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Distribution and virulence of *Orobanche cumana* Wallr. in sunflower weed communities of northeastern Croatia

Rasprostranjenost i štetnost *Orobanche cumana* Wallr. u korovnim zajednicama suncokreta na području sjeveroistočne Hrvatske

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ABSTRACT

Orobanche cumana Wallr. is a parasitic angiosperm distributed in many sunflower-producing countries worldwide, where it can cause a severe yield loss. Field surveys in sunflower fields during July-August in 2017, 2019, and 2021 were conducted in the northeastern part of Croatia to investigate broomrape attack rate, weed community where broomrape was present, and weed management strategies used by the farmers. Although, it was observed the difference between the morphometric characteristics of broomrape in the investigated area (Baranja, Syrmia, and Slavonia), the most concerning fact is a trend of increasing attack rate from 2017 to 2021, recorded in the whole region. Weed communities that co-exist with broomrape consist of a total of 31 species, where late spring broadleaf weeds predominate (58% of the total weed infestation), followed by the late spring grass weeds (16%). The most abundant was *Ambrosia artemisiifolia* followed by *Chenopodium album*, *Xanthium strumarium*, *Setaria viridis*, *Cynodon dactylon*, *Sorghum halepense*, *Convolvulus arvensis* and *Orobanche cumana*. No phytosociological affinities were observed between broomrape and weed communities in sunflowers. The full CCA model explained 82.81%, while the first two axes explained 55.92% of the total variation in community composition. The most important predictors were herbicide and sunflower hybrid choice, while preceding crops, sunflower sowing rate and broomrape attack rate accounted for significantly less variance.

Keywords: root parasitic weed, attacking rate, morphometric characteristics, oilseed crop

SAŽETAK

Orobanche cumana Wallr. je parazitska cvjetnica rasprostranjena u mnogim područjima uzgoja suncokreta gdje pričinjava značajne gubitke u prinosu. Na području sjeveroistočne Hrvatske provedena su istraživanja tijekom srpnja i kolovoza 2017., 2019. i 2021. godine s ciljem utvrđivanja jačine parazitiranja volovoda, korovne zajednice u suncokretu gdje je volovod prisutan, te strategije suzbiljanja korova koje su na tim poljima koristili farmeri. Premda su utvrđene razlike u morfometrijskim obilježjima volovoda na području Baranje, Srijema i Slavonije, ono što zabrinjava je uočeni trend porasta intenziteta štete na cijelom istraživanom području. Korovna zajednica u kojoj je koegzistirao volovod sastojala se od 31 vrste, gdje su dominirali kasnoproljetni širokolisni korovi (58%), a zatim kasnoproljetni uskolini korovi (16%). Najzastupljenija je bila *Ambrosia artemisiifolia*, te zatim *Chenopodium album*, *Xanthium strumarium*, *Setaria viridis*, *Cynodon dactylon*, *Sorghum halepense*, *Convolvulus arvensis* i *Orobanche cumana*. Nije utvrđena fitocenološka povezanost između volovoda i korovne zajednice u suncokretu. CCA modelom objašnjeno je 82,81% varijance, a prve dvije osi čine 55,92% ukupne varijance florističkog sastava. Najznačajniji indikatori bili su herbicidi i odabir hibrida suncokreta, dok su predusjevi, sklop suncokreta i intenzitet napada volovoda sudjelovali sa znatno manje varijance u modelu.

Ključne riječi: parazitski korov korijena, intenzitet napada, morfometrijska obilježja, uljarica

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oil crops worldwide, after palm and soybean. Also, it is the basic oilseed crop in the Republic of Croatia, and one of the most popular among farmers due to favorable trends in oil prices and continuous production needs (Mijić et al., 2022). According to EUROSTAT (2024), the area under sunflower cultivation reached 62.000 ha, with an average grain yield of 2.8 t/ha.

Areas under sunflower production are growing with a continuous demand for a high and stable yield (Kaya et al., 2015; Velasco et al., 2015). This can be achieved by choosing an appropriate hybrid, expansion of crop rotation, and careful crop management (Flenet et al., 2008). However, weeds have been and continue to be a major production constraint in sunflower production (Malidza et al., 2016). To obtain a high yield, it is necessary to effectively control weeds, particularly the late-spring weeds (Pinke et al., 2013). Many authors point out *Chenopodium* spp., *Amaranthus* spp., *Abutilon theophrasti* Medik., *Datura stramonium* L., *Convolvulus arvensis* L., *Polygonum* spp., *Echinochloa crus-galli* (L.) P. Beauv., *Setaria* spp., *Ambrosia artemisiifolia* L., *Cynodon dactylon* (L.) Pers. and *Orobanche cumana* Wallr. as dominant weeds in sunflower production (Szabo et al., 2008; Týr and Vavrík, 2015).

Among them, a sunflower broomrape is an obligate, chlorophyll-lacking root parasitic angiosperm that becomes a severe problem in many sunflower-producing countries around the world. It is mostly present in Mediterranean and sub-tropical climates, but also in all sunflower-growing countries in Central-Eastern and Eastern Europe (Tonev et al., 2020). Once sunflower broomrape is attached to the root system of the sunflower, it reduces capitulum size and kernel ratio, leading to a significant decrease in yield. In a highly infested area, yield losses due to broomrape infestation could be close to 100% (Fernández-Martínez et al., 2015). Moreover, a single sunflower broomrape plant can produce more than 500.000 seeds, and they are persistent in soil and require dual germination requirements of a moist period and a chemical stimulus from the nearby host roots (Kebreab and Murdoch, 2001).

Several studies have provided the effects of sunflower broomrape parasitism and phenology on sunflower growth (Sisou et al., 2021), and some have conducted a competition trial to estimate crop loss due to parasitism (Garcia-Torres et al., 1996).

This study aims to contribute to the understanding of the intensity of sunflower broomrape attacks in sunflower fields of northeastern Croatia and explore if there is a relationship between the intensity of sunflower broomrape attacks and associated crop management and weed community.

MATERIAL AND METHODS

Study site

The study area is located in the far northeastern part of Croatia, in Osijek-Baranja and Vukovar-Syrmia counties, situated between the Sava, Drava, and Danube rivers (Figure 1).

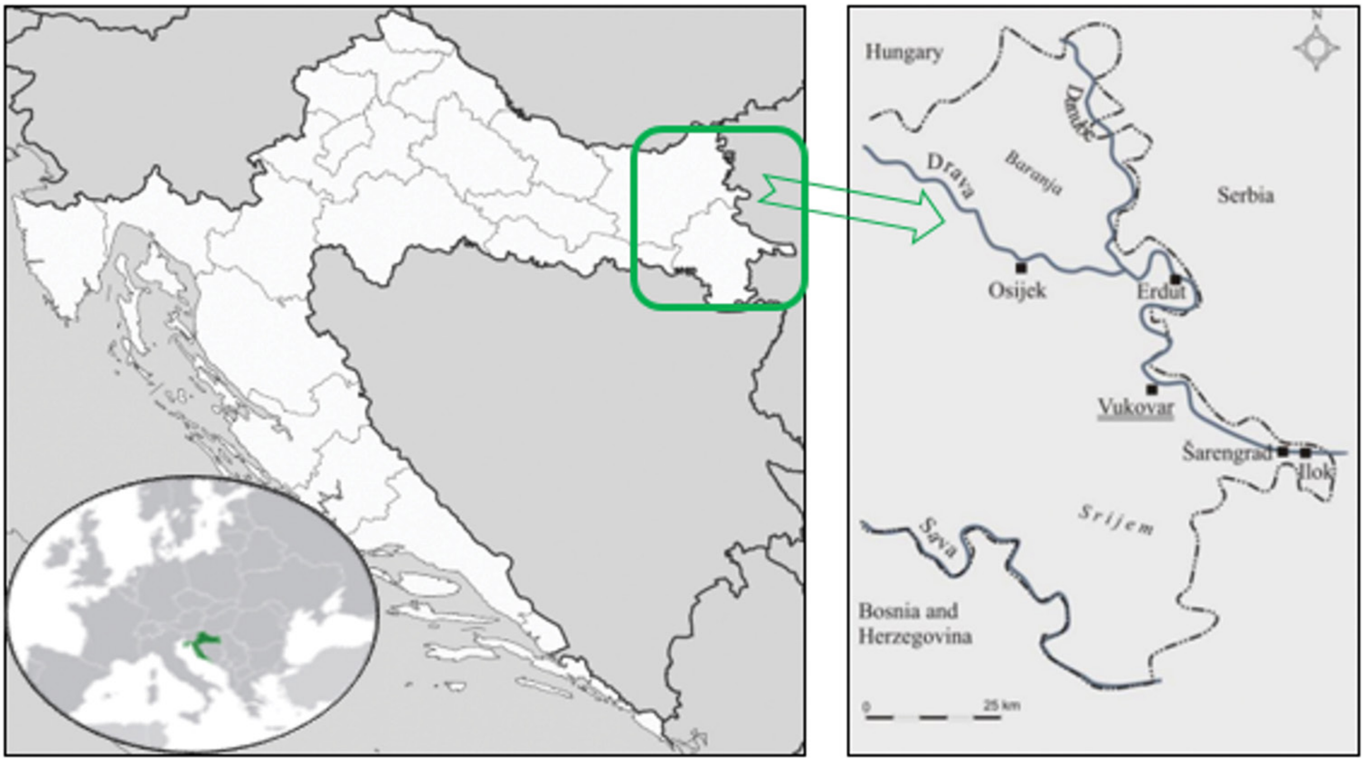

Figure 1. Map of the investigated region (A) with marked Baranja, Syrmia and Slavonia parts (B)

It lies between 45.952 and 44.823 northern latitudes and 17.873 and 19.529 eastern longitudes of the Pannonian plains, representing Croatia's most fertile agricultural region, with the sunflower as one of the main crops.

This area experienced a typical continental climate of warm summers and cold winters, with the average summer temperature amounting to 25 °C and winter 0 °C. Average annual precipitation in the region outreach 700 mm.

Data collection

Field surveys were carried out in sunflower fields during July-August in 2017, 2019, and 2021. Throughout the study period, a sunflower broomrape infection was observed in a total of 34 sunflower fields (13 fields in 2017, 10 fields in 2019, and 11 fields in 2021, respectively), and location information was collected and recorded with GPS.

In each location, an area of 10 m x 10 m was selected for further analysis. The middle sunflower row was used for sunflower broomrape observations, and was evaluated for frequency (F), intensity (I), and attacking rate (AR), according to Kaya et al. (2004), where:

Frequency (F) = (The sunflower plant number infested by sunflower broomrape / Total plants in the row) × 100

Intensity (I) = Total sunflower broomrape / Total plants infested by sunflower broomrape in the row

Attacking rate (AR) = (F × I) / 100.

Then, fifteen sunflower broomrape plants were collected from each field for morphometric measurements (plant height, length of inflorescence, number of flowers).

The weed community inside the selected 10 m x 10 m area (with sunflower broomrape infection) was estimated visually (Mueller-Dombois and Ellenberg, 1974). For the percentage ground cover of weed species and sunflower broomrape in each plot following scale intervals were used: 0-1, 1-5, 5-10, 10-25, 25-50, 50-75, 75-100%.

Finally, a survey questionnaire was developed for the farm owners with questions covering the topics of sunflower cropping practice, weed concerns, and sunflower hybrid use. Farms are located by using ARKOD (https://arkod.hr), and the survey was conducted by personal collection of information from farmers and agronomists or others involved in weed management on farms.

Statistical analysis

Sunflower broomrape morphometric data and attack rate were analyzed with repeated-measure ANOVA with year as a repeated measure, in the SPSS statistical package (IBM® SPSS Statistics for Windows, version 22). Mauchly's criterion test for the compound symmetry of the variance-covariance matrix was used automatically, together with corrected significance levels in case of the rejection of the symmetry assumption (Pallant, 2020).

A multivariate statistic, canonical correspondence analysis (CCA), was run using the CANOCO 5 software for the analysis of the weed community data, sunflower broomrape attack rate, and weed management options (ter Braak and Smilauer, 2012). The ratio of an individual canonical eigenvalue to the sum of all eigenvalues was used to assess the proportion of the explained variation. The effects were tested by Monte Carlo permutation tests with 999 iterations.

RESULTS AND DISCUSSION

The sunflower broomrape attack rate in northeastern Croatian sunflower fields is presented in Table 1. Within a 10 m row, the number of sunflower plants having a sunflower broomrape infestation ranged from 1 to 11. Sunflower broomrape attacks vary in number, frequency, and intensity, and attack rates ranged from 0.03 in 2017 to 1.34 in 2021. Also, different level of tolerance in attack rate was determined in different locations (Figure 1; Baranja, Syrmia, Slavonia) as Kaya et al. (2004) refer to Turkey. Moreover, the authors conclude that sunflower lines and hybrids resistant in one location in the previous year could show susceptibility in the subsequent year, leading to the conclusion that new broomrape races change their reactions depending on year and location.

A similar trend has been observed in the Republic of Moldova (Duca et al., 2019), where broomrape has expanded considerably in new areas in the center and north, but not only in the southern areas.

Sunflower broomrape morphometric observations are shown in Table 2 and Figure 2. Opposite to the attack rate, there were no significant differences between years of investigation (2017, 2019, 2021). However, plant height (P < 0.001) and number of flowers per plant (P < 0.001) significantly differ between locations. Interactions between year and location for plant height and number of flowers per plant were also significantly different (P < 0.032 and P < 0.045).

A significantly higher number of sunflower broomrape plants were observed in Syrmia compared to Baranja and Slavonia, while a number of flowers per sunflower broomrape plant was found significantly higher each study year in the Baranja region. For the length of the inflorescence, no differences were observed throughout the study.

A weed community that was found in sunflower broomrape-attacked areas consists of a total of 31 species (Table 3). Out of the total recorded species, late-spring broadleaf weeds predominate (58% of the total weed infestation), followed by late-spring grass weeds (16%). Tonev et al. (2020) also confirm the dominance of these two functional groups as the most significant in sunflower fields since their mass development and reproduction coincide with the sunflower growing season.

The most abundant weed species throughout the study was *Ambrosia artemisiifolia* (Table 3). This invasive and very noxious weed represents a great problem in sunflower fields of this region (Pinke et al., 2013; Fried et al., 2022; Mijić et al., 2022). Among subdominant weeds that can cause a damage to the crop were *Chenopodium album*, *Xanthium strumarium*, *Setaria viridis*, *Cynodon dactylon*, *Sorghum halepense*, *Convolvulus arvensis* and *Orobanche cumana*. According to Simić et al. (2011), these troublesome weeds in sunflowers usually have an advantage at the outset due to the sunflower's slow initial growth.

Table 1. Sunflower broomrape infection observation inside a 10 m sunflower row during the study period (2017-2021) in north-eastern Croatia

Sample	Year/ Location*	Sunflower number with broomrape	Broomrape number	Frequency (%)	Intensity	Attack rate
1	2017. Bar	2	13	5.71	6.5	0.37
2	2017. Bar	3	20	8.57	6.7	0.57
3	2017. Bar	4	10	11.42	2.5	0.28
4	2017. Bar	2	5	5.71	2.5	0.14
5	2017. Syr	4	14	11.42	3.5	0.39
6	2017. Syr	5	17	14.28	3.4	0.48
7	2017. Syr	4	23	11.42	5.8	0.65
8	2017. Syr	8	10	22.85	1.3	0.28
9	2017. Syr	4	10	11.42	2.5	0.28
10	2017. Syr	2	5	5.71	2.5	0.14
11	2017. Sla	1	1	2.85	1	0.03
12	2017. Sla	2	2	5.71	1	0.05
13	2017. Sla	1	4	2.85	4	0.11
14	2019. Bar	9	25	25.71	2.8	0.71
15	2019. Bar	6	15	17.14	2.5	0.42
16	2019. Bar	3	20	8.57	6.7	0.57
17	2019. Syr	5	16	14.28	3.2	0.45
18	2019. Syr	7	21	20.0	3.0	0.60
19	2019. Syr	7	14	20.0	2.0	0.40
20	2019. Syr	5	8	14.28	1.6	0.22
21	2019. Sla	2	3	5.71	1.5	0.008
22	2019. Sla	1	3	2.85	3.0	0.008
23	2019. Sla	1	2	2.85	2.0	0.05
24	2021. Bar	4	15	11.42	3.8	0.42
25	2021. Bar	5	24	14.28	4.8	0.68
26	2021. Bar	4	17	11.42	4.2	0.48
27	2021. Bar	6	20	17.14	3.4	0.57
28	2021. Syr	11	47	31.42	4.3	1.34
29	2021. Syr	10	45	28.57	4.5	1.28
30	2021. Syr	6	28	17.14	4.7	0.79
31	2021. Syr	8	20	22.85	2.5	0.57
32	2021. Sla	2	2	5.71	1.0	0.57
33	2021. Sla	2	12	5.71	6.0	0.34
34	2021. Sla	3	10	8.57	3.3	0.28

*Location: Bar = Baranja; Syr = Syrmia (Srijem); Sla = Slavonia

Table 2. Repeated-measures ANOVA for the morphometric characteristics of sunflower broomrape in sunflower grown in north-eastern Croatia (2017-2021)

Variable	df	Morphometric characteristics of broomrape						Attack rate	
		Plant height		Length of inflorescences		Number of flowers per plant			
		F	sig	F	sig	F	sig	F	sig
Between-subject source									
Year (Yr)	2	1.668	0.114	0.674	0.842	1.436	0.066	9.131	0.002
Error	4								
Within-subject source									
Location (Loc)	2	513.502	0.000	121.182	0.286	1137.919	0.000	4.569	0.000
Yr x Loc	4	6.196	0.032	0.604	0.970	5.158	0.045	2.223	0.022
Error	84								

Notes: The significance levels (*P < 0.05; ** P < 0.01; *** P < 0.001) of effects are shown for each variable. Geisser-Greenhouse adjusted probabilities were used within-subject analysis. System means and standard errors are shown in Figure 1.

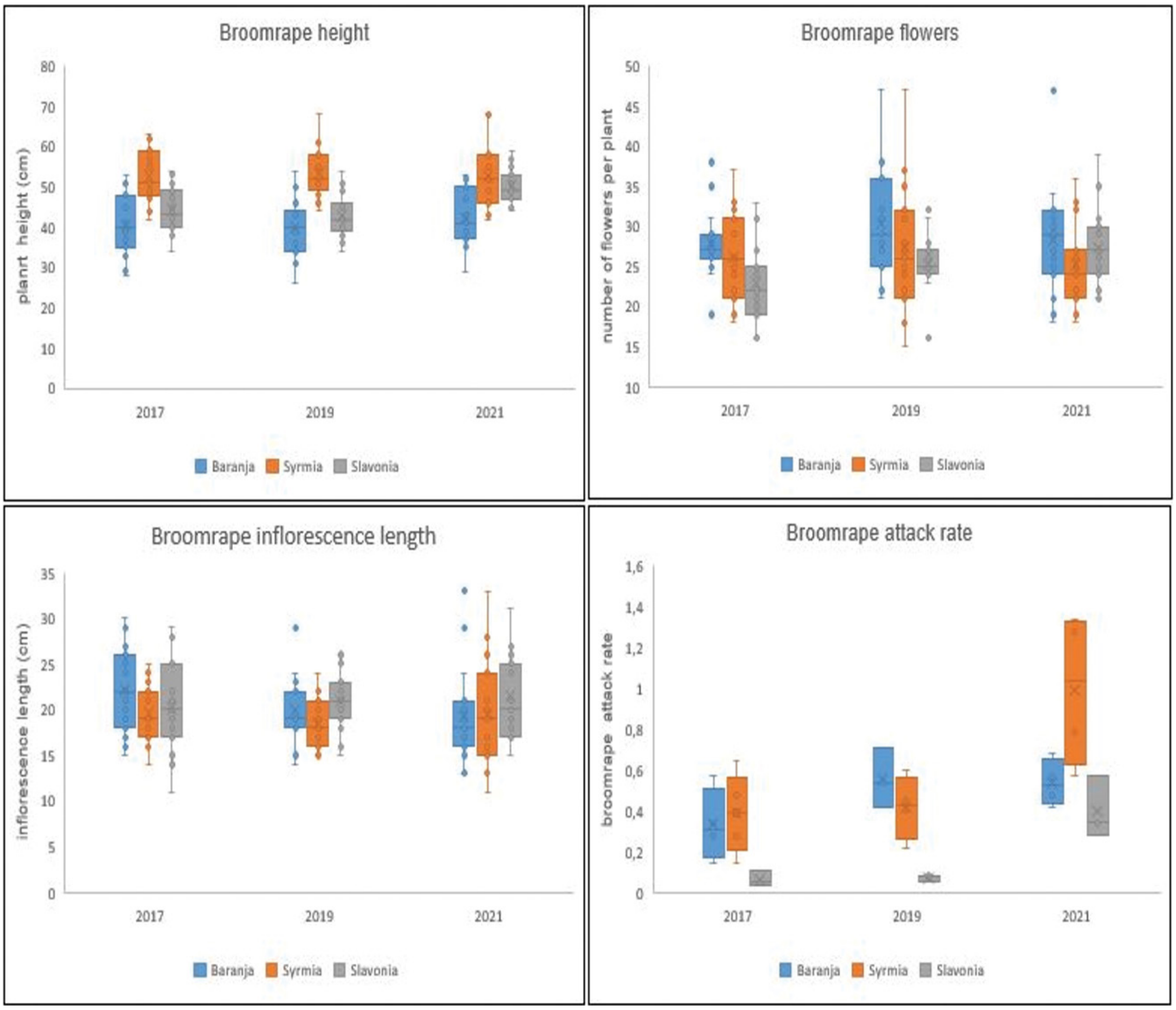

Figure 2. Sunflower broomrape morphometric observation and attack rate in sunflower (Northeastern Croatia, 2017-2021)

Table 3. Weed species mean cover (%) in northeast Croatian sunflower fields from 2017 to 2021

Unctional group	Grass weed		Broadleaf weed	
		Mean cover %*		Mean cover %*
I Annual				
Early spring			*Sinapis arvensis* L.	0.5
Late spring	*Digitaria sanquinalis* (L.) Scop.	3	*Abutilon theophrasti* Med.	3
	Echinochloa crus-galli (L.) Beauv.	3	*Amaranthus retroflexus* L.	3
	Panicum milliaceum L.	0.5	*Ambrosia artemisiifolia* L.	37.5
	Setaria viridis (L.) Beauv.	7.5	*Atriplex patula* L.	0.5
	Setaria glauca L.	3	*Chenopodium album* L.	17.5
			Chenopodium hybridum L.	0.5
			Conyza canadensis L. Cronq.	3
			Datura stramonium L.	3
			Galinsoga parviflora Cav.	0.5
			Hibiscus trionum L.	3
			Lathyrus pratensis L.	3
			Lamium purpureum L.	0.5
			Polygonum aviculare L.	3
			Polygonum persicaria L.	3
			Portulaca oleracea L.	3
			Solanum nigrum L.	3
			Sonchus arvensis L.	0.5
			Xanthium strumarium L.	7.5
II Perennial				
Rhizome	*Sorghum halepense* (L.) Pers.	7.5	*Lathyrus tuberosus* L.	0.5
Root-sprouted	*Cynodon dactylon* (L.) Pers.	17.5	*Cirsium arvense* (L.) Scop.	3
			Convolvulus arvensis L.	7.5
III Cryptogam				
	Equisetum arvense L.	0.5		
IV Parasite				
			Orobanche cumana Wallr.	7.5

* mean percentage of ground cover (2017-2021) according to Mueller-Dombois and Ellenberg (1974)

Sunflower broomrape cover values inside the sunflower crop were between 5 to 10% (mean value = 7.5). During the study period, no phytosociological affinities were observed between broomrape and weed communities in sunflowers. However, Ouallah et al. (2009) reported that some correlation could be established between broomrape (*Orobanche crenata* Forsk.) and some weed species.

Developing an integrated weed management (IWM) plan allows farmers to achieve and maintain low weed numbers while remaining profitable. As Mijić et al. (2022) emphasized, herbicide applications are only a complement to non-chemical measures, which, among many others, in this survey, weed management strategy includes crop rotations, manipulation with sowing rate, herbicide rotations and choice of sunflower hybrid (Figure 3).

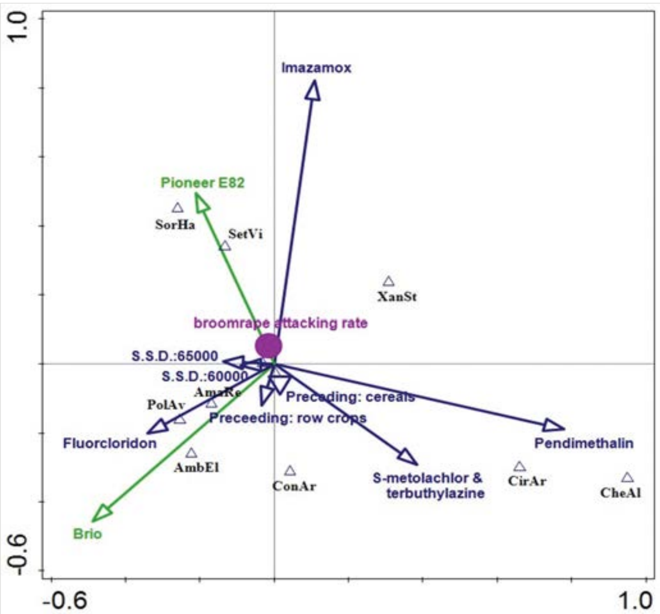

Figure 3. Ordination diagram of the CCA model referring to the survey questionnaire developed for the farm owners and sunflower broomrape attack rate (Bayer codes are used as acronyms for weed species. Only species with the highest importance values are shown (AmbEl = *Ambrosia artemisiifolia*; AmaRe = *Amaranthus retroflexus*; CheAl = *Chenopodium album*; CirAr = *Cirsium arvense*; ConAr = *Convolvulus arvensis*; PolAv = *Polygonum aviculare*; SetVi = *Setaria viridis*; SorHa = *Sorghum halepense*; XanSt = *Xanthium strumarium*)

The full CCA model (Table 4) explained 82.81% of the variance, while the first two axes explained 55.92% of the total variation in community composition.

Table 4. Eigenvalues and cumulative percentage of explained variance of the CCA model

Axis loading	Axes			
	1	2	3	4
Eigenvalues	0.1592	0.1191	0.0768	0.0570
Cumulative percentage of explained variance	31.98	55.92	71.35	82.81
Total inertia				2.172
F - ratio*				5.8
P - value**				0.021

* *F* - ratio – for the test of significance of all canonical axes

** *P* - value – corresponding probability value obtained using the Monte Carlo permutation test (999 permutations)

The most important predictors were herbicide and sunflower hybrid choice (Figure 3). The first axis can be most related to the explanatory variable sunflower hybrids' choices and fluorochloridon herbicide application, while the second axis is strongly correlated with imazamox and pendimethalin herbicides. Negative values along the first axis indicate sunflower hybrid BRIO treated with fluochloridon herbicide with *Ambrosia artemisiifolia*. High axis 1 values tend to be sites growing other sunflower hybrids and mostly treated with pendimethalin, where frequent incidences of *Chenopodium album* and *Cirsium arvense* were noticed. With high values on the second CCA, axes are fields treated with imazamox herbicides where the Pioneer E82 was sown. Those fields are characterized by the presence of *Sorghum halepense* and *Setaria viridis*.

Preceding crops, sunflower sowing rate, and sunflower broomrape attack rate stand for significantly less variance than sunflower hybrids and particularly herbicide options. The lack of significant multivariate relationships in the CCA model for those variables may be attributed to the masking effect due to the highly competitive effects of above mentioned dominant weeds.

Interestingly, the sunflower broomrape attack rate was not significantly correlated with any management variables, but significant attention should be paid since it was observed as an increasing trend during the study (Figure 2). As Duca et al. (2019) concluded, sunflower broomrape presence can be influenced not only by short rotations, type of sunflower hybrids, and soil parameters but also by weather conditions such as higher temperatures and lower humidity. Besides, sunflower broomrape seeds are long-lived and present in soil largely due to the dual germination requirements (Kebreab and Murdoch, 1999). All these, together with their fecundity (Parker and Riches, 1993), make broomrapes extremely difficult to control.

CONCLUSIONS

The parasitic angiosperm *Orobanche cumana*, present in sunflower fields of northeastern Croatia, has been showing an increasing attack rate from 2017 to 2021. Sunflower broomrape differs in morphometric characteristics, and significantly higher plants were observed each study year in Syrmia, while a number of flowers per plant was significantly higher in Baranja. Weed communities that co-exist with broomrape in sunflowers consist of a total of 31 species, and late-spring broadleaf weeds predominate. No phytosociological affinities were observed between broomrape and weed communities in sunflowers. The most important predictors were herbicide and sunflower hybrid choice, while preceding crops, sunflower sowing rate and broomrape attack rate stand for significantly less variance.

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