Postprint: Elimination of Orobanche Soil Seed Bank Using Plant Allelopathy and Trap Crops

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Abstract

Research has demonstrated that allelopathy must satisfy the following four aspects: 1) The donor plant releases allelochemicals that cause continuous and quantitative effects on the receiver plant; 2) Allelochemicals can be isolated and identified from the donor plant, and these allelochemicals can produce effects on neighboring companion plants in natural ecosystems both under laboratory and field conditions; 3) The allelochemicals produced and released by the donor plant can reach neighboring receiver plants at sufficient concentrations under natural conditions; 4) The allelochemicals that reach the receiver plant with sufficient bioactivity can be absorbed and affect the physiological and biochemical processes of the receiver plant, and this effect must exclude the possibility that the receiver plant's growth and development are due to non-allelopathic factors such as competition, animal damage, pathogen infection, and physical environment. Broomrape is a root parasitic plant belonging to the Orobanchaceae family, Orobanche genus, and is a holoparasitic plant that parasitizes the roots of other plants. Over 100 species of Orobanche root parasitic plants have been discovered worldwide; in China, those causing relatively severe damage are Orobanche cumana (sunflower broomrape) and Orobanche aegyptiaca (Egyptian broomrape). Orobanche cumana is mainly distributed in northern Shaanxi, Hebei, Xinjiang, Shanxi, Inner Mongolia, and the three northeastern provinces, primarily harming sunflowers. Orobanche aegyptiaca is mainly distributed in Xinjiang, harming melons, tomatoes, and potatoes. Broomrape weed seeds are small in volume and light in weight (3–6 g), and each plant can produce a large number of seeds. These seeds are numerous and can remain viable in soil for up to 15–20 years. Mature broomrape seeds require a period of after-ripening; after-ripened broomrape seeds need 1–2 weeks of pre-conditioning under certain temperature and humidity conditions before germination. Pre-conditioned broomrape seeds must also obtain a chemical substance from the host to germinate; under natural conditions, this germination stimulant is secreted and provided by the young roots of host or non-host plants. After obtaining this substance, the broomrape

seed' s "germination tube" can emerge from the seed coat within several days; subsequently, under the action of haustorium-inducing substances, it rapidly forms a haustorium, attaches to the host root, penetrates into the root interior, and establishes a connection with the host root's xylem, competitively acquiring water, nutrients, and growth hormones from the host plant. Since Orobanche plants are root parasitic weeds, they have already caused serious damage to crops before emerging above ground, making them difficult to control; an effective approach is to minimize the broomrape seed content in soil. Trap crops refer to crops whose root systems can secrete substances that stimulate Orobanche seed germination, yet are not normally parasitized by broomrape, allowing the trap crop itself to be harvested normally. Since Orobanche seeds have only one life opportunity—if they germinate but cannot parasitize, they die—this type of germination is also called "suicidal germination." This way, broomrape can die before finding a host, thereby greatly reducing the broomrape seed bank in soil. This paper introduces the latest research progress by the author' s project team on utilizing allelopathy and trap crops (wheat, corn, cotton, soybean, etc.) to eliminate the broomrape soil seed bank.

Full Text

Using Allelopathy and Trap Crops to Eliminate the Soil Seed Bank of Broomrape

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Abstract: Research has demonstrated that allelopathy must satisfy four key criteria: 1) Donor plants release allelochemicals that exert continuous and quantitative effects on recipient plants; 2) These allelochemicals can be isolated and identified from donor plants and affect neighboring plants in natural ecosystems under both laboratory and field conditions; 3) The allelochemicals produced and released by donor plants must reach adjacent recipient plants at sufficient concentrations under natural conditions; and 4) The allelochemicals arriving at recipient plants with adequate biological activity must be absorbable and capable of influencing the physiological and biochemical processes of recipient plants, with these effects distinguished from those caused by competition, animal damage, pathogen infection, or physical environmental factors.

Broomrapes (Orobanche spp.) are root holoparasites belonging to the family Orobanchaceae that completely depend on host plants for nutrition. Approximately 100 species of Orobanche have been identified worldwide, with sunflower broomrape (O. cumana) and Egyptian broomrape (O. aegyptiaca) causing the most severe damage in China. Sunflower broomrape is primarily distributed in northern Shaanxi, Hebei, Xinjiang, Shanxi, Inner Mongolia, and the three northeastern provinces, mainly infesting sunflower crops. Egyptian broomrape is mainly found in Xinjiang, where it damages melons, tomatoes, and potatoes.

Broomrape seeds are extremely small and lightweight (3–6 μg), with each plant producing vast quantities of seeds that can remain viable in soil for 15–20 years. After maturation, broomrape seeds undergo a post-maturation period, followed by a 1–2 week pre-conditioning period under specific temperature and moisture conditions before germination. Pre-conditioned seeds must then receive a chemical germination stimulant from host or non-host plant roots. Upon acquiring this substance, the seed' s "germination tube" emerges within days, and under the influence of haustorium-inducing substances, quickly forms a haustorium that attaches to and penetrates the host root, establishing connections with the host' s xylem to competitively extract water, nutrients, and growth hormones. Because broomrape is a root-parasitic weed that causes severe damage before emerging above ground, it is extremely difficult to control. The most effective approach is to reduce the broomrape seed content in soil.

Trap crops are defined as crops whose root systems secrete germination stimulants for broomrape seeds but cannot be normally parasitized by broomrape, allowing the trap crop itself to be harvested normally. Since broomrape seeds have only one life opportunity and die if they germinate without successful parasitization—a process termed "suicidal germination" —this mechanism can significantly reduce the soil seed bank by causing broomrape seeds to die before finding a suitable host. This paper presents the latest research progress from our project team on using allelopathy and trap crops (wheat, corn, cotton, soybean, etc.) to eliminate the broomrape soil seed bank.

Keywords: Plant allelopathy; Trap crop; Broomrape; Root parasitic plant; Soil seed bank

1 Concepts and Basic Characteristics of Plant Allelopathy

Allelopathy was first proposed by Hans Molisch in 1937 to describe the biochemical interactions among all plants (including microorganisms), encompassing both beneficial and harmful relationships. The term "Allelopathy" derives from the Greek roots "Allelon" (mutual) and "Pathos" (suffering). In 1974, American scientist Rice published the classic work *Allelopathy*, defining plant allelopathy as the direct or indirect harmful effects of one plant on another (including microorganisms) through the release of chemical substances into the environment. In the 1984 revised edition, beneficial effects were incorporated into the definition [1].

A crucial aspect of plant allelopathy is that plants add chemical compounds to the environment, which distinguishes it from competition. Plant competition refers to different plants removing or reducing substances from the environment when coexisting in the same habitat. In 2010, Kong Chuihua et al. [2] described that research on plant allelopathy must clarify at least three points: (1) Allelopathy is a naturally occurring phenomenon among coexisting, associated, or ecologically related plant species; (2) Plants must not only synthesize

biologically active substances but also have appropriate pathways—such as natural volatilization, rain leaching, root exudation, and residue decomposition—to release these active substances into the environment; and (3) The active substances released by plants must reach effective concentrations in the environment under the influence of biotic and abiotic factors.

In his 2016 book *Plant Allelopathy (Inhibition and Promotion)* [3], Kong Chuihua further described three basic characteristics of plant allelopathy: (1) The interacting subjects are plants or microorganisms, excluding chemical interactions between plants and insects or other animals; (2) The interacting chemical substances are secondary metabolites that must enter the environment through appropriate pathways, excluding substances that undergo changes and transport within plants or microorganisms; and (3) The released substances primarily affect the growth and development of the plant itself or neighboring plants, excluding chemical communication among plants (such as alarm signals) or environmental pollution (such as volatile emissions from some trees combining with nitrogen oxides to form smog) from the basic definition of plant allelopathy [3].

2 Root Parasitic Plants and Parasitic Weeds

In his review article on allelopathy, Rice described its application in plant pathology, noting that viable parasitic weed seeds [Striga asiatica (L.) O. Kuntze] typically do not germinate unless they are first exposed to warm, moist conditions for several days and then to a chemical substance secreted by host or non-host plant roots [4].

Approximately 3,000 species of angiosperms are parasitic plants [5]. These plants employ different strategies when attacking hosts: those attacking host roots are called root parasites, while those attacking above-ground parts are stem parasites. Both root and stem parasites are further classified as hemiparasites or holoparasites based on the presence or absence of chlorophyll. Parasitism occurs in at least 17 families of flowering plants, with the most important parasitic weeds found in the following eight families (Table 1) [6].

Table 1 Family and genera distribution of plant species with parasitism (including main parasitic weeds)

Family	Important genera
Scrophulariaceae	*Striga*, *Alectra*
Santalaceae	*Santalum*, *Thesium*
Orobanchaceae	*Orobanche*, *Aeginetia*, *Christisonia*
Balanophoraceae	*Thonningia*, *Cynomorium*
Lauraceae	*Cassytha*
Viscaceae	*Phoradendron*, *Arceuthobium*
Convolvulaceae	*Cuscuta* (dodders)

Family	Important genera
Loranthaceae	*Amyema*, *Dendrophthoe*, *Helixanthera*, *Loranthus*

Root-parasitic weeds pose a serious problem in agricultural production, causing crop yield losses in most countries worldwide [7-8]. Broomrape (Orobanche spp., Orobanchaceae) is a holoparasitic plant that attacks host roots, parasitizing species from Asteraceae, Fabaceae, Solanaceae, Cucurbitaceae, Brassicaceae, Cannabaceae, Linaceae, Apiaceae, and Poaceae. Lacking chlorophyll, broomrape must plunder nutrients, water, and growth hormones from hosts to meet its needs [6]. Among over 100 broomrape species, six cause significant crop damage as parasitic weeds: Egyptian broomrape (O. aegyptiaca Pers.), hemp broomrape (O. ramosa L.), nodding broomrape (O. cernua Loefling), small broomrape (O. minor Sm.), sunflower broomrape (O. cumana Wallr.), and bean broomrape (O. crenata Forssk.). Sunflower broomrape is a major factor limiting sunflower (Helianthus annuus L.) production, causing severe economic losses in Mediterranean regions, Eastern Europe, and China, which account for 50% of global sunflower seed production [9-10]. Currently, no effective herbicides or control methods have been developed worldwide for parasitic weeds like sunflower broomrape [8,11].

3 Broomrape Life Cycle and Trap Crops

The interaction between broomrape and its host begins when secondary metabolites secreted by host roots stimulate broomrape seed germination. Under natural conditions, germination stimulants are provided by root exudates from host or non-host plants. After germination, the broomrape radicle elongates approximately 3–4 mm (sometimes called a "germination tube" because this elongating radicle lacks a root cap). However, to complete parasitization, germinated seeds must acquire a haustorium-inducing substance from the host to form a papilla-like adhesive haustorium that attaches to and penetrates the host root, establishing connections with both xylem and phloem to form a parasitic relationship.

Broomrape seeds are small and lightweight (3–6 μg), with each plant producing enormous quantities. Based on our analysis of 21 sunflower broomrape capsules, each plant produces an average of 174,000 seeds (range: 30,000–385,000), with an average of 68 capsules per plant (range: 33–96) and an average seed production of 10.4 mg per capsule (range: 2.9–23.75 mg). The average thousand-seed weight is 4.14 mg (range: 1.48–7.55 mg). Analysis of 21 Egyptian broomrape capsules revealed an average production of 38,000 seeds per plant (range: 5,000–81,000), with an average of 25 capsules per plant (range: 16–41) and average seed production of 17.3 mg per capsule (range: 1.27–30.78 mg). The average thousand-seed weight is 12.27 mg (range: 1.88–32.23 mg) [12]. These seeds accu-

mulate in farmland, forming massive soil seed banks. Measurements show that in Xinjiang Production and Construction Corps fields, seed density reaches 2.8 billion seeds per hectare (in 0–10 cm soil, 4 g of soil contains over 130 broomrape seeds, with the 0–10 cm soil layer weighing 135 $t \cdot hm^{2}$ and Egyptian broomrape thousand-seed weight averaging 12.3 mg), with a second plot containing 1.25 billion seeds. In saline-alkali soils of Dingbian County, Shaanxi Province, sunflower broomrape seed numbers reach 280 million per hectare [13].

These vast quantities of seeds can remain viable in soil for 15–20 years. After maturation, broomrape seeds require a post-maturation period, followed by 1–2 weeks of pre-conditioning under specific temperature and moisture conditions before germination. Pre-conditioned seeds must then receive a chemical substance from the host to germinate; under natural conditions, this germination stimulant is secreted by young roots of host or non-host plants. After acquiring this substance, the seed's "germination tube"emerges within days, and under the influence of haustorium-inducing substances, quickly forms a haustorium that attaches to the host root, penetrates it, and establishes connections with the host' s xylem to competitively extract water, nutrients, and growth hormones.

Because broomrape is a root-parasitic weed that causes severe damage before emerging above ground, it is extremely difficult to control. The most effective approach is to minimize broomrape seed content in soil. Trap crops are defined as crops whose root systems secrete germination stimulants for broomrape seeds but cannot be normally parasitized by broomrape, allowing the trap crop itself to be harvested normally. Since broomrape seeds have only one life opportunity and die if they germinate without successful parasitization—a process termed "suicidal germination" —this mechanism can significantly reduce the soil seed bank by causing broomrape seeds to die before finding a suitable host [14].

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4 Materials and Methods

For over a decade, we have conducted experiments on broomrape seed germination stimulation using traditional Chinese medicinal herbs and crops including wheat (Triticum aestivum L.), corn (Zea mays L.), cotton (Gossypium sp.), and soybean (Glycine max L.) to investigate trap crop control of broomrape.

4.1.1 Seeds and Reagents

Sunflower broomrape and Egyptian broomrape seeds were collected annually starting in 2008 from infested sunflower fields in Dingbian County, Shaanxi Province, and tomato (Lycopersicon esculentum Mill.) fields in Xinjiang Uygur Autonomous Region, respectively. Small broomrape seeds were provided by Professor Koichi Yoneyama of Utsunomiya University, Japan. The germination stimulant GR24 (a strigolactone analog) was provided by Professor Binne Zwanenburg of Radboud University, The Netherlands.

Six hundred six traditional Chinese medicinal herbs were purchased from herbal medicine stores across mainland China' s provinces, municipalities, and autonomous regions. The medicinal parts were air-dried, ground using a pulverizer (FW135-177, Tianjin Taisite Instrument Co., Ltd.), and passed through a 0.45 mm sieve for later use.

4.1.3 Wheat Varieties

Wheat seeds were provided by Researcher Deng Xiping from the Institute of Soil and Water Conservation and Professor Ji Wanquan from the Wheat Center, College of Agronomy, Northwest A&F University. Sunflower broomrape seeds were collected from Farm 51, Division 12 of the Xinjiang Production and Construction Corps, while Egyptian broomrape seeds were collected from Hejing County, Xinjiang. Strigol was provided by Professor Koichi Yoneyama of Utsunomiya University, Japan. Different wheat genotypes included: diploid wild einkorn and cultivated einkorn; tetraploid wild emmer and cultivated emmer; and hexaploid 'Shaan 139' and 'Shaan 253' .

4.1.4 Corn Varieties

Four hybrid combinations and their parental lines, along with 200 F1 generation corn seeds for stimulating sunflower broomrape germination, were provided by Professor Mao Jianchang from the College of Agronomy, Northwest A&F University (Table 2). These combinations were obtained through crosses between inbred lines. Among these inbred lines, '3026' was developed from a cross between 'Ye 478' and '89-1' , with '89-1' derived from 'P87599' (a Pioneer hybrid). '340X' represents Chinese 'Lüda Red Cob' germplasm. 'Zheng 58' was selected from off-type plants in 'Ye 478' , and '5212' belongs to Chinese 'Tang Sipingtou' germplasm. 'Jun 20' is a hybrid derived from foreign materials. '3255' was selected from foreign hybrids, and '335' is a Pioneer inbred line. Ten commercially promoted corn varieties for stimulating Egyptian broomrape were purchased from Yangling Seed Company ('Changcheng 799' , 'Changdan 48' , 'Luyu 13' , 'Nongda 364' , 'Shandan 2001' , 'Tiancheng 288' , 'Yuyu 22' , 'Zhengda 12' , 'Zhengdan 958' , and 'Zhengyu 203').

4.1.5 Cotton Varieties

Twenty cotton varieties ('P08-C4' , 'P08-B12' , 'P08-B7' , 'P08-A4' , 'P08-E4' , 'P08-E5' , 'P08-C11' , 'P08-A7' , 'P08-B10' , 'P08-E6' , 'P08-C8' , 'P08-A12' , 'P08-E7' , 'P08-B6' , 'P08-A10' , 'P08-A9' , 'P08-A13' , 'P08-D5' , 'P08-A11' , and 'P08-F3') were provided by Associate Researcher Zhao Junxing from the College of Agronomy, Northwest A&F University. Additionally, parental lines and F1 hybrids of 'Zhongmiansuo 48' (male parent, female parent, and 1F1), 'Zhongmiansuo 51' (male parent, female parent, and 2F1), 'Zhongzi 101' (male parent, female parent, and 3F1), '8 Tiao-083113' (male parent, female parent, and 4F1), and '7 Tiao-083001' (male parent, female parent, and 5F1) were

purchased from the Cotton Industry Science and Technology Co., Ltd., Chinese Academy of Agricultural Sciences.

4.1.6 Soybean Varieties

Ten soybean varieties widely promoted in Northeast China ('Beidou 18', 'Suinong 10' , 'Fengdou 3' , 'Heinong 28' , 'Dongdou 339' , 'Zhonghuang 13' , 'Hefeng 55' , 'Kenjiandou 35' , 'Heinong 44' , and 'Kengfeng 16') were selected for the study.

4.2.1 Surface Sterilization and Pre-conditioning of Broomrape Seeds

Broomrape seeds were surface-sterilized by soaking in 1% sodium hypochlorite solution for 3 minutes, then rinsed with deionized water. Seeds were placed on moist 8 mm glass fiber filter paper (GFFP, Whatman, GE Healthcare UK LTD, Buckinghamshire, UK) and pre-conditioned at 25°C in darkness for 3–8 days.

4.2.2 Laboratory Germination Tests

One hundred milligrams of plant sample powder was weighed into a 2 mL centrifuge tube, 1.5 mL deionized water added, and after ultrasonic treatment for 30 minutes, centrifuged (Millipore Cat. No. XX42 CF0, 60 Lot No. N8JMB042A, Nihon Millipore LTD. Yonezawa, Japan) to obtain the supernatant as the stock solution. The stock solution was diluted 10-fold and 100-fold with deionized water. These aqueous extracts were used directly for bioassays, with 15 µL of extract applied to 8 mm diameter glass fiber filter paper containing broomrape seeds, then placed in petri dishes. Before adding extracts, the glass fiber filter paper containing broomrape seeds was placed on filter paper (Shuangquan GB/T1914–2007, Hangzhou Wohua Filter Paper Co., Ltd.) for a moment to remove excess water. For methanol extracts, the extraction and dilution methods were the same as above. Fifteen microliters of methanol extract was first applied to glass fiber filter paper without seeds. After methanol evaporation, the filter paper containing seeds was placed on top and 30 µL deionized water added. Treated seeds were incubated at 25°C in darkness, and germination rates were observed after 8 days. GR24 and deionized water treatments served as positive and negative controls, respectively, with four replicates per treatment. Germination rates were observed under a 20X microscope, with seed germination defined as the emergence of the germination tube through the seed coat.

4.2.3 Collection of Root Exudates

Plant seeds were surface-sterilized with 75% (v/v) ethanol for 2 minutes, then treated with 1% (v/v) sodium hypochlorite solution for 2 minutes. After thorough rinsing with sterile distilled water, sterilized seeds were placed in containers on filter paper moistened with distilled water and germinated at 25°C in darkness for 1 day. Germinated seedlings (approximately 500) were transplanted into a frame (33 cm × 22 cm × 8 cm) with a layer of gauze at the bottom and

slits in the base. This container was placed inside a slightly larger box (33 cm × 23 cm × 8.5 cm) without slits, containing 1 L tap water as culture medium, and maintained in a growth chamber at 120 μmol·m ^{2}·s ^{1} light intensity, 25°C/23°C day/night temperature, and 14 h/10 h light/dark cycle. After 6 days of growth in tap water, the seedling frame was transferred to a larger container (40 cm × 30 cm × 10 cm) with 4 L tap water and phosphorus-free 1/2 Tadano and Tanaka nutrient solution [15] for 6 days, after which the culture solution was collected.

4.2.4 Pot Experiments

Experiments were conducted at the Institute of Soil and Water Conservation, Chinese Academy of Sciences. Soil from the 0–20 cm cultivated layer of previously cropped land was crushed, sun-dried, and passed through a 1 cm sieve. It was mixed with earthworm castings at a 4:1 ratio as potting soil and placed in rocket pots (25 cm height, 20 cm diameter, Zhongke Environmental Engineering Co., Ltd.). Plant rhizosphere soil and plants were collected, with roots, stems, and leaves separated, freeze-dried at low temperature, ground after freeze-drying, and passed through a 50-mesh sieve, with three replicates per variety.

4.2.5 Stimulation of Broomrape Seed Germination by Methanol Extracts of Plant Rhizosphere Soil

Five grams of crop rhizosphere soil was weighed, 10 mL methanol added, ultrasonicated for 30 minutes, left to stand for 5 minutes, then filtered to obtain the filtrate as the stock solution. After 10-fold and 100-fold dilution, 20 μL of each of the three concentrations was applied to 1 cm diameter glass fiber filter paper. After 30 minutes of methanol evaporation, glass fiber filter discs containing sunflower broomrape seeds (20–40 seeds) were placed on the dried filter paper, and 40 μL distilled water added. Each concentration had three replicates, with 1 mg·L ^{1} strigol treatment and methanol extract of soil without cotton planting as controls. Petri dishes were sealed with Parafilm and incubated at 25°C for 10 days, after which germination rates were observed and counted under a 20 × 16 magnification microscope.

4.2.6 Stimulation of Broomrape Seed Germination by Methanol Extracts of Plant Roots, Stems, and Leaves

One hundred milligrams of plant sample (roots, stems, and leaves) was placed in a 1.5 mL centrifuge tube, 1 mL methanol added, ultrasonicated for 30 minutes, then centrifuged at 6,400 r·min ^{1} for 2 minutes to obtain the supernatant as the stock extract. Dilution and culture methods were the same as above, with 1 mg·L ^{1} strigol or GR24 (synthetic strigol) treatment and distilled water treatment as controls.

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5 Results

5.1 Chinese Medicinal Herbs Stimulating Broomrape Seed Germination

Traditional Chinese medicine comprises natural drugs used for disease prevention and treatment under the guidance of traditional Chinese medical theory. With thousands of years of research history, Chinese scientists have identified the distribution of active components within these medicinal plants. Leveraging this rich theoretical and practical knowledge to identify germination stimulants for Orobanche seeds represents a unique advantage for Chinese agricultural scientists. By utilizing China' s abundant traditional medicinal herb resources and conducting germination induction experiments on broomrape seeds, we can screen non-parasitic traditional Chinese medicinal herbs to identify substances with high activity and stability that stimulate or inhibit Orobanche seed germination, thereby opening new avenues for medicinal herb utilization.

Chinese medicinal herbs are widely cultivated in broomrape-infested regions. Our research targeting these areas revealed that among 606 collected medicinal herbs, 20 species produced aqueous extracts that stimulated small broomrape germination rates exceeding 60% at three different concentrations. Rush (*Juncus effusus* L. var. *decipiens* Buchen) showed the strongest stimulation, achieving 90.3% germination. Most crude aqueous extracts showed weak stimulation, with only rush reaching 72.7%. Eighteen medicinal herb extracts stimulated sunflower broomrape germination rates exceeding 30%, with white-flowered hogfennel (*Peucedanum pracruptorum* Dunn) and green foxtail extracts showing relatively high stimulation at 10-fold dilution, achieving 49.2% and 48.8% germination, respectively. Twenty-one medicinal herb extracts stimulated Egyptian broomrape germination (>30%), with *Hedyotis diffusa* Willd. 10-fold dilution achieving the highest rate at 81.9%. Four herbs—*Arenaria kansuensis* Maxim., wild chrysanthemum (*Chrysanthemum indicum* L.), *Phyllanthus urinaria* L., and green foxtail—stimulated germination of all three broomrape species. Screening of 69 traditional Tibetan medicinal herbs identified 16 species whose methanol or aqueous extracts induced 20% germination in Egyptian broomrape seeds and 21 species that induced 20% germination in sunflower broomrape seeds. Six herbs—sweet wormwood (*Artemisia annua* L.), *Meconopsis horridula* Hook. f. et Thoms. var. *racemose* (Maxim.) Prain, *Millettia dielsiana* Harms ex Diels., *Dracocephalum ruyschiana* L., *Nardostachys jatamansi* DC., and *Lamiophlomis rotata* (Benth.) Kudo.—induced >20% germination in both broomrape species, indicating these herbs contain highly active or concentrated broomrape germination-inducing substances [16-17].

5.2 Wheat Stimulating Broomrape Seed Germination

As the second most important food crop, wheat could potentially be used in rotation with host crops like sunflower and tomato to reduce broomrape damage. Six years of research yielded the following main findings: 1) Pre-conditioning

sunflower and Egyptian broomrape seeds with distilled water can break dormancy, requiring a minimum of 3 days pre-conditioning, with germination rates stabilizing after 1–2 weeks; 2) Wheat root exudates at different concentrations stimulated germination in the order: 100 mg·L^{1} > 10 mg·L^{1} > 1 mg·L^{1}. At all three concentrations, germination rates increased progressively with wheat ploidy level, with significant differences ($P<0.05$) among varieties; 3) Rhizosphere soil from different wheat genotypes at the seedling stage directly stimulated germination of both broomrape species, indicating that wheat naturally secretes germination stimulants at sufficient concentrations. At the jointing stage, germination stimulation by rhizosphere soil and extracts increased with ploidy level, with hexaploid genotypes showing the highest rates. Germination stimulants are synthesized in wheat roots, with positive correlations between root and shoot extract stimulation rates. At the heading stage, 'Shaan 253' roots achieved the highest stimulation rate for sunflower broomrape (32.4%), while Egyptian broomrape germination rates ranged 20–30%. Hexaploid 'Shaan 139' and 'Shaan 253' stem methanol extracts achieved the highest Egyptian broomrape germination rates of 49% and 51.1%, respectively. Comparing different wheat organs, leaf aqueous extracts showed the highest stimulation rates for sunflower broomrape, with roots, stems, and spikes showing similar activity and positive correlations among roots, stems, leaves, and spikes. At maturity, rhizosphere soil stimulation rates decreased while above-ground organ stimulation increased, with 'Shaan 139' stem aqueous extracts achieving 28.1% stimulation and methanol extracts >30%. 'Shaan 253' stem extracts achieved 27.7% (aqueous) and 23.7% (methanol) stimulation. Cultivated einkorn leaf methanol extracts achieved 45.9% stimulation of sunflower broomrape, while hexaploid 'Shaan 253' leaf extracts achieved 24% (aqueous) and 29% (methanol) stimulation of Egyptian broomrape. 'Shaan 253' glume extracts achieved 26.8% (aqueous) and 37.6% (methanol) stimulation of sunflower broomrape, with methanol extracts achieving 33.2% stimulation of Egyptian broomrape. Positive correlations existed between roots and stems/grains; 4) With increasing wheat ploidy, germination stimulation by extracts (aqueous and methanol) from different organs at seedling, jointing, heading, and maturity stages showed a progressive upward trend, with wheat allelopathic effects on both broomrape species gradually strengthening; and 5) Planting 'Shaan 253'and 'Shaan 139'as previous crops reduced sunflower broomrape parasitism to 3.2 and 4.6 plants per sunflower, respectively (1/6 and 1/4 of control levels), significantly reducing parasitism rates. Subsequent sunflower plants showed 0.2 mm greater stem diameter and 10 cm greater height than controls. Parasitized sunflower fresh and dry weights were only 46% and 37% of non-parasized plants. Preceding winter wheat cultivation increased subsequent sunflower stem diameter, height, and biomass while significantly reducing broomrape parasitism, indirectly increasing sunflower yield [18-21].

5.3 Corn Stimulating Broomrape Seed Germination

Corn is widely cultivated in China. Using corn as a trap crop in regions where sunflower or tomato are grown should similarly reduce broomrape damage. Three years of repeated experiments revealed significant differences among corn hybrid combinations in stimulating sunflower broomrape germination, with germination stimulants produced during early corn germination. In pot and field experiments, corn rhizosphere soil, root extracts, and above-ground extracts all stimulated sunflower broomrape germination. Significant differences existed among different corn inbred lines and F1 generations. The '3255' × '335' F1 hybrid and its parents showed strong stimulation effects. During breeding, corn stimulation of sunflower broomrape germination has not been used as a selection criterion, necessitating genetic analysis of these allelochemicals. Breeders have developed corn lines with higher resistance to Striga through simple breeding programs, suggesting that superior trap crop varieties for broomrape control could be developed through alternative breeding approaches [22].

Building on corn stimulation of sunflower broomrape, we investigated corn effects on Egyptian broomrape. Using nationally promoted corn varieties, 'Changcheng 799' and 'Zhengdan 958' showed the strongest stimulation, while 'Luyu 13' and 'Zhengyu 203' showed weaker effects. Pot experiment results were consistent with these findings, providing a basis for future field promotion trials.

In both sunflower and Egyptian broomrape experiments, corn root extracts showed higher stimulation rates than above-ground extracts, consistent with research showing that strigolactones are primarily synthesized in roots and transported to shoots. Methanol extracts also showed higher stimulation rates than aqueous extracts [22].

Our preliminary experiments indicated that corn cannot be parasitized by broomrape. Recent results confirm that the '3255' × '335' combination shows the strongest stimulation of sunflower broomrape, while 'Changcheng 799' most strongly stimulates Egyptian broomrape, making them viable trap crops. This research systematically verified stimulation effects of different corn lines/varieties on both broomrape species, confirming corn as a broomrape trap crop and providing new control strategies for these "malignant weeds." Using corn allelopathy to reduce or eliminate field weeds increases corn yield, reduces herbicide use, and decreases environmental pollution from pesticides.

Following publication in *Crop Science*, the journal' s website featured this research as the top news story in January 2013 (https://www.crops.org/science-news/corn-could-help-farmers-fight-devastating-weed). The news highlighted that corn, widely grown worldwide as food, animal feed, and energy material with flexible management and diverse uses, may soon have a new purpose: Chinese researchers discovered corn' s ability to induce "suicidal germination" in a devastating weed. Professor Ma Yongqing and his team from Northwest A&F University proposed developing specialized corn lines as trap crops to control

broomrape while also serving as forage crops [23-24].

5.4 Cotton Stimulating Broomrape Seed Germination

Xinjiang is China' s main cotton production region and also a province severely affected by broomrape, with both Egyptian and sunflower broomrape present. Using 43 cotton varieties/lines as donors and sunflower broomrape and small broomrape as receptors, we employed hydroponic, pot, and field experiments to analyze allelopathic effects based on germination induction rates. Cotton roots secrete strigol, which can stimulate broomrape seed germination, with significant differences among varieties/lines in inducing small broomrape germination. Rhizosphere soil and methanol/aqueous extracts of cotton plants induced small broomrape germination, with the following pattern: roots > stems > leaves. Significant linear correlations existed between rhizosphere soil and stem extract stimulation rates ($R^2 = 0.3622$) and between rhizosphere soil and leaf extract stimulation rates ($R^2 = 0.3070$), while root and stem extract stimulation rates showed a correlation coefficient of 0.5237 [25-26].

Fifteen cotton varieties were used in pot experiments to induce sunflower broomrape germination at three seedling stages (two-leaf, four-leaf, and six-leaf stages). Results showed the highest induction rates at the six-leaf stage, indicating stronger induction during this period. Different cotton organs showed similar effects on sunflower broomrape as on small broomrape, with the pattern: roots > stems > leaves [25].

Field experiments with 15 cotton varieties showed that throughout the growth period, methanol extracts of roots, stems, and leaves followed the pattern: roots > stems > leaves, consistent with pot experiments and further validating that root > stem > leaf distribution of secondary metabolites. Among three extract concentrations, rhizosphere soil extract at original concentration showed the highest stimulation, while root, stem, and leaf extracts at 100-fold dilution were most effective. Cotton organ methanol extracts showed a "low promotion, high inhibition" pattern on sunflower broomrape germination. Rhizosphere soil methanol extracts showed the highest and most stable stimulation at the budding stage, indicating higher root exudate levels during this period. Root extracts showed highest activity at the six-leaf stage, while stem and leaf extracts peaked at the two-leaf stage, suggesting higher activity of germination-inducing substances during seedling stages [27].

5.5 Soybean Stimulating Broomrape Seed Germination

All three northeastern Chinese provinces experience broomrape damage, with sunflower broomrape being most severe. As Northeast China is also a major soybean production region, we investigated soybean-induced broomrape germination. Soybean roots release germination stimulants for both sunflower and Egyptian broomrape seeds, with different organs showing varying effects: roots > stems > leaves. Pot experiments using 14 soybean varieties widely promoted

in Northeast China were conducted with sampling at V1, V3, V5, R2, and R4 stages, including rhizosphere soil, roots, stems, and leaves. Results showed significant differences among soybean varieties in stimulating germination of both broomrape species.

Soybean rhizosphere soil effectively stimulated germination of both broomrape species. 'Zhonghuang 13' rhizosphere soil showed high stimulation of sunflower broomrape, indicating that germination stimulants can persist stably in soil with varying effects among varieties. The V3 stage showed the highest induction rates. Different organs showed the pattern: roots > stems > leaves. Ten percent root extract stimulation rates showed significant positive linear correlations with 10% stem and leaf extracts, indicating stimulant transport from roots to shoots. Ten percent and 1% root extract stimulation rates at V3 showed significant power-function correlations with nodule diameter and weight, suggesting a relationship between stimulant production and nodule formation [28-29].

Three soybean varieties showing high, medium, and low stimulation in pot experiments were selected for field planting, with sampling at V3, V5, and R4 stages and extraction using water and methanol. Aqueous extracts showed low stimulation rates, suggesting methanol is a better solvent for verifying allelopathic potential differences among soybean varieties. Field rhizosphere soil stimulated germination of both broomrape species, confirming soybean as a potential trap crop. V3 stage samples showed higher stimulation than V5 and R4 stages. The pattern roots > stems > leaves was consistent with pot experiments [30]. LC-MS/MS analysis of freeze-dried root samples of 'Zhonghuang 13' at V3 from pot experiments identified a strigolactone compound, orobanchyl acetate, in soybean roots.

In summary, this study employed different methods to investigate effects of different soybean varieties on germination of sunflower and Egyptian broomrape seeds, demonstrating that soybean (rhizosphere soil and plant organs) can induce broomrape germination. Based on these results, future field rotation and other agronomic practices between soybean and broomrape host plants could be implemented in suitable soybean-growing regions to further validate soybean' s effectiveness as a trap crop for both broomrape species.

Although Chinese researchers in plant allelopathy have increased substantially in recent years, those simultaneously working on allelopathy and broomrape control are extremely rare. Searching "broomrape + germination" on CNKI (http://epub.cnki.net/kns/brief/default_result.aspx) yields 846 records, but most concern medicinal applications. Except for three records from Professor Zhou Weijun' s team at Zhejiang University, the top 40 publications are all from Ma Yongqing' s team. Searching "Orobanche trap crop" on Web of Science yields only 28 relevant publications, indicating that international researchers in broomrape control are also scarce. Chinese scientists primarily focus on medicinal components and bioactivity, with limited clinical application [31]. As previously noted, broomrape is a root-parasitic weed that produces massive seed quantities, remains viable in soil for over 10 years, and causes severe

damage before emergence, making it extremely difficult to control. Currently, no effective herbicides exist worldwide, and broomrape control remains a global challenge.

Our project team has conducted trap crop research for broomrape control since 2008. Although this method is environmentally friendly and practical, progress has been slow because few existing crop varieties secrete sufficient germination stimulants, as breeding programs have not targeted high strigolactone secretion. Notably, the Science and Technology Bureau of Xinjiang Production and Construction Corps Division 2 recognized the severity of the problem and organized a corps-level project application in 2016, marking the first direct application of our research results in production practice and representing the first step toward translating our publications into practical solutions.

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Note: Figure translations are in progress. See original paper for figures.