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Original article

## Study of New Hosts of Sunflower Broomrape (*Orobanche Cumana* Wallr.)

Maria Petrova <sup>a\*</sup>, Hristo Stoyanov <sup>b</sup>

<sup>a</sup> Agricultural Academy – Sofia, Dobrudzha Agricultural Institute – General Toshevo, 9521, General Toshevo, Bulgaria

### Abstract

Weed flora serves as a persistent reservoir for phytopathogenic fungi and parasitic plants, sustaining their survival and spread even via non-cultivated hosts. Pathogens like *Diaporthe helianthi* and *Plasmopara halstedii* can infect both sunflower (*Helianthus annuus*) and diverse weedy species, maintaining continuous inoculum sources. Studies have shown that *Phomopsis* spp. colonize various non-cultivated plants, where they form necrotic lesions and viable conidia. Experimental inoculations confirmed cross-infectivity between sunflower and several weeds, indicating epidemiological links. Regarding the obligate root holoparasite *Orobanche cumana*, natural infections appear mostly confined to Asteraceae, especially *Artemisia* spp. However, in controlled conditions, *O. cumana* shows strict specificity to *H. annuus*, with no successful parasitism of non-*Helianthus* species. Given its impact on sunflower production, identifying potential alternative hosts is vital for early detection and management. In this study, 23 accessions from nine Asteraceae species were evaluated for *O. cumana* susceptibility. Plants were grown in soil infested with quantified broomrape seed loads, and root systems were assessed 45 days after sowing. High susceptibility was recorded in all four tested *Arctium lappa* accessions, confirming its role as a potential alternative host under favorable conditions. These findings highlight the importance of integrated disease management, including resistant hybrids, targeted fungicide use, and weed host control, to limit inoculum pressure and reduce disease outbreaks in sunflower crops.

**Keywords:** Sunflower, Alternative hosts, *Arctium lappa*

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\* **Corresponding author:**

Maria Petrova, Agricultural Academy – Sofia, Dobrudzha Agricultural Institute – General Toshevo, 9521, General Toshevo, Bulgaria  
Email: maria.petrovaa.1994@gmail.com

## INTRODUCTION

Sunflower broomrape (*Orobanche cumana* Wallr.) is a holoparasitic plant species that significantly limits sunflower (*Helianthus annuus* L.) production across Europe, Asia, and increasingly in parts of Africa (Fernández-Martínez et al., 2015; Amri et al., 2012; Nabloussi et al., 2018). In natural ecosystems, the parasite primarily infects wild Asteraceae species, especially *Artemisia* spp., whereas in cultivated systems it exhibits strict host specificity, targeting only sunflower (literature has needed for both cases as wild and crop). Severe infestations can result in yield losses in sunflowers exceeding 50% in susceptible hybrids and may reach complete crop failure under high-pressure conditions (Cvejić et al., 2020). The rapid spread and adaptability of *O. cumana* make it a formidable pest with significant agronomic and economic consequences.

The dispersal of *O. cumana* is facilitated by its production of numerous microscopic, long-lived seeds that can remain viable in the soil for up to two decades (Pineda-Martos et al., 2014; Habimana et al., 2014). Seed germination is triggered by specific chemical signals, including strigolactones and sesquiterpene lactones, released by host roots (Raupp and Spring, 2013). Following germination, the radicle grows toward the host root and forms a prehaustorium, whose invasive cells penetrate the host tissue. This is followed by the development of the haustorium, which establishes vascular connections that allow the parasite to extract water and nutrients from the host (Delavault, 2015; Yoshida et al., 2016; Krupp et al., 2019). The parasite develops underground tubercles that serve as nutrient storage, from which shoots emerge, flower, and produce seeds, replenishing the soil seed bank and ensuring further dissemination (Molinero-Ruiz et al., 2015).

*Orobanche cumana* is naturally distributed from Central Asia to European Russia, including the Black Sea coast. It is later introduced over whole Blacksea coast (including Bulgaria, according to Duca and Bivol, 2023), where it commonly parasitizes *Artemisia maritima* L. (Pineda-Martos et al., 2014a; Fernández-Martínez et al., 2015;). In recent decades, the parasite has expanded to other regions, including Spain, Turkey, Israel, Iran, Kazakhstan and China. Long-distance dispersal is primarily facilitated by human-mediated activities, machinery, water, wind, and attachment to sunflower seeds, while local spread is enhanced by the persistence of the soil seed bank (Molinero-Ruiz et al., 2015).

Genetic analyses of *O. cumana* populations have revealed the existence of distinct gene pools, with populations in Spain divided into the Guadalquivir Valley and Cuenca province. Founder effects contribute to low intra-pool diversity, but recombination between gene pools can generate highly virulent populations capable of overcoming resistance in sunflower (Pineda-Martos et al., 2013). Understanding the structure and connectivity of these populations is essential for predicting the evolution of virulence and for implementing effective management strategies.

The sunflower – *O. cumana* interaction follows a gene-for-gene model, in which dominant resistance genes in the host recognize corresponding avirulence genes in the parasite. This interaction gives rise to distinct physiological races of *O. cumana*, historically classified as races A–E in Eastern Europe, followed by more virulent races F, G and H in response to selection pressure from resistant cultivars (Vranceanu et al., 1980; Kaya et al., 2004, Duca and Bivol, 2023). The emergence of these virulent races illustrates the dynamic co-evolution between host and parasite and underscores the importance of continuous monitoring and characterization of parasite populations.

Wild *O. cumana* populations may serve as reservoirs of genetic diversity, providing alleles that can contribute to the evolution of new virulent races in cultivated fields. However, the genetic structure and virulence potential of these wild populations remain poorly understood, particularly regarding their ability to infect cultivated sunflower and the potential for gene flow between wild and weedy populations (Vaz Patto et al., 2007; Botanga et al., 2008).

Sunflower is inherently resistant to broomrape species in its area of origin, North America, and the first infections by *O. cumana* occurred after the rapid expansion of sunflower cultivation in Russia at the end of the 19th century. Early breeding programs focused on identifying resistant germplasm from landraces and wild Helianthus species, particularly *H. tuberosus* (Pustovoit, 1966). Resistance to races A-E is typically controlled by single dominant genes, facilitating conventional backcrossing into commercial cultivars (Vranceanu et al., 1980, Škorić et al., 2006; Fernández-Martínez et al., 2010).

Resistance to race F is more complex, involving either recessive alleles or dominant genes influenced by modifier loci that can alter expected dominance patterns (Rodríguez-Ojeda et al., 2001; Akhtouch et al., 2002; Pérez-Vich et al., 2004a; Velasco et al., 2006). Introgression from wild perennial species poses additional challenges due to hybrid embryo abortion and sterility in early generations, which can be mitigated by advanced techniques such as embryo rescue and chromosome doubling (Jan & Fernández-Martínez, 2002). Molecular mapping has positioned the Or5 gene, conferring resistance to race E, in a telomeric region of sunflower linkage group 3, likely part of an NBS-LRR gene cluster (Tang et al., 2003; Radwan et al., 2008). Resistance to race E is further supported by multiple minor QTLs contributing to quantitative resistance, while resistance to race F is controlled by several QTLs with small to moderate effects (Pérez-Vich et al., 2004a; Pérez-Vich et al., 2004b; Labrousse et al., 2001; Letousey et al., 2007).

The predominant use of single, race-specific dominant resistance genes in commercial hybrids has accelerated the emergence of new virulent races, demonstrating the need for integrated breeding strategies. Approaches such as pyramiding multiple resistance genes, combining vertical and horizontal resistance, or alternating resistance sources are essential to achieve durable and sustainable resistance (Molinero-Ruiz et al., 2015; Fernández-Martínez et al., 2015). Further, understanding the

role of wild populations as reservoirs of genetic diversity is critical for anticipating the emergence of virulent races and designing effective integrated pest management strategies.

Despite significant progress in characterizing weedy *O. cumana* populations and resistance mechanisms in cultivated sunflower, several knowledge gaps remain. The genetic diversity, population structure, and virulence potential of wild *O. cumana* populations, and their contribution to the emergence of new races, are poorly understood. Addressing these gaps is crucial for elucidating host – parasite co-evolution, improving sunflower breeding programs, and developing sustainable management strategies to mitigate the impact of this aggressive holoparasite in agricultural ecosystems. The aim of this study is to assess the capacity of selected *Asteraceae* species to function as alternative hosts for *Orobanche cumana*, thereby refining current understanding of its host specificity.

### MATERIALS AND METHODS

In the present study, the susceptibility of 23 accessions from 9 Asteraceae species was evaluated. The taxonomic identity and origin of the accessions are presented in Table 1.

**Table 1.** Asteraceae accessions by species and origin

No	Accession code	Species name	Origin
1	OA-1	<i>Onopordum acanthium</i>	Stozher, Bulgaria
2	OA-2	<i>Onopordum acanthium</i>	Stozher, Bulgaria
3	OA-3	<i>Onopordum acanthium</i>	Stozher, Bulgaria
4	OA-4	<i>Onopordum acanthium</i>	General Toshevo, Bulgaria
5	OA-5	<i>Onopordum acanthium</i>	General Toshevo, Bulgaria
6	OA-6	<i>Onopordum acanthium</i>	General Toshevo, Bulgaria
7	OA-7	<i>Onopordum acanthium</i>	Etropole, Bulgaria
8	OA-8	<i>Onopordum acanthium</i>	Etropole, Bulgaria
9	OA-9	<i>Onopordum acanthium</i>	Etropole, Bulgaria
10	CA-1	<i>Carduus acanthoides</i>	Stozher, Bulgaria
11	CA-2	<i>Carduus acanthoides</i>	General Toshevo, Bulgaria
12	CA-3	<i>Carduus acanthoides</i>	Varna, Bulgaria
13	CA-4	<i>Carduus acanthoides</i>	Debrene, Bulgaria
14	AL-1	<i>Arctium lapa</i>	Stozher, Bulgaria
15	AL-2	<i>Arctium lapa</i>	Dobrich, Bulgaria
16	AL-3	<i>Arctium lapa</i>	Varna, Bulgaria
17	AL-4	<i>Arctium lapa</i>	Debrene, Bulgaria
18	EP-1	<i>Echinacea purpurea</i>	Commercial seeds, cultivated
19	MC-1	<i>Matricaria chamomila</i>	Commercial seeds, cultivated
20	CO-1	<i>Calendula officinalis</i>	Commercial seeds, cultivated
21	CS-1	<i>Cosmos sp.</i>	Commercial seeds, cultivated
22	DH-1	<i>Dahlia sp.</i>	Commercial seeds, cultivated
23	GS-1	<i>Gazania sp.</i>	Commercial seeds, cultivated
24	HA-1	<i>Helianthus annuus</i>	DAI – General Toshevo

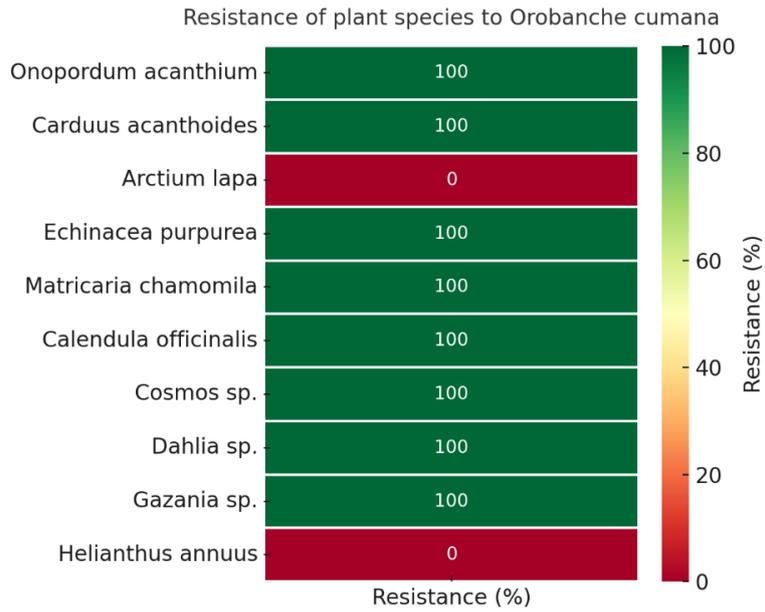
Cultivated sunflower HA-1-25-12 was used as a standard control in the experimental setup. Plants were grown following standard methodology (Panchenko, 1973) in containers filled with a 1:1 mixture of sand and soil, which was inoculated with a defined concentration of broomrape (*Orobanche cumana*) seeds. The experiment was conducted in two replicates, each consisting of 10 plants per accession. After 45 days, the soil was removed, and the presence or absence of tubercles on the host root system was recorded.

## RESULTS AND DISCUSSION

The host range evaluation of *O. cumana* revealed distinct interspecific differences in the responses of the tested Asteraceae species. Among the ten analyzed taxa, eight exhibited complete resistance (100%), whereas two species – *Helianthus annuus* and *Arctium lappa* – were found to be fully susceptible (0% resistance). In all resistant species (*Onopordum acanthium*, *Carduus acanthoides*, *Echinacea purpurea*, *Matricaria chamomilla*, *Calendula officinalis*, *Cosmos sp.*, *Dahlia sp.*, and *Gazania sp.*), no attachment or tubercle formation by *O. cumana* was observed, indicating full incompatibility between host and parasite (Table 2; Figure 1).

**Table 2.** Resistance of different *Asteraceae* accessions to *Orobanche cumana* parasite

Species	Number of grown plants	<i>Orobanche cumana</i> susceptibility		
		Resistant plants number	Susceptible plants number	Resistance, %
<i>Onopordum acanthium</i>	180	180	0	100
<i>Carduus acanthoides</i>	80	80	0	100
<i>Arctium lapa</i>	80	0	80	0
<i>Echinacea purpurea</i>	20	20	0	100
<i>Matricaria chamomila</i>	20	20	0	100
<i>Calendula officinalis</i>	20	20	0	100
<i>Cosmos sp.</i>	20	20	0	100
<i>Dahlia sp.</i>	20	20	0	100
<i>Gazania sp.</i>	20	20	0	100
<i>Helianthus annuus</i>	20	0	20	0



**Figure 1.** Heatmap illustrating percentage resistance of various Asteraceae accessions to *Orobanche cumana*

In contrast, both *H. annuus* and *A. lappa* supported successful haustorial penetration and the formation of visible tubercles, confirming the establishment of a functional parasitic connection and physiological compatibility (Figure 2).





**Figure 2.** Presence of tubercles and emerging shoots on the roots of *Arctium lappa*

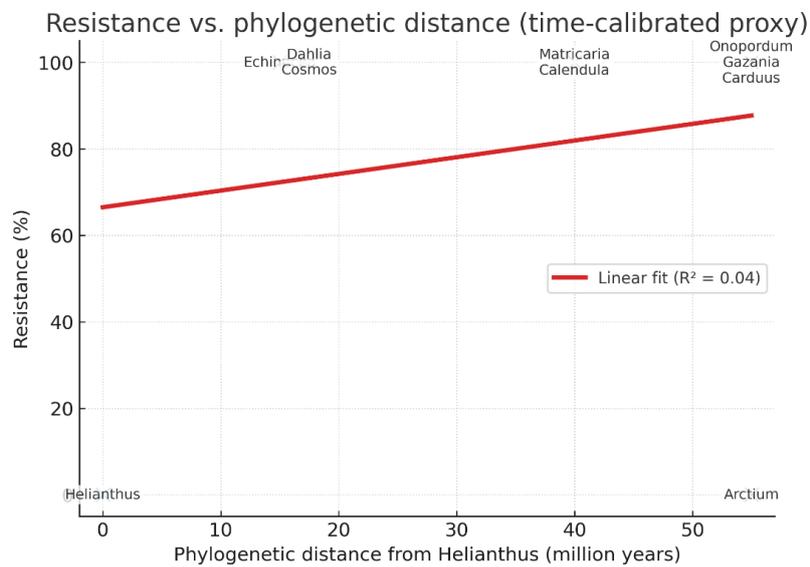
From a phytopathological perspective, the complete resistance observed in most of the tested non-host species is likely associated with the absence of germination stimulants (such as strigolactones) in their root exudates, which are essential for *O. cumana* seed germination. Additionally, histological incompatibility between host and parasite may prevent haustorial penetration and vascular connection. The accumulation of phenolic compounds and localized cell death (hypersensitive response) could further reinforce these defense barriers, as previously described in resistant sunflower genotypes.

The full susceptibility of *H. annuus* was expected, given its role as the natural host of *O. cumana*. However, the fact that *A. lappa* (burdock) also supported parasite attachment and development is particularly noteworthy. This phenomenon may be explained by the presence of similar biochemical signals and compatible root anatomy, reflecting a phylogenetic proximity within the Asteraceae family. This observation is of considerable scientific importance, as **the formation of *O. cumana* tubercles on *A. lappa* has been documented here for the first time.** Until now, the sunflower broomrape has been considered a highly host-specific parasitic plant, infecting exclusively the cultivated sunflower (*H. annuus*) and, in rare cases, closely related wild *Helianthus* species. The newly discovered ability of the parasite to recognize and successfully infect *A. lappa* – a species of the same family but of a different genus – represents a significant expansion of the known host range of *O. cumana* and suggests a potential shift in its host specificity.

From an evolutionary standpoint, this finding may indicate a **potential broadening of the ecological amplitude of *O. cumana*.** The ability to parasitize *A. lappa* could reflect an adaptive change within *O. cumana* populations, possibly driven by genetic variability or recombination among races that have evolved under selection pressure in cultivated fields. This phenomenon has important

epidemiological implications, as it could enable the persistence of the parasite in non-cultivated environments, forming a potential inoculum reservoir and facilitating gene flow between wild and cultivated populations

The relationship between resistance to *Orobanche cumana* and phylogenetic divergence from *Helianthus annuus* was evaluated using time-calibrated distances derived from molecular chronograms of Asteraceae. The family originated around 83 Mya, with major subfamily radiations during the Eocene (Mandel et al., 2019; Zhang et al., 2024). Divergences among tribes of Asteroideae generally occurred 55–40 Mya, while *Echinacea* (Heliantheae) diverged more recently, about 15 Mya. These estimates served as proxies for evolutionary distance in a regression model of Resistance (%) versus Distance (Mya).



**Figure 3.** Relationship between resistance to *Orobanche cumana* and phylogenetic distance from *Helianthus annuus*.

The linear model (Figure 3) showed a positive but weak correlation ( $R^2 = 0.04$ ), reflecting the binary nature of the data, where most species were either fully resistant (100%) or fully susceptible (0%). Despite the low explanatory power, the direction of the trend supports the host–parasite escape hypothesis, which suggests that specialization of *O. cumana* on sunflower limits its ability to infect more distantly related taxa (Poulin & Keeney, 2008). Resistance in distant tribes such as Anthemideae and Calenduleae likely arises from divergence in root chemical profiles and haustorial recognition mechanisms (Joel et al., 2013).

Thus, although the statistical relationship is weak, the evolutionary signal remains clear: resistance predominates in lineages that diverged from *Helianthus* tens of millions of years ago. This pattern emphasizes the evolutionary constraint of host range in *O. cumana* and highlights distant Asteraceae taxa as promising resistance donors in breeding programs (Louarn et al., 2016).

## CONCLUSIONS

This study confirms the strict host specificity of *O. cumana* within the Asteraceae but reveals, for the first time, its ability to parasitize *Arctium lappa* in addition to sunflower. Most tested species showed complete resistance, likely due to the absence of germination stimulants and histological incompatibility. The infection of *A. lappa* suggests a possible shift in host specificity and an expansion of the parasite's ecological range. Although phylogenetic distance correlated weakly with resistance, the trend supports the host-parasite escape hypothesis, indicating that susceptibility is confined to closely related taxa. These findings underscore the importance of monitoring broomrape evolution and exploiting distant *Asteraceae* relatives as potential resistance sources in sunflower breeding programs.

### Conflicts Of Interest

The author declares no conflicts of interest.

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