Root parasitic plants belonging to the genus *Orobanche* (broomrapes) lack chlorophyll and depend totally on their host plants to provide water, assimilates, and inorganic nutrients. *Orobanche* spp. cause dramatic damage to vegetable and field crops in Asia, Africa, and southern and eastern Europe (Foy et al., 1989; Parker and Riches, 1993; Joel et al., 2007). *Orobanche cumana* Wallr. parasitizes sunflower (*Helianthus annuus* L.), one of the main oil crops in China. Sunflowers were grown on about 839,000 ha in China in 2004 (Li et al., 2006). In Dingbian County of Shaanxi Province, at present sunflower plantation covers around 10,000 ha, and 64% of the field is parasitized (Chen, 2010). Elsewhere in China the heaviest infestations are in Hebei, Xinjiang, Shanxi, Inner Mongolia, Heilongjiang, Liaoning, and Jilin provinces. Infestation by *O. cumana* can reduce sunflower yield by as much as 50% (Wang and Zhu, 1981; Song and Yang, 1991; Wang et al., 2003; Chen, 2010; Wang, 2010).

To germinate, *Orobanche* spp. seeds must be exposed to certain environmental conditions (i.e., preconditioning) as well as a chemical germination stimulant (Parker and Riches, 1993). Thirteen germination stimulants for *Orobanche* spp. have been identified from root exudates of host and nonhost plants. These stimulants (e.g., strigol, strigol acetate, sorgolactone, alectrol, and orobanchol) all belong to a chemical class referred to as strigolactones.
(Joel et al., 1995; Yokota et al., 1998; Yoder, 2001; Bouwmeester et al., 2003; Awad et al., 2006). Synthetic strigol analogs (e.g., GR5, GR7, and GR24) have also been synthesized and among these, GR24 has the highest activity (Bergmann et al., 1993).

Several means, including chemical, biological, and cultural methods, have been used for Orobanche spp. control (Foy et al., 1989; Parker and Riches, 1993). Rotation with “trap crops” (i.e., plants that stimulate germination of parasite seeds but do not allow normal attachment and parasite development) can also be used to decrease the Orobanche spp. seed bank in soil (Foy et al., 1989; Parker and Riches, 1993). However, the control of Orobanche spp. is difficult and only partially effective due to the complex relationship between parasite and host. No single control method is both effective and economically feasible for small scale farms.

Maize is a nonhost plant for at least two Orobanche species, O. ramosa L. and O. minor Sm. (Sunderland, 1960a, 1960b; Zehhar et al., 2003). However, there are no reports about the ability of maize to induce O. cumana germination. The objective of the experiment was to screen four maize hybrids and their parental lines for their ability to induce O. cumana germination. Through a cut-root assay and pot and field experiments, some inbred and hybrid lines that induced higher germination rates of O. cumana could be identified. The ultimate aim is to develop a maize line that could be used as a trap crop for the control of O. cumana.

MATERIALS AND METHODS
Preparation of Seeds and Chemicals

Seeds from four maize hybrids and their parental lines were supplied by Professor Jianchang Mao in 2009 (College of Agricultural Science, Northwest A & F University, China) (Table 1). These four hybrid lines were derived through breeding by crossing different inbred lines. Of these inbred lines, 3026 was selected by cultivation of selected plants of Ye478 × 89-1; 335 belongs to the Lvda Red Cob Group germplasm in China. Xun20 and 3255 were selected from inbreeding progenies of non-Chinese hybrids, but the source is unknown. 335 belongs to the Tangsipingtou Group germplasm in China. Xun20 and 3255 were selected from inbreeding progenies of non-Chinese hybrids, but the source is unknown. 335 belongs to the Lvda Red Cob Group germplasm in China. 5212 belongs to the Tangsipingtou Group germplasm in China. Xun20 and 3255 were selected from inbreeding progenies of non-Chinese hybrids, but the source is unknown. 335 belongs to the Lvda Red Cob Group germplasm in China. 5212 belongs to the Tangsipingtou Group germplasm in China. Xun20 and 3255 were selected from inbreeding progenies of non-Chinese hybrids, but the source is unknown. 335 belongs to the Lvda Red Cob Group germplasm in China.

<table>
<thead>
<tr>
<th>Female parent</th>
<th>Male parent</th>
<th>F1 generation</th>
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<tbody>
<tr>
<td>Xun20 × 5212</td>
<td>Xun20 × 5212</td>
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<tr>
<td>Zheng58 × 340X</td>
<td>Zheng58 × 340X</td>
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<tr>
<td>Xun20 × 3255</td>
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Table 1. The maize hybrids and their parental lines that were used in this study.

Experiment 1: Cut Root Assay

The cut-root assay method was used to determine the effect of maize roots on O. cumana germination (Van Mele et al., 1992; Botanga et al., 2003). Briefly, surface-sterilized maize seeds (10 seeds) of each hybrid or parental line were placed in sterile petri dishes lined with moistened filter paper. The petri dishes were wrapped in aluminum foil and incubated at 25°C for 96 h. The seedlings were removed from the petri dishes and the seedling roots were cut into 0.5-cm segments (~0.5 g). The segments were put in the center of petri dishes lined with filter paper (one segment per dish). Orobanche cumana seeds (20–50 seeds) were arranged in three concentric circles around each root segment. These circles were 1, 2, and 3 cm from the root segment and will be referred to as the inner, middle, and outer circles, respectively. A piece of aluminum foil encircled the sides of each root segment so that there was no direct contact between the root segment and the O. cumana seeds. Sterile distilled water (1.5 mL) was slowly poured over the top of the root segment. The water ran down the segment and then spread out evenly across the bottom of the petri dish. The petri dishes were sealed with parafilm, wrapped in aluminum foil, and incubated at 25°C for 10 d. Each treatment was replicated three times. The experiments were repeated twice and the results were consistent, so one set of data is used in this paper.

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 synthetic strigolactone analog GR24 is the standard germination agent for O. cumana. Three GR24-treated disks (20 μL of 0.1 mg GR24 L1 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.

Experiment 2: Root Exudate Assay

After the seedlings were removed from the petri dishes in Exp. 1, 16 glass fiber filter paper disks (7-mm diam.) with 20 to 50 O. cumana seeds per disk were put in the area where the maize roots had grown. The petri dishes were sealed with parafilm, wrapped in aluminum foil, and incubated at 25°C for 10 d. Germination rates were determined microscopically as described above.

Experiment 3: Pot Experiment

A pot experiment was conducted at the Institute of Soil and Water Conservation, Yangling, Shaanxi Province, China, in April 2009 and April 2010. Eight kilograms soil was put into plastic pots (25 cm high by 20 cm diam.). The soil, which was collected from a cultivated field near the research institute, is classified as silty loam (Eum-Orthic Anthrosol). The soil tests gave the following mean values: soil pH was 7.98, organic matter content was 13.97 g kg1, NO3–N was 48.3 mg kg1, Olsen
RESULTS

The germination stimulant GR24 induced *O. cumana* germination rates of 60 to 90%. Distilled water induced no significant germination. So the *O. cumana* seeds were viable in our studies.

**Experiment 1: Cut-Root Assay**

Germination rates of *O. cumana* ranged from 0 to 30% (Fig. 1). Germination rate generally increased as the distance between the *O. cumana* seed and the maize root segment increased. Among the hybrids, 3255 × 335 generally induced the highest germination rates (19.2 to 30.1%) followed by Zheng58 × 5212 (17.6 to 26.5%). Among the inbred lines, 5212 generally induced the highest germination rates (4.6 to 22.6%) followed by 3255 (4.8 to 21.6%). The hybrids 3255 × 335 and Zheng58 × 5212 induced significantly higher germination rates than their parental lines. The hybrid Xun20 × 5212 induced germination rates that were intermediate between its parental lines. There was no significant difference among germination rates induced by the hybrid 3026 × 340X and its parental lines.

**Experiment 2: Root Exudate Assay**

Root exudates of all hybrids and parental lines induced *O. cumana* germination, although the hybrid 3026 × 340X induced germination rates <6%. Among the hybrids, 3255 × 335 induced the highest germination rate (36.5%) followed by Zheng58 × 5212 (25.7%) (Fig. 2). Among the inbred lines, 3255 and 335 induced the highest germination rates (25.7% to 36.5%) followed by 3026 × 340X (19.2% to 26.5%). The hybrids 3255 × 335 and Zheng58 × 5212 induced significantly higher germination rates than their parental lines. The hybrid Xun20 × 5212 induced germination rates that were intermediate between its parental lines. There was no significant difference among germination rates induced by the hybrid 3026 × 340X and its parental lines.

**Statistical Analyses**

The SPSS 10.0 software (SPSS Institute, 1999) was used to perform one-way analysis of variance. Treatment means were compared using least significant difference tests at the 5% level of probability.
rates (35.3 and 30.9%) followed by Xun20 (30.2%) and 5212 (26.4%). The hybrids 3255 × 335 and Zheng58 × 5212 generally induced higher germination rates than their parental lines but the differences were not significant. In contrast, the germination rates induced by the hybrid Xun20 × 5212 were significantly lower than those induced by its parental lines. The hybrid 3026 × 340X and its parental lines induced low germination rates (<15%) (Fig. 2).

Experiment 3: Pot Experiment
Rhizosphere soil of all three hybrids and their parental lines induced *O. cumana* germination; however, for the inbred line 5212, only rhizosphere soil collected at the four-leaf stage induced germination (Fig. 3). Rhizosphere soil from hybrid 3255 × 335 induced the highest germination rates (17.8 to 22.3%) and these rates were significantly higher than those induced by rhizosphere soil from its parental lines. Rhizosphere soil from the hybrid Zheng58 × 5212 also induced germination rates that were higher than those induced by its parental lines; one of its parents (5212) induced negligible germination. The hybrid Xun20 × 5212 induced low germination rates (<15%) (Fig. 2).

Extracts of rhizosphere soil also induced significant *O. cumana* germination (Fig. 4A, 4B, 4C, and 4D). Aqueous extracts of rhizosphere soil collected at the four-leaf stage induced *O. cumana* germination whereas aqueous extracts of rhizosphere soil collected at the six- and eight-leaf stages did not induce germination (Fig. 4A). The 10-fold dilutions of the aqueous extracts generally induced the highest germination rates. Methanol extracts of rhizosphere soil collected at all three growth stages induced *O. cumana* germination (Fig. 4B, 4C, and 4D). The undiluted methanol extracts induced the highest germination rates. At the four- and six-leaf stage, the inbred line Xun20 induced the highest germination rates. At the eight-leaf stage, the hybrids lines 3255 × 335 and Zheng58 × 5212 induced the highest germination rates. The germination rates induced by both hybrids were higher than those induced by their parental lines.

Extracts of maize roots collected at all three growth stages induced *O. cumana* germination (Fig. 5). The number of lines inducing germination generally increased as the plants grew older. Ten-fold dilutions of the root extracts generally induced higher germination rates than the 100-fold dilutions, although this was not true for the methanol extracts of roots collected at the six-leaf stage (Fig. 5E). The hybrid 3255 × 335 and its parental lines generally induced the highest germination rates among the lines in this study.

Extracts of shoots collected at the eight-leaf stage induced *O. cumana* germination whereas extracts of shoots collected at the four- and six-leaf stages did not (Fig. 6). For the aqueous shoot extracts, the hybrid 3255 × 335 induced the highest germination rate and this rate was significantly higher than those induced by its parental lines (Fig. 6A). For the methanol shoot extracts, the inbred line...
335 induced the highest germination rate. There were no significant differences in germination rates induced by the hybrid 3255 × 335 and its parental lines (Fig. 6B).

Furthermore, correlation analysis was performed to attempt to find the relationship between different dilutions among the genotypes at different growth stages. There were only significantly positive correlations among undiluted extracts with 10-fold dilution ($r = 0.744$, $P < 0.05$) (Fig. 7A) and undiluted extracts with 100-fold dilution ($r = 0.621$, $P < 0.05$) (Fig. 7B) in case of distilled water extracts of rhizosphere soil from three maize hybrids and their parental lines at the four-leaf stage whereas there were not statistically significant correlations existed among other treatments at the six- and eight-leaf stages.

**Experiment 4: Field Experiment**

Results from Exp. 1, 2, and 3 indicated that 3255 × 335 generally induced the highest $O. \text{cumana}$ germination rates among the maize lines in this study. Therefore, this hybrid and its parental lines were used in a field experiment.

Rhizosphere soil collected at all three growth stages induced $O. \text{cumana}$ germination (Fig. 8). The germination rates ranged between 7.0 and 34.9%. The hybrid 3255 × 335 induced higher germination rates than its parental lines. Aqueous extracts of rhizosphere soil collected at the four-leaf stage induced germination rates of 7.2 to 24.7% whereas extracts from rhizosphere soil collected at the six- and eight-leaf stages induced negligible germination (Fig. 9A). Methanol extracts of rhizosphere soil collected at all three growth stages induced $O. \text{cumana}$ germination, with germination rates ranging between 3.5 and 36.7% (Fig. 9B).
For both aqueous and methanol extracts, the germination rates induced by the hybrid 3255 × 335 were higher than those induced by its parental lines. Maize root extracts generally induced *O. cumana* germination; however, the ability of maize root extracts to induce germination tended to decrease as the plants matured (Fig. 10). The methanol extracts generally induced higher germination rates than the aqueous extracts. At the four-leaf stage, root extracts from the inbred line 335 induced higher germination rates than root extracts from the hybrid 3255 × 335. At the eight-leaf stage, the hybrid line induced higher germination rates than either of its parents. Extracts of shoots collected at the eight-leaf stage induced *O. cumana* germination whereas extracts of shoots collected at the four- and six-leaf stages did not (Fig. 11A and 11B). At the eight-leaf stage, methanol extracts of the hybrid 3255 × 335 induced higher germination rates than either of its parents. Comparison among the aqueous

Figure 5. Effect of root extracts from three maize hybrids and their parental lines on *Orobanche cumana* germination in the pot experiment. (A), (B), and (C) Aqueous extracts from maize roots collected at the four-, six-, and eight-leaf stages, respectively. (D), (E), and (F) Methanol extracts from maize roots collected at the four-, six-, and eight-stages, respectively. The significant differences at 5% (LSD, $P < 0.05$) are marked by the small letters, respectively.
extracts indicated no significant difference between the hybrid and its parental lines.

Meanwhile, correlation analysis was also performed to study the relationship between different dilutions among the genotypes at different growth stages. But no statistically significant correlations existed between any treatments among the genotypes.

**DISCUSSION**

Results from these four experiments indicated that maize can induce *O. cumana* germination. Joel et al. (2007) observed that a sesquiterpene lactone produced by sunflower induced *O. cumana* germination; however, there are no reports indicating that maize produces sesquiterpene lactone. At least some maize lines produce strigol, a potent germination stimulant for *Orobanche* spp. (Siame et al.,
This explains why maize induces the germination not only of *O. cumana* but also *O. minor* and *O. ramosa* (Sunderland, 1960a, 1960b; Zehhar et al., 2003). We observed that root extracts generally induced higher germination rates than shoot extracts in both pot and field experiments. Kohlen et al. (2011) reported that strigolactones were mainly synthesized in roots and transported to shoots (Kohlen et al., 2011). We also found that methanol extracts generally induced higher germination rates than distilled water extracts. This is consistent with our predictions, because it was reported that maize mainly produces strigolactones, which are not water soluble chemicals when purified (Siame et al., 1993). Furthermore, through correlation analysis between different dilutions among the genotypes at different growth stages, we didn’t find any consistent relationships between them. Only at the four-leaf stage in the pot experiment were there positive correlations among genotypes between different dilutions in the case of distilled water extracts of rhizosphere soil. This showed that the stimulants in water extracts of rhizosphere soil were stable and high enough to induce *O. cumana* to germinate although they were further diluted. By contrast, this correlation didn’t exist among other treatments at different growth stages in both pot and field experiments. This phenomenon is consistent with the observations of others who have been working on controlling for *Orobanche* spp., have found that *Orobanche* spp. seed could germinate within a certain concentration range, and have described a “hormesis effect” (Jin et al., 2008; Dong et al., 2009; Lang et al., 2011). So in our study, the *O. cumana* seed germinated at certain concentrations, and between the different concentrations there generally didn’t exist correlation relationships.

The ability to induce germination varied significantly among maize hybrid and inbred lines. The hybrid 3255 × 335 and both of its parents generally induced the highest *O. cumana* germination rates. It is important to note that the ability to induce germination of *O. cumana* had not been

Figure 9. Effect of rhizosphere soil extracts from one maize hybrid and its parental lines on *Orobanche cumana* germination in the field experiment. (A) Aqueous extracts of rhizosphere soil collected at the four-leaf stage. (B), (C), and (D) Methanol extracts of rhizosphere soil collected at the four-, six- and eight-leaf stages, respectively. The significant differences at 5% (LSD, *P* < 0.05) are marked by the small letters, respectively.
a selection criterion in the breeding program from which these maize lines were derived. The genetic mechanism for the production of allelochemicals that induce O. cumana germination needs to be analyzed. Botanga et al. (2003) reported that the ability of cotton (Gossypium hirsutum L.) to induce the germination of another root parasitic weed, Striga hermonthica (Delile) Benth., is a qualitatively inherited trait. Furthermore, the gene controlling this trait is monogenic and simply inherited. Similarly, Jamil

Figure 10. Effect of root extracts from one maize hybrid and its parental lines on Orobanche cumana germination in the field experiment. (A), (B), and (C) Aqueous extracts of roots collected at the four-, six-, and eight-leaf stages. (D), (E), and (F) Methanol extracts of roots collected at the four-, six-, and eight-leaf stages. The significant differences at 5% (LSD, P < 0.05) are marked by the small letters, respectively.
et al. (2011) reported genetic variation in strigolactone production by rice (*Oryza sativa* L.).

Recent studies have focused on the identification and assessment of potential trap crops for *Orobanche* spp. (Lins et al., 2006; Fenández-Aparicio et al., 2007; Khan et al., 2002) and the possibility of breeding for increased production of germination stimulants (Botanga et al., 2003). We propose that a breeding program could be developed to produce maize varieties with greater ability to induce *O. cumana* germination. Plant breeders have used a similar approach to develop maize lines with greater resistance to another root parasitic weed, *S. hermonthica*. The resultant lines have performed well in research and on-farm trials in west and central Africa (Kim, 1991; Carsky et al., 1998; Lagoke et al., 1997).

**CONCLUSIONS**

Intercropping with nonhost plants that induce “suicidal germination” and/or are allelopathic to root parasites is a promising approach that warrants continued efforts to identify potential trap crops and improve their efficacy. Through our pot and field trials, we conclude that maize is a potential trap crop for the root parasitic weed *O. cumana*. If maize were intercropped or rotated with sunflower, it might be possible not only to control *O. cumana* infestation but also to harvest a forage crop for feeding livestock. Future studies need to be done to confirm that maize can significantly reduce the *O. cumana* seed bank under field conditions.

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**References**


