	0 d	0 c
Hybrid 3	0 d	0 b	0 e	0 d	0 e	0 e	0 d	0 c
Male 4	0 d	1.8 b	30.0 bc	0 d	2.2 e	2.2 e	0 d	0 c
Female 4	0 d	0.5 b	0 e	0 d	12.8 cde	12.8 cde	0 d	0 c
Hybrid 4	0 d	4.9 b	6 de	20.1 b	45.2 b	45.2 b	0 d	7.9 bc
Male 5	40.7 bc	0 b	7.2 de	7.2 cd	12.3 cde	12.3 cde	0 d	0 c
Female 5	49.6 b	11.4 b	0 e	1.4 d	0 e	0 e	1.8 d	0.9 c
Hybrid 5	3.3 d	48.9 a	3.2 e	0 d	19.4 cd	19.4 cd	0 d	0 c

that the extracts from each growth stage induced *O. cumana* germination, we conclude that strigol is continuously produced during the entire growth stage.

Dor et al.[26] found the fast-neutron-mutagenized tomato mutant, SL-ORT1 was highly resistant to various *Phelipanche* and *Orobanche* spp., but no toxic activity or inhibition of *Phelipanche* germination was detected in the SL-ORT1 root extracts. They concluded that the SL-ORT1 resistance was due to its inability to produce and secrete natural germination stimulants into the rhizosphere[26]. This conclusion suggested that resistance to the parasites was due to the inability of the host plant to produce germination stimulants. Recently, it was reported that the resistance of sunflower to *O. cumana* might be associated with a hypersensitive reaction which was activated by exogenous salicylic acid treatment[27]. In contrast, in our experiments all the tested cotton cultivars were able to produce *O. cumana* germination stimulants, albeit with significant cultivar interactions (Table 2; Table 5; Table 6). Importantly, however, it was observed that not all cotton cultivars were able to induce *O. cumana* germinate under the pot and field experiment conditions.

Strigolactones, including strigol, can stimulate branching of arbuscular mycorrhizal fungi[28] and inhibit shoot branching in plants[29,30], and so the consensus is that most of the strigolactone-producing plants (80% of all land vegetation) could be used as trap crops for broomrapes. In reality, the situation is far more complex. Broomrape germination induced by strigolactones is an allelopathic phenomenon. Therefore the strigolactones produced in planta have to be released into the soil in suitable quantities to reach concentrations able to induce germination of parasitic weeds under various environmental stresses. As we gain greater knowledge of allelopathy and trap cropping for broomrape control, new questions arise. Further research to understand the required concentration and 3-dimensional structure of strigolactones; how they are released into the environment; their stability in any given environment; how they are translocated; how they are degraded in soil or fixed in organic soil matter; the effect of the stage of host and/or non-host plant production; local soil temperature, moisture, and mineral conditions; and how strigolactones are adsorbed by broomrape seeds, is required. Answering these questions is of paramount importance. In addition, the criteria used to recommend crop cultivars to farmers as trap crops for broomrape is complicated by the fact that each crop have numerous cultivars, and many of them may not be able to induce broomrape germination. Transferring knowledge about trap crops and allelopathy for broomrape weed control to individual farmers remains a major challenge.

4 Conclusions

The pot experiment indicated that there were significant interactions with cotton cultivar. Specifically, germination induced by the samples collected at the six-leaf stage was the highest, followed by the four-leaf stage and the two-leaf stage. The field experiment showed that extracts over the whole growth period of the cotton, rhizosphere and tissues were capable of inducing *O. cumana* germination,

and that there were significant interactions with cultivar with the flowering-boll stage being the least active. These results show that cotton can be used as a trap crop to control *O. cumana*.

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