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Assessing the Performance of Maize (*Zea mays* L.) as Trap Crops for the Management of Sunflower Broomrape (*Orobanche cumana* Wallr.)

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Abstract: Sunflower broomrape (*Orobanche cumana* Wallr.) is a weedy root parasite that causes huge damage to sunflower (*Helianthus annuus* L.). Trap crop can stimulate parasitic seed germination without attachment in trap crop, and it was considered as economic methods for controlling the parasite. In this study, co-culture and pot experiments were conducted to assess the allelopathic activity of maize (*Zea mays* L.) to stimulate broomrape seeds germination and to evaluate the performance of maize rotation for reducing sunflower broomrape infection. All the tested maize cultivars could induce sunflower broomrape germination, and the most active maize cultivar was N314, which induced approximately 40% broomrape seeds germination. Rotation of maize significantly reduced broomrape infection on subsequent sunflower and increased sunflower biomass. After cultivated N314 for 3 years, broomrape attachment on sunflower was the lowest (0.8 attachment plant^{-1}), and the dry mass of sunflower shoot (28.7 g plant^{-1}) was approximately twice higher than the sunflower without previous crop. The effectiveness of broomrape management was significantly different among various maize cultivars. Meanwhile, the differences among cultivars were decreased with the years of maize cultivated prolonged. Our results confirmed that maize was significantly effective in reducing the sunflower damage caused by sunflower broomrape and suggested that maize rotation could be included in the integrated management of sunflower broomrape.

Keywords: sunflower broomrape; maize; crop rotation; weed management

1. Introduction

Broomrapes (*Orobanche* and *Phelipanche* spp.) and witchweeds (*Striga* spp.) are holoparasites that completely depend on the host, due to the lack of chlorophyll and functional roots. Sunflower broomrape (*Orobanche cumana* Wallr.) exhibits a restricted host range and essentially attacks sunflower (*Helianthus annuus* L.). It spreads over a vast area from central Asia to south-eastern Europe [1]. It is also present in large parts of northern China and has become a serious constraint, causing substantial yield losses of sunflower [2]. Areas of sunflower affected (and yield losses) have been estimated at about 20,000 ha (20–50% losses) in China and in Bayan Nur City, Inner Mongolia. Approximately 40% of sunflower fields are infested by sunflower broomrape, resulting in 25–40% sunflower yield reductions [3].

As an obligate parasite, the life cycle of sunflower broomrape is strongly cued to that of its host. In nature, the germination of sunflower broomrape requires chemical signals from the roots of host plants [4]. The first germination stimulant, namely strigol, was isolated from root exudates of cotton (*Gossypium hirsutum* L.) [5]. In subsequent years, a series of germination stimulants, containing similar structural features with strigol, have been identified and collectively named as strigolactones (SLs) [6]. Besides the germination synchronized with host cultivation, several factors contribute to the fact that the eradication of broomrape seed bank is extremely difficult. Every season *Orobanche cumana* produce large amounts of long-living seeds which contribute to the build-up of parasite populations in sunflowers [7]. Several measures, including particular cultural practices and chemical and biological options, as well as suicidal germination and catch and trap crops, can contribute to seedbank reduction [8]. In the case of sunflower broomrape, the most effective control strategy is by far the use of genetically resistant hybrids, although the effectiveness and durability of this option depends on the appearance and spread of new parasite races [7].

Trap crop is regarded as a valuable tool in developing integrated *Orobanche* control systems, especially in low input agriculture [9]. A non-host plant could exudate germination stimulants to the soil, the broomrape would germinate but would not survive for lack of nutritional support. Thus, the non-host could be exploited in suicidal germination control strategies and commonly referred to as "trap crop" [10]. Previous studies revealed that the root exudates from switchgrass induced sunflower broomrape germination. Promising results for stimulating sunflower broomrape seeds germination have also been reported in sorghum, Sudan grass, and common millet under laboratory conditions [11]. Red clover (*Trifolium pratense* L.) showed fewer clover broomrape (*O. minor* Sm.) attachments after wheat [12]. Rhizosphere soil of foxtail millet (*Setaria italica* L.) had a germination-inducing activity to sunflower broomrape seeds and the rotation with foxtail millet reduced sunflower broomrape infection on sunflower [13]. Unfortunately, the use of trap crop rotation to combat parasitic seed bank is a slow process, which requires long term management to avoid seed bank replenishing [14]. Although many promising studies reported the efficacy of trap crops for controlling *Orobanche* spp., little information about the effectiveness of this measure under continuous cropping is available [15].

Maize (*Zea mays* L.) is a host plant of *Striga* but a non-host plant for at least three broomrape species, including ramose broomrape (*O. ramose* L.), sunflower broomrape and clover broomrape [16–18]. The germination stimulants identified and characterized from maize root exudates are mainly SLs and the typical examples are strigol, 5-deoxy-strigol, sorghumol, zealactone and zeapyranolactone [19–22]. Maize has been ranked as the most widely planted crop in China and it also plays an important role in food security in semiarid Northwest China [23]. Therefore, maize may theoretically be a good trap crop against the sunflower broomrape infested field in China. Our previous work revealed the extracts and exudates of some hybrid maize lines could induce sunflower broomrape germination in the laboratory [16]. In this study, we continued our previous study and mainly focused on: (1) screening the sunflower broomrape germination stimulating the ability of commercial maize cultivars under co-culture conditions; (2) the effectiveness of maize rotation on broomrape control, and (3) how the rotation years affect broomrape control effectiveness. The results of this study will be of value to develop further utilization of maize as trap crops in an integrated broomrape management approach.

2. Materials and Methods

2.1. Experiment 1. Maize-Broomrape Coculture Experiment

2.1.1. Plant Materials and Chemicals

Seeds of 15 different maize cultivars, Neidan 314 (N314), Fengtian 6 (F6), Zhengdan 958 (Z958), Yayu 318 (Y318), 32D22, Dongyue 116 (D116), Zeyu 19 (Z19), Lianshneg 1 (L1), Haoyu 19 (H19), Cangyu 18 (C18), Xinyu 12 (X12), Jinong 107 (J107), Qingzhu 67 (Q67), Yuhe 988 (Y988), and Zhongbeiheng 6 (Z6) were purchased from Sanqing Seed Company in Yangling, Shaanxi Province, China. These cultivars adapted well to the climate and soil nutrients and were popular in Northwest China. Seeds of sunflower broomrape were collected from infested sunflower fields in Xinjiang Uygur Autonomous Region of China in 2012. GR24, a germination inducing stimulant and a synthetic analog of strigol, was kindly provided by Professor Binne Zwanenburg (the University of Nijmegen, The Netherlands).

2.1.2. Experiment Design

In Experiment 1, sunflower broomrape was co-cultured with seedlings of various maize cultivars in a cell culture plate. The maize seeds were sterilized with 75% ethanol for 1 min, rinsed three times with sterile deionized water, further sterilized with 10% NaOCl for 3 min, and again rinsed three times. Prior to use, all sunflower broomrape seeds were disinfected firstly with 1% (W/V) NaOCl solution for 3 min and then with 75% (V/V) ethanol for 3 min [24]. Then, the seeds were thoroughly rinsed with distilled water three times and air-dried. Approximately 150–300 sunflower broomrape seeds were sown onto 20 mm glass fiber filter disks (Whatman GF/A). The disks with sunflower broomrape seeds were carefully transferred in each well of 12-well cell culture plate. Sterilized vermiculite (0.5 g) was added to each well and one maize seed was planted on vermiculite. 3 mL distilled water was added to each well to keep it moist. The same treatment without maize seeds were used as the negative control, and seed disks were treated with sterilized vermiculite and 3 mL GR24 (1 mg L^{-1}, synthetic analog of strigol) and then served as the positive control. All culture plates were then placed in the incubator at a constant temperature of 25 °C. Distilled water was added to each well every other day to maintain moisture. Germination rates of sunflower broomrape were scored at 15 d after maize seeds germinated. A completely randomized design was utilized (seven replications of 15 maize cultivars) and the experiment was done twice. The data were combined for analyses. The experimental system is illustrated in Figure S1.

2.2. Experiment 2. Maize Rotation Experiment

2.2.1. Plant and Soil Materials

The pot experiments were conducted in Guyuan Ecological Station of the Institute of Soil and Water Conservation, Chinese Academy of Sciences located in Hechuan Village (35°99′ N, 106°44′ E), Guyuan City, Ningxia Hui Autonomous Region of China. The elevation of the site is 1676 m, and mean annual temperature and precipitation are 6.2 °C and 438 mm, respectively. In-season air temperature measured on-site by a HOBO weather station (Onset, Bourne, MA, USA) during this time period is shown in Figure S2. Based on Experiment 1, two cultivars with high allelopathic effect (N314 and H19) and two cultivars with low allelopathic effect (Q67 and Z6) were selected in this experiment. The susceptible host to sunflower broomrape, a commercial hybrid cultivar of sunflower (T33), was provided by Associate Professor Enshi Xiao, Northwest A&F University, China. The test soil without any sunflower broomrape seeds was collected from a cultivated field near the research institute, is common dark loessial soil and the soil properties were as the following: pH of 7.41, a soil organic matter of 9.41 g kg^{-1}, a nitrogen total of 280 mg kg^{-1}, available phosphorus (p) of 2.28 mg kg^{-1}, and available potassium (K) of 206 mg kg^{-1}. The sand, brought from Guyuan, was sieved medium-sized natural river sand. The soil used in this experiment was thoroughly mixed with sand at a 1:1 ratio.

2.2.2. Experiment Design

The experiment was a randomized complete block design arrangement with five replications of three or two plants of maize or sunflower, respectively. The treatments consisted of different crop rotation cycles: maize (trap crop)-sunflower (host), maize-maize-sunflower, maize-maize-maize-sunflower and were conducted from 2012 to 2015. Plants were grown in 7.8-L plastic pots containing 8 kg of dry soil mix that was artificially infested with 32 mg of sunflower broomrape seeds (about 8000 seeds per pot). The same treatments without maize were used as the negative control (NC). Pots without sunflower broomrape were used as the positive control (PC). All pots were placed in a sunny outdoor area and the plants were watered every other day. All crops were planted without fertilizer. Five seeds were sown into each pot. Five days after maize/sunflower emergence, the maize seedlings were thinned to three per pot and the sunflower seedlings were thinned to two per pot. The pots were kept weed-free throughout the growing season by hand weeding except for broomrape. The experimental details about the rotation system are shown in Table 1. At the end of the sunflower rotation in each cycle, sunflower roots were washed and separated from the attached broomrape. Broomrape emergence and attachment were quantified. The shoot, root and head mass of sunflowers from harvest times were determined after drying for 48 h at 70 °C.

Table 1. Cropping rotation system management of Experiment 2.

Year	Artificially Infested with Broomrape Seeds						Sowing Date	Harvest Date
2012	No crop	No crop	No crop	Maize	Maize	Maize	5 May	14 September
2013	Sunflower	No crop	No crop	Sunflower	Maize	Maize	24 April	29 September
2014		Sunflower	No crop		Sunflower	Maize	10 May	26 September
2015			Sunflower			Sunflower	1 May	2 October
	Without Broomrape Seeds							
2012	No crop	No crop	No crop				5 May	14 September
2013	Sunflower	No crop	No crop				24 April	29 September
2014		Sunflower	No crop				10 May	26 September
2015			Sunflower				1 May	2 October

2.2.3. Statistical Analyses

Data processing was done with excel 2016 and SPSS (IBM SPSS Statistics v20). The germination data of sunflower broomrape in co-culture experiment were arcsin transformed before analysis and proportions of 0/n were replaced with 1/4n to improve the arcsine transformation [25]. The back-transformed data are presented in figures. Two-way ANOVA was conducted to test the effects of maize cultivars and planting years on broomrape infection and sunflower growth. Tukey's honest significant difference (α = 0.05, Tukey's Honestly Significant Difference (HSD) test) was used to compare the means. The correlation analysis was performed to determine possible relationships between the sunflower and broomrape traits using Pearson's correlation analysis.

3. Results

3.1. Germination Responses of Sunflower Broomrape to Various Maize Cultivars

The broomrape seeds germinated at a high rate (89.5%) after treating with GR24, whereas none of them germinated when treated with sterilized vermiculite just wetted with water. These data indicated that distilled water did not induce sunflower broomrape germination and that interference of sterilized vermiculite was eliminated. All the seedlings of commercial maize cultivars co-cultured with broomrape induced germination, but the subsequent attachment of broomrape on their roots did not occur (Figure 1), which demonstrates that maize is a non-host for sunflower broomrape. The differences among 15 maize cultivars were significant ($p < 0.05$) and sunflower broomrape germination due to maize ranged from 15% to 40%. The most active maize cultivars were N314, which induced 41.0% broomrape germination, followed by F6 (37.0%) and Z958 (37.8%). Three maize cultivars (Q67, Y988, Z6) induced significantly lower germination of broomrape seeds (<20%) compared with other cultivars.

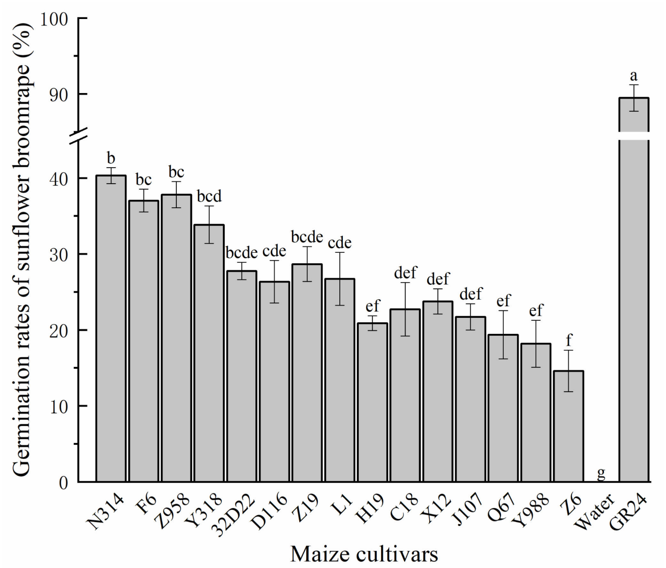

Figure 1. Germination of sunflower broomrape seeds co-cultured with maize seedlings. Figure plots are means ± standard error (SE) from seven replicates. Different letters indicate significant differences between maize cultivars at $p < 0.05$ (Tukey's HSD test).

3.2. Broomrape Infection Levels after Rotation with Maize

In a 4-year rotation experiment, no broomrape infection on any maize roots was observed (data not shown). Sunflower without rotation (NC) exhibited a significantly higher number of broomrapes attachment and emergence than that of rotation system. Broomrape emergence and attachment on sunflower differed with years ($p < 0.05$). The number of attached broomrapes per sunflower was 12.6, 19.4, and 23.2 for NC from 2013 to 2015, respectively (Figure 2). After 1-year rotation, a large reduction was found in the number of emerged and attached broomrapes in N314 rotation system, which was 68% and 76% of NC on broomrape emergence and attachment, respectively (Figure. 2). After 2-year and 3-year maize rotation, all tested cultivars greatly reduced the emergence and attachment of sunflower broomrape relative to NC. N314 Rotation had the best performance, with a reduction in broomrape attachment by 90.7% and emergence by 96.5% of NC. In contrast with the results of Experiment 1, neither the number of attached nodules nor the number of emerged stems of *O. cumana* depended on the maize cultivars.

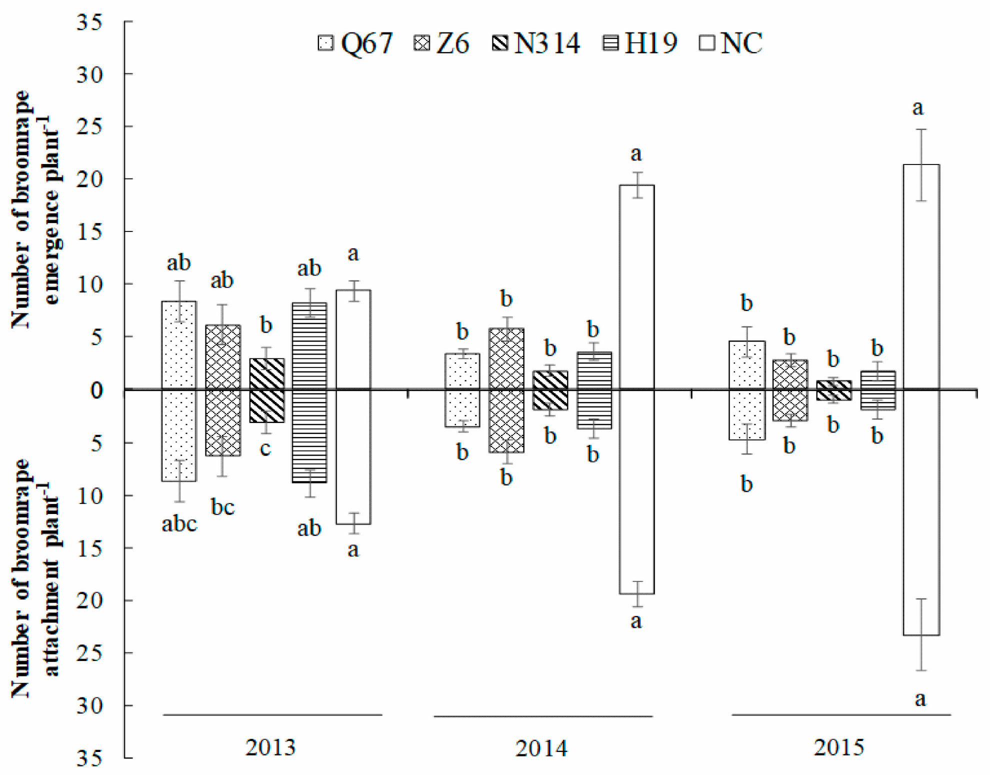

Figure 2. Numbers of broomrape attachments and emerged broomrape shoots in sunflower grown after four different cultivars of maize. Different letters indicate significant differences between maize cultivars at $p < 0.05$ (Tukey's HSD test).

3.3. Sunflower Performance after Rotation with Maize

The growth of sunflower was significantly different among various rotation systems and planting years (Table 2). Sunflower without rotation (NC) had the lowest biomass in contrast to other treatments. The inhibitory effect of broomrape infection was most pronounced in dry weight of the sunflower head. The head dry weight of infected sunflower (NC), which ranged from 0.46 to 1.48 g plant^{-1} and were significantly lower than those of PC (7.78 to 10.70 g plant^{-1}). Sunflower grown after maize significantly produced higher head dry weight than that of NC that the average head weight was 3.9-, 8.3-, and 3.3-fold increase compared with NC after 1-, 2-, and 3-year rotation with maize, respectively (Figure 3). Crop rotation significantly affected sunflower seedling height only in 2015. The highest sunflower seedling height was obtained for sunflower grown after H19 (128.9 cm) and followed by the seedlings grown after N314 (112.9 cm) (Table 3). Broomrape infection dramatically reduced shoot dry weight of sunflower as there were 4.4 times of average shoot dry weight in parasite-free control (PC, 29.9 g plant^{-1}, averaged across years) compared with the infected sunflower (NC, 6.8 g plant^{-1}, averaged across years) (Table 3). All rotations improved maize shoot dry weight, since the average shoot weight of sunflower grown following maize (22.86 g plant^{-1}) was significantly higher than that of NC. A 3-year rotation of N314 had shown the best potential of reducing broomrape damage since negligible differences between N314 (28.7 g plant^{-1}) and PC (29.5 g plant^{-1}) were observed. However, there was no significant difference in sunflower root dry mass among all treatments. Only heavily broomrape infection in 2015 resulted in a significantly lower root dry mass (2.0 g plant^{-1}, NC) in contrast to other treatments (3.3 g plant^{-1}, averaged across the four cultivars). The root dry mass was not significantly affected by maize cultivars. Table 4 shows a significantly negative correlation (r = −0.638) between broomrape attachment and sunflower shoot dry mass. The same condition was also observed in sunflower head dry mass which means by decreasing the amount of broomrape attachment, the aboveground dry mass of sunflower increases.

Table 2. ANOVA results of the effects of previous crop and planting years on broomrape infection levels and sunflower growth.

Source	*df*	*F*-Value		*df*	*F*-Value			
		No. of Emerged Sunflower Broomrape	No. of Attached Sunflower Broomrape		Seedling Height	Shoot Dry Weight	Root Dry Weight	Sunflower Head Dry Weight
Previous crop	4	49.52 **	13.11 **	5	1.35	21.18 **	2.77 *	10.46 **
Planting years	2	0.37	6.76 **	2	4.19 *	2.95	1.73	5.07 **
Interaction effect	8	7.79 **	4.01 **	10	1.04	0.66	0.59	1.58

Degree of freedom (df) and F-values from ANOVA are given in the table. * $p < 0.05$, ** $p < 0.01$.

Table 3. Effect of maize rotation on sunflower seedling height and biomass grown under broomrape infection.

Maize Cultivars	Seedling Height (cm)			Shoot Dry Weight (g plant^{-1})			Root Dry Weight (g plant^{-1})		
	2013	2014	2015	2013	2014	2015	2013	2014	2015
Q67	80.2 ± 4.9 a	78.6 ± 1.0 a	104.8 ± 12.1 ab	21.2 ± 3.1 a	20.6 ± 1.6 ab	19.5 ± 0.9 b	2.8 ± 0.4 a	3.0 ± 0.4 a	3.5 ± 0.2 a
Z6	78.8 ± 2.3 a	76.9 ± 5.2 a	98.2 ± 11.3 ab	20.4 ± 5.0 a	20.1 ± 2.6 ab	20.3 ± 1.2 b	2.7 ± 0.3 a	2.3 ± 0.4 a	2.9 ± 0.1 ab
N314	86.7 ± 2.4 a	78.9 ± 5.3 a	112.8 ± 28.3 ab	24.0 ± 3.1 a	21.4 ± 1.3 ab	28.7 ± 1.2 a	3.0 ± 0.6 a	2.8 ± 0.4 a	3.6 ± 0.2 a
H19	75.2 ± 7.6 a	64.0 ± 4.2 a	128.8 ± 47.3 a	21.6 ± 1.2 a	17.4 ± 2.1 b	27.3 ± 0.5 a	2.9 ± 0.3 a	2.6 ± 0.3 a	3.0 ± 0.5 ab
NC	77.4 ± 11.5 a	61.8 ± 6.4 a	56.6 ± 5.5 b	5.3 ± 0.6 b	5.1 ± 0.2 c	9.9 ± 0.5 c	2.4 ± 0.2 a	2.5 ± 0.1 a	2.0 ± 0.2 b
PC	81.2 ± 5.8 a	81.6 ± 7.9 a	81.6 ± 6.1 ab	29.3 ± 6.5 a	31.0 ± 2.7 a	29.5 ± 1.6 a	2.9 ± 0.4 a	3.0 ± 0.3 a	3.4 ± 0.4 ab

Only the means in the same column were compared. The means marked with different letters differed significantly at the 95% confidence level, according to a one-way ANOVA. Data represent the mean ± SE ($n = 5$).

Table 4. Correlation analysis between sunflower and broomrape traits.

Pearson Correlation	Seedling Height	Shoot Dry Mass	Root Dry Mass	Head Dry Mass	No. of Emergent Broomrape
Shoot dry mass	0.197				
Root dry mass	0.293 **	0.215 *			
Head dry mass	0.232 *	0.795 **	0.219 *		
No. of Emergent broomrape	−0.271 **	−0.616 **	−0.374 **	−0.620 **	
No. of Attached broomrape	−0.266 *	−0.638 **	−0.377 **	−0.633 **	0.990 **

Significant level set at 0.05, * $p < 0.05$, ** $p < 0.01$.

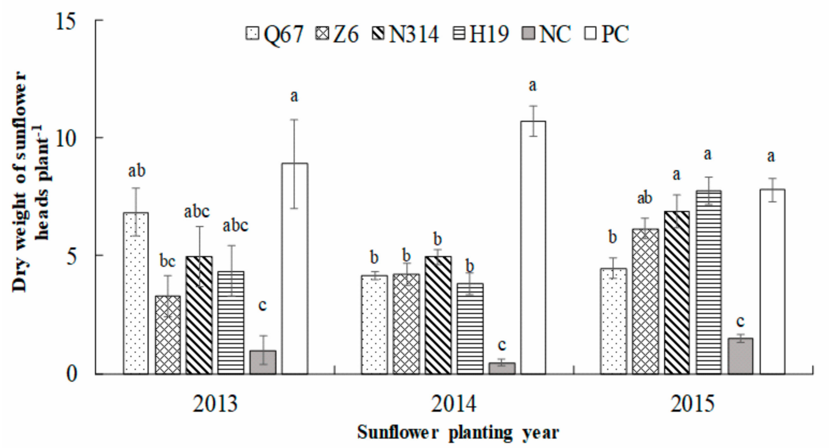

Figure 3. Sunflower head dry weight as affected by maize rotation. Different letters indicate significant differences between maize cultivars at $p < 0.05$ (Tukey's HSD test).

4. Discussion

Generally, crop rotation was effective in integrated weed management, which changed features of the field including resources competition and soil disturbance and resulted in an unstable environment for weeds [26]. Considering the complex life cycle of broomrape, effective broomrape control should target the underground mechanisms of crop parasitism, especially the germination phase [14,27]. Thus, rotations with trap crops that stimulate suicidal germination of parasite seeds are frequently described as a cultural method for broomrape control and play a major role in integrated control or eradication of these malignant weeds [28,29]. In the present work, all tested maize cultivars promoted sunflower broomrape germination but were not parasitized. By 3-year rotation with maize, the number of attached and emerged sunflower broomrape was generally decreased and biomass of sunflower greatly increased, which could get support from Ma et al. [16] who showed that maize could be used as trap crops for the control of this devastating root parasitic weed.

Different plant species or cultivars have been reported to be significantly different in their germination stimulatory activity towards broomrape [30,31]. Differences between the efficiency of trap crops were expected since differing in taxonomy, physiology, but mainly the ability to produce germination stimulants [32,33]. In the maize-sunflower rotation experiment, only sunflower grown after N314 showed the lowest number of broomrape attachments. All 15 maize cultivars induced germination of sunflower broomrape in the co-culture experiment, indicating that maize produces germination stimulants at the early development stage. Even 5-day-old maize seedlings have produced three allelochemicals which may affect the growth or germination of other plant species [34]. Similar results have been reported for wheat (*Triticum aestivum* L.) that the maximum broomrape germination inducing capacity peaked at the wheat seedling stage [15]. Considering the high variability among species or cultivars in stimulating parasitic seeds germination, it is desirable to develop a simple and accurate method to evaluate the broomrape seed-germination activity of hosts or nonhosts. Several means of evaluating are available, including the use of excised fresh pieces of plant roots and stems [35], root exudates [36], extracts of rhizosphere soil [37], and "cut-root assay" described by Berner et al. [38]. In the present work, we developed an easy and fast method for measuring broomrape seed germination activity based on a crop-broomrape co-culture system. The important feature of this co-culture system is that additional preconditioning of broomrape seeds was not needed as other methods [39,40]. The conditioning of broomrape seeds was synchronized with the germination and emergence of trap crop that was close to reality. In addition, the results of co-culture assay gave a good correlation ($p < 0.01$) with the results of root exudates, extracts of rhizosphere soil, organ exudates, and xylem sap (detailed germination data tested by other methods presented by Ye [41], and the correlation analysis were shown in Table S1). It is therefore recommended that this co-culture assay is adapted to high-throughput evaluating of broomrape seed germination inducing capacity of hosts or nonhosts.

The use of trap crops is generally considered to be of limited efficacy due to a large number of seeds present in infested agricultural soils, and the sustained cultivation of trap crops may not be economically feasible [42]. The use of cereals could be a solution, due to the high frequency with which they are cultivated and their importance for human consumption. Our results were in concordance with previous reports that maize stimulated germination of sunflower broomrape but without offering it any chance for further growth and development of the parasite in the host crop. Relative to sunflower without previous crop, there was a significantly lower parasite infection on sunflower grown after maize. The results further confirmed that the maize was a true trap since it was not parasitized and greatly reduced parasite infection on the followed host crop [43]. The differences obtained in sunflower broomrape infection among various maize cultivars may be related to the cultivars' germination inducing capacity in Experiment 1 (Figures 2 and 3). In addition, the differences among cultivars were decreased with the years of maize cultivated prolonged. Even after a 3-year rotation with N314, the infection of sunflower broomrape remained. This was similar to the previous results of Al-thahabi [43], who observed that after 4 years of continuous cropping with wheat, small broomrape (*O. minor* Sm.) attachment still remained.

Generally uninfected sunflower plants had a significantly higher aboveground biomass than those infected by sunflower broomrape. Our results further confirmed that broomrape parasitizment significantly increased the shoot/root ratio of infected sunflowers, which could get supports from other parasitize-host systems, such as *Orobanche minor* and red clover [44]; *Striga hermonthica* and sorghum [45]; *S. hermonthica* and maize [46]. All these indicated that more dry matter was partitioned to root in the infected host than in controls. However, in *Mikania micrantha* infected by *Cuscuta campestris*, the infected seedlings increased resource allocation to the above-ground parts and decreased allocation to roots. The differences in resource allocation strategy in the host may be due to the different types of parasitism (stem parasite or root parasite) [47]. The dry mass of sunflower head and shoot were highly related to infection severity (Table 4), which could get support from Fernández-Aparicio [48], who proposed an exponential model to explain the relationship between aboveground dry matter reduction of faba bean and infection severity of *O. crenata*. The reduction of the host growth caused by the diversion of water and nutrients to the parasite is widely reported for broomrapes [49] and, particularly, for *O. cumana* [50]. Surviving infected plants have smaller capitula, as well as low number and small seeds; plant death is also very frequent [51]. Moreover, sunflower growth reduction when parasitism is visible aboveground, is irreversibly caused during underground parasite stages due to a probable decreased photosynthesis [52] as well as to metabolic imbalance and water flow impairment [53]. Our results suggest that damage in sunflower is directly attributed to broomrape infection.

5. Conclusions

The tested maize cultivars promoted sunflower broomrape germination but were not parasitized by broomrape. Maize rotation significantly suppressed sunflower broomrape infection in subsequent sunflower and improved sunflower growth. Accordingly, maize might be used as trap crops to effectively and commercially reduce sunflower broomrape damage and lead to an integrated and biologically-based strategy for broomrape control. Further research should be done in sunflower broomrape infested fields to confirm the efficacy of using maize as a trap crop under field conditions.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/1/100/s1, Figure S1: Diagram of the maize broomrape co-culture assay procedure. Figure S2: Meteorological data of experimental site during trails obtained from a HOBO weather station (Onset, Bourne, MA, USA). The bars represent maximum and minimum temperature (in °C) whilst the line diagram represents monthly average temperature (in °C), Table S1: Correlation coefficients between sunflower broomrape germination of various maize cultivars in different experiments.

Author Contributions: Conceptualization, X.Y. and Y.M.; methodology, X.Y.; data curation, X.Y. and M.Z. (Meng Zhang); writing—original draft preparation, X.Y. and M.Z. (Manyun Zhang); writing—review and editing, X.Y. and Y.M.; funding acquisition, X.Y. and Y.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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