RESEARCH ARTICLE

Effect of an aggressive new race broomrape (*Orobanche cumana* L.) on agronomical and technological traits of the sunflower in Mediterranean environments

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Kilic Y, Goksoy AT. Effect of an aggressive new race broomrape (*Orobanche cumana* L.) on agronomical and technological traits of the sunflower in Mediterranean environments. AGBIR.2024;40(6):1338-1342.

Sunflower (*Helianthus annuus* L.) is the most important edible oil crop in Turkey as 85% of the edible oil consumed is made from it. A major limiting factor in sunflower-producing countries is infestation by the parasitic plant *Orobanche cumana*. In all regions of Turkey where sunflower is grown, sunflower plants exhibiting various degrees of infestation by this parasite and various invasive groups of this parasite have been observed. The aim

of this study was to assess the tolerance of sunflower varieties to the new aggressive parasitic race and to determine its effect on agronomical and technological traits of the sunflower. Our results over a two-year study of agronomically important traits indicate that the hybrids, HA-4 and HA-5 are fully resistant to the parasite under field conditions and had significantly higher seed yield and oil content than the standards. Therefore, new sources of genetic resistance and herbicide-resistant sunflower hybrids are required against highly invasive races for this region.

Key Words: Broomrape; Oil content; Oleic acid; Seed yield; Sunflower

INTRODUCTION

Sunflower broomrape (*Orobanche cumana* Wallr.) is a holoparasitic plant occurring in the wild from south-eastern Europe to central Asia and parasitizes few species belonging to the family Asteraceae, mainly *Artemisia* spp. It parasitizes sunflower in many sunflower-producing countries, such as central and eastern Europe, Spain, Turkey, Israel, Iran, Kazakhstan and China and is one of the crops most serious production constraints [1]. The Mediterranean region is one of the most important centers of biodiversity; new records indicate that the genus *Orobanche* is represented by 39 species in Turkey [2]. After infecting sunflower roots, the parasite easily takes nutrients and water from the soil through the plant's roots, thus damaging the crop and reducing yields by up to 100% [3]. This leads to considerable decrease in yield and low quality of produce [4].

Over two decades, the aggressiveness of the parasite increased substantially with the appearance of new physiological races after a relative stable period of broomrape race E. For the host spots infested with race G, it is not recommended to plant race E resistant hybrids because of the drastic yield decreases. Hybrids resistant to race F also register drastic yield decreases in these areas. Broomrape, thus, substantially reduced seed and oil production. Moreover, it is not recommended to grow sunflower hybrids with resistance genes for race E or F in locations infested with race G or G+ [5]. Southeast Romania is infested with the most invasive races of O. cumana (G, H and others). Recent studies show that the most aggressive Orobanche race, resistant to the varieties and hybrids commonly used in production, occurs in Adana on Mediterranean coast of Turkey [6].

The invasion of 11 populations of the parasites in Hungary, Romania, Spain and Turkey was assessed after the infection of sunflower inbred lines to differentiate races of the parasite under glasshouse conditions. Analyses of molecular diversity detected race F in Hungary, Spain and Turkey and the most invasive race G in Turkey [7]. Increasingly invasive populations classified as races G and H are becoming predominant in countries surrounding the Black Sea. The new broomrape races detected in the sunflower crop in Turkey seem to be more invasive than the races occurring in other countries [8].

The eastern Mediterranean region of Turkey has very fertile soils and has experienced a gradual increase in acreage and production of sunflower because of the economic interest the crop has stimulated in local farmers. Although no *Orobanche* has been detected in sunflower fields in the region

so far, infestation is possible as the parasite naturally occurs in this region. *Orobanche ramose*, O. *aegyptiaca*, and O. *crenata* were identified infecting tomato, lentil and fava bean fields in this region respectively. It is important to train growers on how to prevent the broomrape infestation from spreading to sunflower fields in the eastern Mediterranean region [9]. In countries, such as Romania, Turkey, Ukraine, Bulgaria and Russia, Broomrape races A-E overcoming all the known resistance genes were also identified and have been named F, G and H [10-12]. Broomrape epidemic breaks out every 20 years (1960, 1980 and 2000) and overcomes the resistance of sunflower cultivars grown in that region. The parasitic interaction between sunflower and O. *cumana* generally follows a gene for gene model, with resistance in sunflower [13].

Sunflower grows widely in Turkey and in other countries as it is highly adaptable, the cultivation is highly mechanizable, it is easy to market and it is one of the most preferred vegetable oil for consumption in Turkey. Weeds and the broomrape parasite are the devastating biotic stress factors that limit seed and oil yield in sunflower production [14].

Estimation of the effects of broomrape infestations on the sunflower under field conditions is difficult as non-infected control plots are necessary [15].

The aim of this study was to evaluate the effect of newly-discovered highly-invasive *O. cumana* races that started to appear on the Mediterranean coast of southern Turkey on the yield and quality of sunflower varieties.

MATERIALS AND METHODS

Genetic material, experiment location and design

Nine sunflower hybrids were used as genetic material in this experiment and two were registered hybrids that were used as check varieties. Field experiments of the research study were conducted at two locations in Adana, Turkey with a randomized complete block design and four replicates. The field experiments were conducted in the spring of 2018 and 2019 in Sagkaya District (37°09'55.8"N 35°43'11.0"E and 37°11'22.1"N 35°41'45.1"E) and Dedeler Disctrict (37°04'48.8"N 35°33'15.6"E and 37°02'51.6"N 35°33'17.0"E) in Adana Province, Turkey. The Adana Province is located on the Mediterranean cost and has an average annual rainfall of 668,1 mm and a mean monthly temperature of 19°C. In this region, the total rainfall received in the growing period of sunflower (March-July) constitutes about 29% of the annual precipitation (Table 1).

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Received: 25-Oct-2024, Manuscript No. AGBIR-24-152637; Editor assigned: 28-Oct-2024, Pre QC No. AGBIR-24-152637 (PQ); Reviewed: 12-Nov-2024, QC No. AGBIR-24-152637; Revised: 20-Nov-2024, Manuscript No. AGBIR-24-152637 (R); Published: 27-Nov-2024, DOI:10.35248/0970-1907.24.40.1338-1342



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TABLE 1
Some meteorological parameters in the experimental area in Adana in 2018 and 2019

	`Ave	rage tempera	ture (°C)	Tota	al precipitatio	n (mm)	Relative humidity (%)			
Months	2018	2019	Last Year Average (LYA)	2018	2019	Last Year Average (LYA)	2018	2019	Last Year Average (LYA)	
March	16	13.1	13.3	38.4	71.2	85.9	71.2	71	64.7	
April	19.2	16.3	17.2	38.2	59.4	45.6	59.3	69.7	64.4	
May	23.3	23.3	21.5	38	1	46.8	64.5	57.8	63.9	
June	25.5	26	25.4	33.4	63	25.6	70.7	72.6	65.4	
July	28.3	27.1	28.2	0.2	63.6	7.25	69.5	73	66.5	

Cultural practices, measurement parameters and statistical analysis

At all locations, optimal agro technical practices were applied for sunflower cultivation. The experimental varieties and checks were planted in a well prepared soil in four rows with a length of 7.5 m. Plant spacing was 70 cm between rows and 30 cm between plants within a row, resulting in a total plot area of 21 m². The two rows in the middle of each plot were selected for the hybrids for observations and harvest. For each hybrid, head diameter and seed yield were measured. Oil content as a percentage was determined in the laboratory by Bruker minispec XL Nuclear Magnetic Resonance (NMR). Oleic acid content was also measured by Spinlock SLK-200 NMR. Variance analysis was performed for all data obtained from the field experiment using the JMP-7 software.

Observations on broomrape included Frequency (F), Intensity (I) and Attacking Rate (AR) based on the pustovoit method. The plants were regarded as resistant if frequency was 0%-10% and AR values were 0-1 [13].

Frequency of plants with Orobanche (F) was calculated as a percentage as follows:

$$F\% = \frac{Number\ of\ plants\ infected\ with\ Orobanche}{Total\ number\ of\ plants\ in\ a\ row} \times 100$$

The mean number of Orobanche per infested plant was calculated as follows:

$$I = \frac{Total \ number \ of \ Orobanche}{Total \ number \ of \ plants \ infested \ with \ Orobanche \ in \ the \ row}$$

 $AR = The number of Orobanche in one plant in the row = F \times I / 100$

RESULTS AND DISCUSSION

Seed yield

The results indicated that seed yield was significantly affected by year, variety, and location \times year interaction (p \leq 0.01) (Table 2). Analysis of variance showed statistically significant location \times variety \times year interaction. The second year (2019) had lower seed yield in sunflower infected with broomrape than the first year (2018). Observed broomrape frequency was higher in 2019 than in 2018 in Dedeler (Table 3).

Infection of sunflower with broomrape affected yield (Figure 1). The Pustovit method showed that varieties Check2 and HA-7 had high tolerance against broomrape attacks in 2018; however, both varieties were susceptible in 2019 due to very high broomrape infection frequency. Varieties Check1, HA-8 and HA-9 were susceptible to broomrape in both years in the broomrapeinfected location. Grenz et al., recorded similar reductions in sunflower yield due to Orobanche infection [16]. The low broomrape infestation could cause 5%-20% loss of yield and high infestation could cause over 50% loss of yield. Prodan et al., reported that the hybrids with good resistance gave good seed yield due to their behavior during the broomrape attack; although, the degree of broomrape attack was higher in 2020 than in 2021 [17]. Molinero-Ruiz et al., reported that an increase in sunflower yield because of the use of resistant hybrids was most noticeable during high field infestations [18]. Stevan et al., observed that hybrids exhibited different susceptibilities and variable responses to broomrape attack [19]. The seed yield loss in infected sunflower plants varied from 2.6% to 70%. The intensity of damage depends on severity of attack and decrease of the seed yield depends on number of broomrape plants per sunflower plant.

Results presented in Table 3, show that in Dedeler in 2018 and 2019, races H and overcame broomrape resistance standard checks. The effect of the attack depended on genotype, which conferred different tolerances to broomrape.

In this study, broomrape AR ranged between 0.00% and 7.12% in 2018 and between 0.00% and 23.92% in 2019. Gospodinov (1960) reported that if the number of broomrape plants per sunflower plant is from 1 to 10, the seed yield decreases by 13.8%, and if the number of broomrape plants per sunflower plant are from 111 to 130 seed yield decreases by 70%.

Oil content

The John's Mathematical Program (JMP) based variance analysis results showed that oil content was significantly affected by year and variety interactions. Additionally, there was a significant effect of location \times year \times variety interactions on the oil content (Table 2).

Broomrape infection of sunflower affecting oil content was high in 2019 in Dedeler because of high broomrape infection rate. Thus, we concluded that the oil contents decreased in direct proportion to broomrape infection rate and not because of the locations where the infection was either low or absent (Figure 2).

Similar results have also been found by Gisca who observed that oil content in the hybrids favorit, Performer and LG5661 was affected to some extent by the degree of parasite infection [20]. The difference in oil content in sunflower seeds between the uninfected/poorly infested fields and those heavily infested was about 2%-3% (favorit, LG5661) and 5%-6% (performer), respectively. Shindrova et al., reported that the decrease in oil content of sunflower plants infected by broomrape is directly proportional to the intensity of infection [21]. Liu et al., observed that oil content in seeds of the plants infected by broomrape drops by 3%-18% [22].

Head diameter

Location, varieties, location \times variety, year and location \times year interactions had significant effects on head diameter (p \le 0,01). Head diameter decreased significantly with broomrape infestation in both trial years. The lowest head diameter in the second year was 9.75 cm in broomrape infested fields, whereas it was 11.50 cm in the first year (Figure 3). In 2018, only a few varieties were affected by broomrape due to low infestation. The hybrids HA-4, HA-5 and HA-6 were an exception, as they had higher diameter than non-infected plants. This could be attributed to the relatively low frequency of broomrape infection in addition to other factors that influence head diameter. Some differences may occur owing to differences in genotypes and environmental differences. Moreover, in 2019, most of the varieties were affected by higher rate of broomrape infection. The most pronounced effect on head development under broomrape stress was found in the HA-9 variety. In this case, the sunflower head diameter was significantly lower (-34.4%) than that of the checks (Figure 3).

Broomrape infection directly affects yield components, e.g. decreases of up to 20.40% in the sunflower head diameter compared to check varieties [23]. Similarly, lowest values of head diameter ranging from 13.4 to 17.4 cm were recorded under infection conditions by Gisca et. al., [24]. Shindrova et al., reported that in sunflower plants infected by broomrape, decrease in head diameter directly depends on the intensity of infection [21]. Duca reported that broomrape has a negative effect on sunflower development; the infected plants are smaller, the sunflower head diameter is reduced and up to 80% of yield losses are observed [25].

TABLE 2
Combined analysis of variance for yield and quality traits under *Orobanche* spp. infection (across two years)

				Seed y	rield Oil content			Head diameter			High oleic			
Source	DF¹	DF ²	2018	2019	2018-2019	2018	2019	2018-2019	2018	2019	2018-2019	2018	2019	2018-201
Loc	1		ns	**		**	*		**	**		*	**	
Loc*var	8		**	**		**	ns		**	**		ns	ns	
Rep (loc) and ran	6		ns	ns		ns	ns		ns	*		ns	ns	
Year		1			**			**			**			ns
Loc (year)		2			*			**			**			**
Rep (year, loc)		12			ns			ns			**			ns
Var	8	8	**	**	**	**	ns	**	**	**	**	**	**	**
Year*var		8			ns			ns			**			**
Loc*var (year)		16			**			*			**			ns
Error	48	96												

Note: ns: DF¹: Degrees of freedom for variance analysis of individual years; DF²: Degrees of freedom for combined analysis of variance not significant; *: significance level (p \leq 0.05); **: significance level (p \leq 0.01).

TABLE 3
Soil infection by *O. cumana* in some fields affected yield and limited development of the crop in the Adana region

Entry No	Hybrids		20	18	2019			
		Rep	Sagkaya	Dedeler	Sagkaya	Dedeler % (Absence of resistance)		
			% (Absence of resistance)	% (Absence of resistance)	% (Absence of resistance)			
1	Check 1	1	0	4.09	0	11.83		
1	Check 1	2	0	2.58	0	17.16		
1	Check 1	3	0	3.95	0	10.59		
1	Check 1	4	0	3.06	0	7.06		
2	Check 2	1	0	0.05	0	11.35		
2	Check 2	2	0	0.85	0	16.4		
2	Check 2	3	0	0.36	0	15.28		
2	Check 2	4	0	0.66	0	16.63		
3	HA-3	1	0	0.02	0	0.8		
3	HA-3	2	0	0.28	0	0.11		
3	HA-3	3	0	0.04	0	0.13		
3	HA-3	4	0	0.12	0	0.13		
4	HA-4	1	0	0	0	0		
4	HA-4	2	0	0	0	0		
4	HA-4	3	0	0	0	0		
4	HA-4	4	0	0	0	0		
5	HA-5	1	0	0	0	0.05		
5	HA-5	2	0	0	0	0.6		
5	HA-5	3	0	0	0	0.2		
5	HA-5	4	0	0	0	0.14		
6	HA-6	1	0	0	0	0		
6	HA-6	2	0	0	0	0.01		
6	HA-6	3	0	0	0	0		
6	HA-6	4	0	0	0	0.01		
7	HA-7	1	0	0.04	0	8.54		
7	HA-7	2	0	0.89	0	5		
7	HA-7	3	0	0.68	0	4.71		
7	HA-7	4	0	0.54	0	6.08		
8	HA-8	1	0	5.32	0	18.14		
8	HA-8	2	0	4.62	0	10.5		
8	HA-8	3	0	5.24	0	16.04		
8	HA-8	4	0	6.25	0	17.84		

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9	HA-9	1	0	7.12	0	7.13
9	HA-9	2	0	6.64	0	23.73
9	HA-9	3	0	6.4	0	23.92
9	HA-9	4	0	5.69	0	11.2

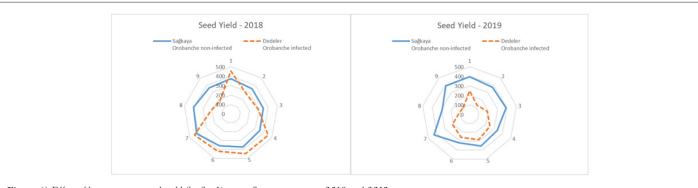
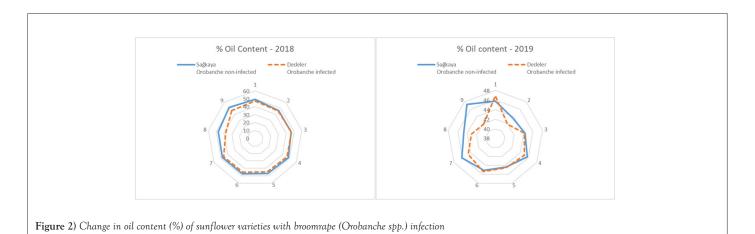
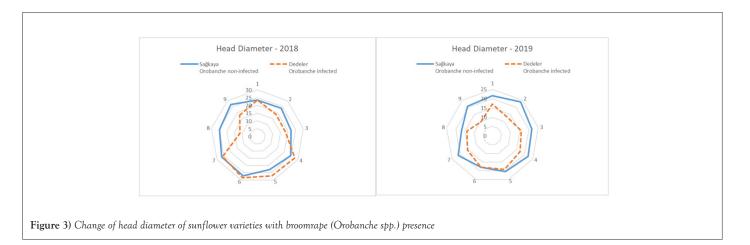


Figure 1) Effect of broomrape on seed yield (kg/ha-1) in sunflower varieties in 2018 and 2019



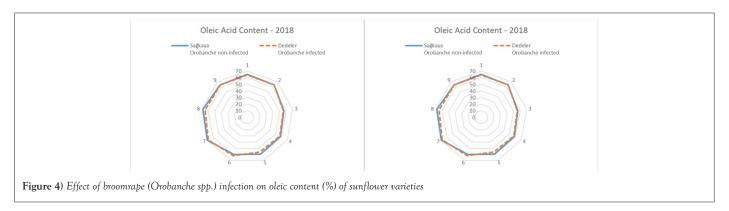


Oleic acid content

The year's effect on oleic acid content was not significant; although, there was a significant effect of year × variety interactions on oleic acid content. There was no significance in the location × variety × year interaction in both years and locations (Table 2). However, location and location × year interactions had significant effects on oleic acid content. Oleic acid content is determined by Ol genes and genetic factors and is also highly influenced by environmental factors [26]. Fatty acid content varies with genetic, climatic, ecological, morphological, physiological and cultural practices (Figure 4) [27].

Oleic acid content of sunflower varieties was not influenced by broomrape infestations. Similar results were reported by Shindrova et al., who found no changes in composition quality of the analyzed fatty acids under broomrape

infection [21]. Hosni et al., failed to identify relationship between fatty acid composition and resistance to broomrape infestation [28]. According to Gisca, the content of oleic acid in the hybrids Sandrina, HS5034 and HS3045, with mean resistance and varieties LG-3, Favorit and HS3655 with a high resistance to broomrape, varies mainly in relation to the genotype and cultivation conditions and depends less on infection by the parasite [20]. Sumalan et al. reported that broomrape parasitism on sunflower plants did not show any impact on palmitic and stearic acid contents, the differences being nonsignificant; however, oleic and linolenic acid levels increased in parasitized plants [29]. Akar et al., emphasized that besides having high oleic acid content, sunflower cultivars should also have high yield, high oil content and resistance to *Orobanche (Orobanche cumana Wallr.)* and mildew in Turkey [30].



CONCLUSION

The values of the analyzed productivity indices vary a lot depending on the genotypes of sunflower. Some hybrids (e.g. the hybrid HA-3, HA-4, HA-5 and HA-6) are resistant to broomrape and endure its attack with low losses; however, hybrids HA-8 and HA-9 with the highest degree of infection, were the most affected, showing significantly decreased indices for all the studied characteristics.

Our results indicate that tested hybrids expressed different susceptibility and variable response in seed yield, oil content and head diameter to broomrape parasitism. The influence of environmental conditions, locations, production years and broomrape presence on oleic acid content depended on the variety. By testing the new hybrids for broomrape resistance, seed yield and oil content simultaneously, the introduction of new high yielding hybrids resistant to broomrape was possible. Additionally, further research and breeding programs should be undertaken urgently to limit O. Cumana's spread.

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