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RESEARCH ARTICLE OPEN ACCESS

Influence of environmental conditions on the virulence and distribution of *Orobanche cumana* Wallr. in the Republic of Moldova☆

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Abstract – The parasitic angiosperm *Orobanche cumana* is present in the main sunflower-producing countries around the world. In recent years, more aggressive races of broomrape have evolved and the parasite has rapidly spread to new areas. A similar trend has been observed in the Republic of Moldova. At the beginning of 1950s, broomrape was detected in Moldova, especially in the southern areas. Currently, all the known races of *Orobanche* are present in the country and the parasite has expanded considerably on new areas in the center and north. Based on these results and the data reported by other authors, related to influence of climate change on the phytosanitary situation, we studied the interdependence between the climate and the distribution of *Orobanche cumana* Wallr. over different parts of the Republic of Moldova. Prevalence of broomrape infection mainly in the southern and central part of the Republic of Moldova and its sporadic presence in the northern part can be influenced, not only by short rotations, type of sunflower hybrids grown and soil parameters, but also by the weather conditions such as higher temperatures and lower humidity in the south and center. Based on multiannual data and trends observed in recent years, characterized by an increase in temperature and decrease of relative humidity, we conclude that climate change will create favorable conditions for infecting sunflower plants in all the areas where sunflowers are grown, including the expansion of broomrape to the north of Moldova.

Keywords: *Orobanche cumana* Wallr / sunflower / climate change / spreading / infestation

Résumé – Influence des conditions environnementales sur la virulence et la distribution de l'*Orobanche cumana* Wallr. en République de Moldavie. L'angiosperme parasite *Orobanche cumana* est présente dans les principaux pays producteurs de tournesol du monde. Ces dernières années, des races d'Orobanches plus agressives sont apparues et le parasite s'est rapidement étendu à de nouvelles zones. Une tendance similaire a été observée en République de Moldova. Au début des années 1950, l'Orobanche a été détectée en Moldavie, en particulier dans les régions du Sud. Actuellement, toutes les races connues d'Orobanche sont présentes dans le pays et le parasite s'est considérablement développé dans de nouvelles zones du Centre et du Nord. Sur la base de ces résultats et des données rapportées par d'autres auteurs, liées à l'influence du changement climatique sur la situation phytosanitaire, nous avons étudié l'interdépendance entre le climat et la distribution d'*Orobanche cumana* Wallr. sur différentes régions de la République de Moldova. La prévalence de l'Orobanche principalement dans le Sud et le Centre de la République de Moldova et sa présence sporadique dans la partie nord peuvent être influencées non seulement par les courtes rotations, le type d'hybrides de tournesol cultivés et les paramètres du sol, comme des températures plus élevées et une humidité plus faible dans le Sud et le Centre. Basé sur des données pluriannuelles et des tendances observées ces dernières années, caractérisé par une augmentation de la température et la diminution de l'humidité relative, nous concluons que le changement climatique créera des conditions favorables d'infection du tournesol par l'orobranche dans toutes les zones de cultivation, y compris l'expansion au nord de la Moldavie.

☆ Contribution to the Topical Issue "Sunflower and climate change / Tournesol et changement climatique"
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1 Introduction

Sunflower is the basic oilseed crop in the Republic of Moldova and one of the most popular among farmers. The favorable trend in oil prices, sustained by continuous production demands, has maintained increases in the areas sown to sunflower. Currently, sunflower has consistent planting areas, exceeding the admissible limits in crop rotations (Duca, 2015). This significant expansion is leading to an increase in the frequency and aggressiveness of various pathogens and especially of broomrape (*Orobanche cumana* Wallr.). Broomrape successfully competes with the sunflower for nutrient sources, thereby reducing sunflower yield by up to 50%. These losses are dependent on genotype resistance, ontogenetic stage, duration of infection and climate conditions, which influence the infestation level (Dominguez, 1996; Alcántara *et al.*, 2006).

Considering the economic importance of sunflower, technological management of *Orobanche* was and it still is – a high priority for Moldovan sunflower researchers. The role of crop rotation, soil adaptability, fertility management, climatic influence, crop water use, disease incidence etc. were studied at the Research Institute of Field Crops *Selecţia, ASUM, MSU,* making important scientific recommendations (Sharova, 1977; Buciuceanu *et al.*, 1994; Vronschih *et al.*, 2002; Duca *et al.*, 2009; Rotarenco, 2010). Moreover, several breeding programs are conducted by private companies. As a result, during the last decades, in the Catalogue of Plant Varieties of the Republic of Moldova, 26 hybrids were registered, around 30% being resistant to broomrape. However, the use of resistant cultivars, generally carrying single major genes for resistance, has been followed by the appearance of more virulent physiological races of parasite that overcome all known resistance genes (Fernández-Martínez *et al.*, 2010). The long-term success of breeding for broomrape resistance requires breeders to take into account geographical distribution, genetic variation and evolution of *O. cumana*.

The first documents that attest to the presence of broomrape in Moldova date back to 1863 just after the start of the sunflower crop in 1840. At the beginning of 1970s, a new broomrape race, called Moldovan race or race C, was identified for the first time by Sharova (Sharova, 1977). Furthermore, a short historical investigation revealed the appearance of new physiological races of *O. cumana* in the Eastern part of Europe (former USSR) between 1970 and 1980 and only later the parasite migrated to Western Europe (Antonova, 2014). This data demonstrates co-evolution of the broomrape with its host, sunflower.

Russia was for many years the center of intensive breeding activities and VNIIMK varieties were cultivated (Škoric, 2016). Once Leclercq (1969, 1971) and Kinman (1970) discovered the cytoplasmic male sterility source and the corresponding restorer genes, the centers of intensive breeding programs are moved to Europe, subsequently broomrape became a limiting factor for sunflower development in Romania, Serbia, Bulgaria, Turkey and Spain (Molinero-Ruiz and Delavault, 2015).

Due to its geographical position at the border of former USSR and Western European countries, the Republic of Moldova represents a region of bidirectional flow of sunflower varieties and broomrape seeds. Currently, all the known races of *Orobanche* have been detected in Moldova. The areas affected by these parasitic plants expanded considerably across the territory of the country (Duca *et al.*, 2017). At the beginning of 1950s, broomrape was detected in the southern areas of Moldova, migrating further to some fields in the center of the country (Duca, 2015). Based on the latest data, the parasite can also be found in newer areas in the center and north (Duca *et al.*, 2015).

According to several authors, parasitic spread may be explained by the influence of biogeographical parameters, environmental factors (temperature, average rainfall, soil fertility) and the nutrients availability (Mohamed *et al.*, 2006; Spallek *et al.*, 2013). Considering that Moldova has a relatively small area (33 850 sq. km), without major geographical barriers (rivers, mountains) and with short distances between sunflower fields, it was both interesting and challenging to investigate and uncover the factors influencing the differential distribution and virulence of *Orobanche cumana* Wallr. in Moldova.

2 Materials and methods

The investigations were conducted in July–August, 2014, in a variety of locations from center, south and north of Moldova. Sampling was conducted using a mobile GPS. Each field investigated was divided into 10×10 m square plots. Nine randomly selected plots from each group were analyzed. The number of plants attacked by broomrape and the number of broomrape stems per host plant were recorded in each plot. The frequency and intensity of the broomrape attack in natural conditions were calculated as described Kaya *et al.* (2004).

During the expeditions, fresh tissue and mature seeds of more than 40 populations of *O. cumana* parasitizing sunflower were collected. Simultaneously, soil samples were collected and the geographic coordinates of each sampling point were specified. Also, the state of sunflower farm fields was documented (type of hybrids grown, crop rotation, precedants, etc.) on the base of farmers' sociological survey. In order to establish the current racial structure of broomrape in Moldova, seeds collected were analyzed in greenhouse experiments, which were carried out using one susceptible genotype sunflower and 5 homozygous differentials carrying *Or 5, 6* and *7* resistance genes as previously described (Duca *et al.*, 2017).

To assess current and future risks of broomrape expansion, we analyzed the daily surface temperature and humidity data for all regions of Moldova between May and July 2014, as well as multiannual (1961–2014) data recorder by State Hydrometeorological Service of the Republic of Moldova. Geographic Information Systems were used as a research tool. For the interpolation of weather-climatic data the ArcGIS Program within the Spatial Analyst extension (the coordinate system is UTM WGS84 area 35N with central meridian 27) was applied. A multivariate regression analysis software package, STATGRAPHICS Centurion XVI was used. Among the physical and geographic factors taken into account (geographic latitude and longitude, absolute and relative elevation, slope orientation and exposure), the absolute elevation and slope were significant for the obtaining of regression models. Automatically generated maps were overlapped with the digital map representing points of broomrape outbreaks.

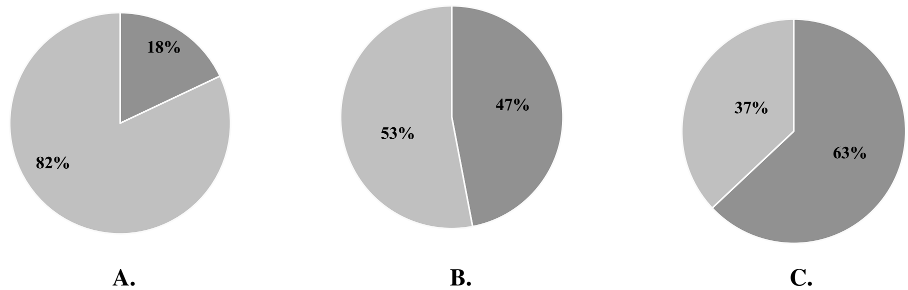


Fig. 1. The rate of fields naturally infected by *Orobanche cumana* Wallr. in different parts of Republic of Moldova (A – North, B – Centre and C – South).

3 Results and discussion

During the expeditions conducted in July 2014 in 25 administrative districts from different parts of the country, it was concluded that broomrape infection was most widespread in central and southern regions of Moldova, the frequency and intensity of broomrape attack being higher in the south: broomrape was present in 14 of 22 localities analyzed (63%) from the south, 17 of 36 localities (47%) from the central part and only in 4 of 22 localities (18%) in the northern regions (Fig. 1).

Furthermore, in about 50% of southern locations analyzed, the frequency of attack was 60–80%, while in central part the maximum frequency was 40%, in most cases about 10% (Duca, 2015; Duca *et al.*, 2015, 2016). So, the presence and intensity of broomrape attack in sunflower fields decrease from southern to northern parts (Fig. 2). In the south the broomrape infestation was very high, causing critical damage to sunflower production.

Our previous results, based on phenotypic screening, revealed that populations collected from the central part of the republic were less virulent, mostly belonging from race E or ≤ E (about 65%), while populations in the southern and northern regions were characterized by increased virulence, more than 60% of them being classified as aggressive races G and H (Fig. 3) (Duca *et al.*, 2017).

This variation in race distribution and degree of attack for each field analyzed may result from control measures involving well-organized crop rotation, use of herbicides and of broomrape resistant hybrids, which are known to be effective in protection against the parasite (Habimana *et al.*, 2013).

According to the farmers' social survey data, the rules of crop rotation are generally respected so more intense sunflower cropping and reduction of theoretically based crop rotations of 6–7 years to 1–3 years have been introduced only in 15% of fields analyzed in all three regions, including 4% when sunflower was grown where it had been grown the year before (Fig. 4).

It is important to highlight that a majority of hybrids grown, produced by Pioneer, Saaten Union, Syngenta, NARDI Fundulea, are known to be resistant to broomrape.

Considering the importance of pedoclimatic factors in spread and evolution of broomrape in sunflower crop, the physical and chemical parameters of the soil (humus, nitrogen, phosphorus and mineral elements content, pH, humidity, etc.) were analyzed. In most fields, the frequency of broomrape was positively influenced by high humus content and pH and negatively impacted by high potassium concentration. Samples from southern districts were characterized by higher pH (7.8–8.3), compared to 7.3–8.0 in the central part (Duca *et al.*, 2016). It is known that slightly acid to neutral pH offers best conditions for broomrape development (Lyra *et al.*, 2016), which could explain the preferential distribution of the parasite in the south. At the same time Miladinovic *et al.* (2012) concluded that soil texture, fertility and pH were not generally critical for broomrape presence.

Some authors reported that seed germination and development of *O. cumana* are highly dependent on climate (Song *et al.*, 2005; Ephrath and Eizenberg, 2010) and actual climate change, characterized by increased temperatures and absence of rainfall could influence the expansion of *Orobanche* to large areas (Mohamed *et al.*, 2006; Maširevic and Medic-Pap, 2009; Vear, 2016). Temperature requirements for parasite development differ among species, *e.g.* for *O. crenata* the optimum of temperature was found to be around 18 °C, lower and higher values decreased germination (Kasasian, 1973). Higher value of optimal temperature for conditioning and germination were reported in the case of *O. cumana:* 20–25 °C (Kaya, 2016), *O. cernua*: 23–26 °C and up to 28 °C for *O. ramosa* (Nandula *et al.*, 1996). The duration of temperatures required to promote seed conditioning usually are described in a range of 4–12 days at a temperature of 19–23 °C, in dark and humid conditions (Gibot-Leclerc *et al.*, 2004; Lechat *et al.*, 2012). Temperature significantly influences, also, the elongation of broomrape radicle (Nandula, 1998).

According to Sukno *et al.* (2001), broomrape populations are able to infect sunflower at a wide range of temperatures below 27 °C, with variation depending on the sunflower line–broomrape population combination. Eizenberg *et al.* (2003) reported that effects of temperature on *O. cumana* are complex. At higher temperatures (27 °C) the resistant varieties were more resistant to broomrape attack than at lower temperatures. Lower temperatures (15 °C) slowed parasite development in both resistant and susceptible varieties (Eizenberg *et al.*, 2003).

Thus, the development and higher level of infection in south and central part of the Republic of Moldova could be due to the increased temperature in these regions compared to northern parts. The annual growth rate of broomrape varied

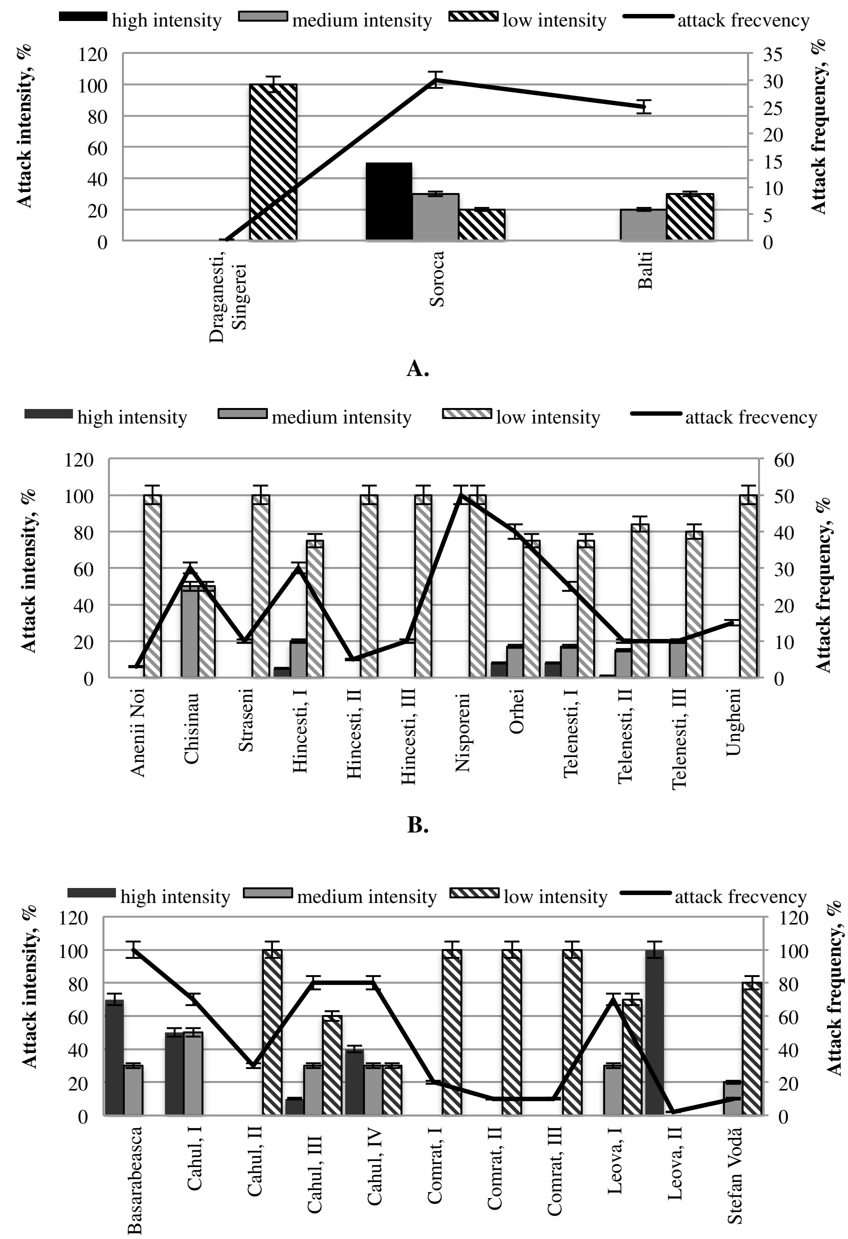


Fig. 2. Virulence of broomrape collected from different part of the Republic of Moldova (A – North, B – Centre and C – South).

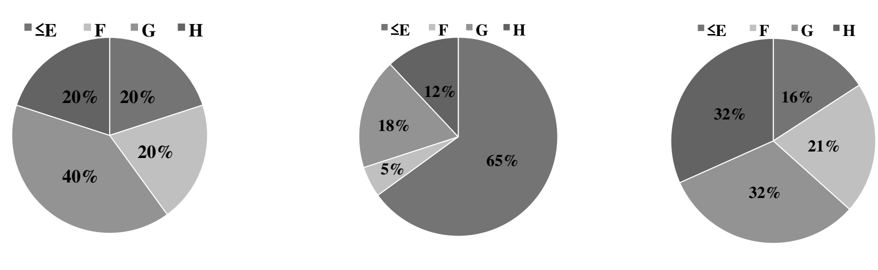


Fig. 3. Race l structure of broomrape from the territory of Republic of Moldova (A – North, B – Centre and C – South).

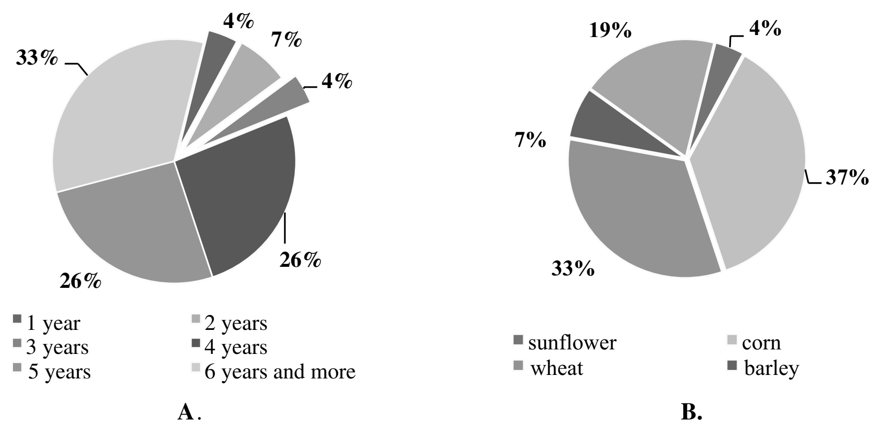
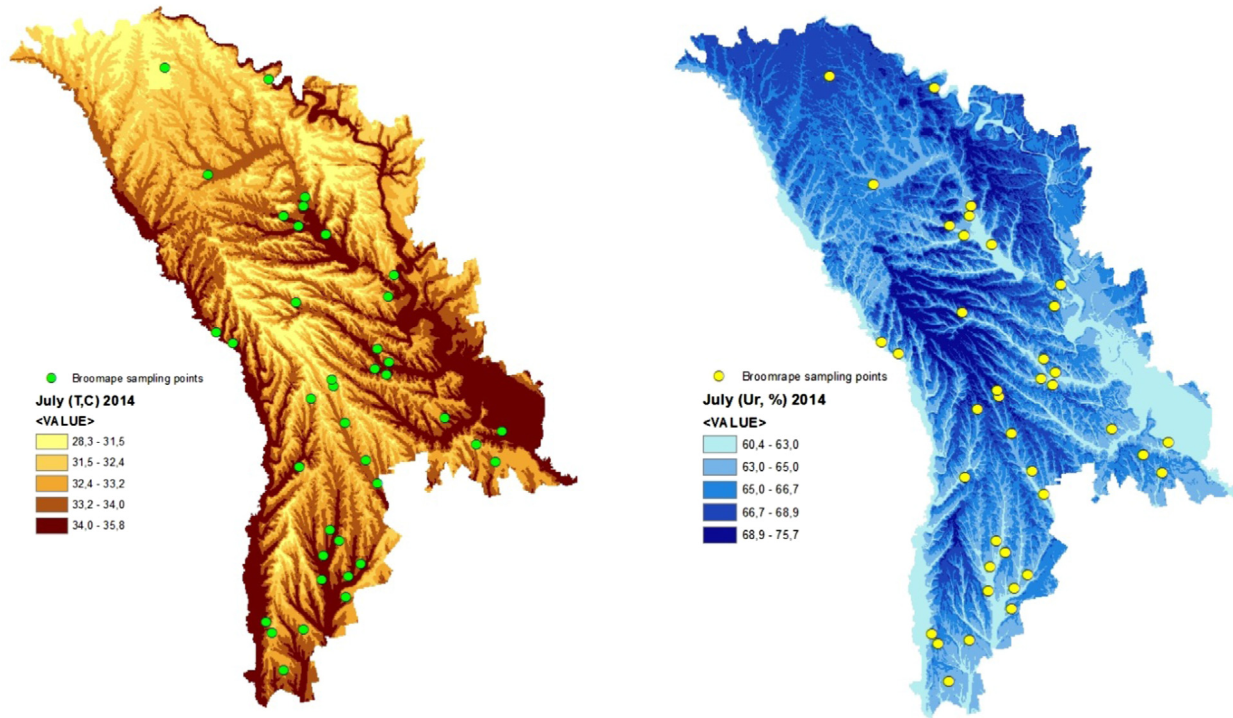


Fig. 4. Rotation of sunflower in the infected fields (A – Crop rotation, B – Precedants).

Table 1. The variation of air temperature and humidity values in different region of Moldova during May–July 2014.

			Temperature, °C			Humidity,%		
Month	10-day period	Zone	Minim	Maxim	Average	Minim	Maxim	Average
May	1	North	8.4	15.6	12.9	47	86	57.8
		South	9.7	16.5	13.6	56	91	73.5
		Center	10.5	16.1	13.5	46	89	65.1
	2	North	11.9	18.5	14.6	64	90	71.9
		South	12.3	19.8	16.1	59	88	74.5
		Center	12.1	20.6	15.8	52	87	69.8
	3	North	13.4	22.1	19.3	48	95	65.3
		South	14.8	22.3	19.6	68	93	75.4
		Center	16.8	23.8	20.6	48	89	64.9
June	1	North	12.5	22.5	18.5	62	92	75
		South	15.5	23.9	20.2	59	92	73.5
		Center	14.3	24.4	20.5	49	82	62.6
	2	North	15.0	20.5	17.4	54	75	62.2
		South	16.0	22.2	18.9	61	95	73.3
		Center	15.5	21.9	19.0	50	86	59.6
	3	North	13.2	20.6	16.7	51	90	65.7
		South	16.7	22.0	19.6	56	77	66.9
		Center	18.4	21.0	19.0	47	78	56.7
July	1	North	16.9	23.3	19.7	53	80	65.0
		South	18.3	24.2	22.1	50	80	65.3
		Center	18.4	24.7	22.2	45	76	56.0
	2	North	17.6	21.7	19.7	65	91	78.0
		South	20.7	23.0	21.8	63	86	76.2
		Center	20.8	23.4	22.2	57	77	66.8
	3	North	18.0	24.2	21.5	50	92	69.4
		South	21.1	26.3	24.1	54	86	64.9
		Center	20.9	26.6	24.4	32	76	51.3

between years and was significantly correlated with temperatures in certain months (Cohen *et al.*, 2017). We therefore analyzed climate data (temperature and humidity) for all regions of Moldova between May and July 2014 – the period of sunflower growth and the most favorable period for broomrape development (Tab. 1).



A. **B.**

Fig. 5. Cartographic modeling of temperature (A) and relative humidity (B) in July 2014.

In the first and middle part of May, mean temperature varied between 12.9–16.1 °C with maximum in the south and minimum in the north part of the country. At the end of the month, the highest average value – 20.6 °C was observed in the center of Moldova, followed by 19.6 °C in south and 19.3 °C in the north. A similar tendency was typical also for the first and middle part of June, as well as for July, in the south and center part the temperature being higher with 2.0–2.9 °C than in the north. Thus, in June the average temperature ranged between 16.7–18.5 °C – in north, 18.9–20.2 °C – in south and 19.0–20.5 °C – in Center and in July the temperature varied as follow: 19.7–21.5 °C – in north, 22.4–24.4 °C – in Center and 21.8–24.1 °C – in south. The maximal temperature in these months (24.4 °C in June and 26.6 °C in July) also was observed in Central part. southern and Central parts of Moldova are also characterized by lower humidity comparative to north part.

The higher temperature and lower humidity observed in south and center could improve germination and attachment of broomrape to the sunflower roots and could be an explanation of differential level of infestation between different zones (Fig. 1). Similarly, Louarn *et al.* (2016) supposed that higher level of broomrape infection in Cordoba in 2015 compared to 2014 could be due by the increased temperature (Louarn *et al.*, 2016). Several studies have shown that *Orobanche cumana* attack of sunflower is positively correlated with temperature during germination, attachment and tubercle-production stages. Parasite development is enhanced at high temperatures and delayed by low temperatures (Ephrath and Eizenberg, 2010).

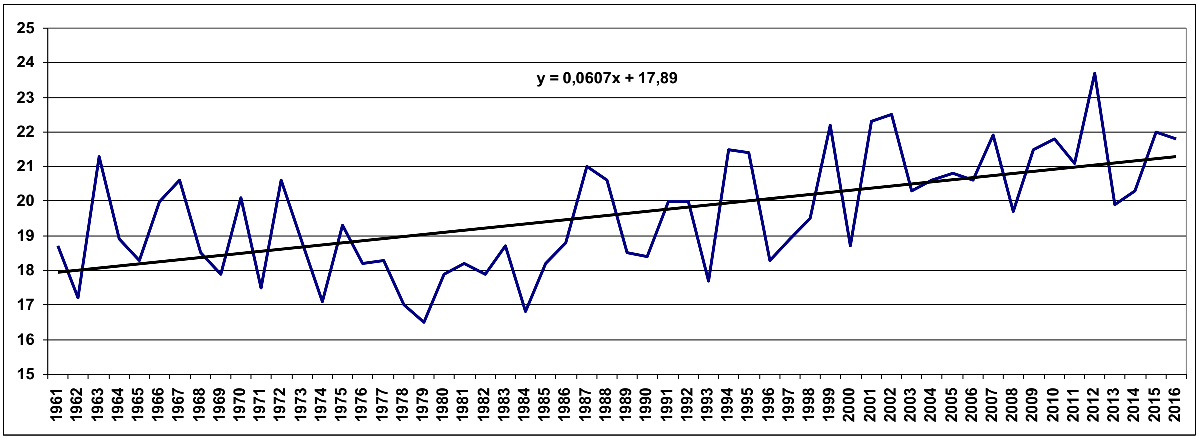
To permit visualization of broomrape populations in different areas, we mapped spatial distribution using computerized techniques (Fig. 5). We overlapped the outbreaks *O. cumana* with the distribution of temperature and humidity across the Republic of Moldova. As a result, we established that the main areas where *O. cumana* is spread coincide with higher temperatures and lower humidity.

Maps representing the spatial distribution of the broomrape outbreaks could be useful for correct zonation of sunflower hybrids and parasite control and monitoring.

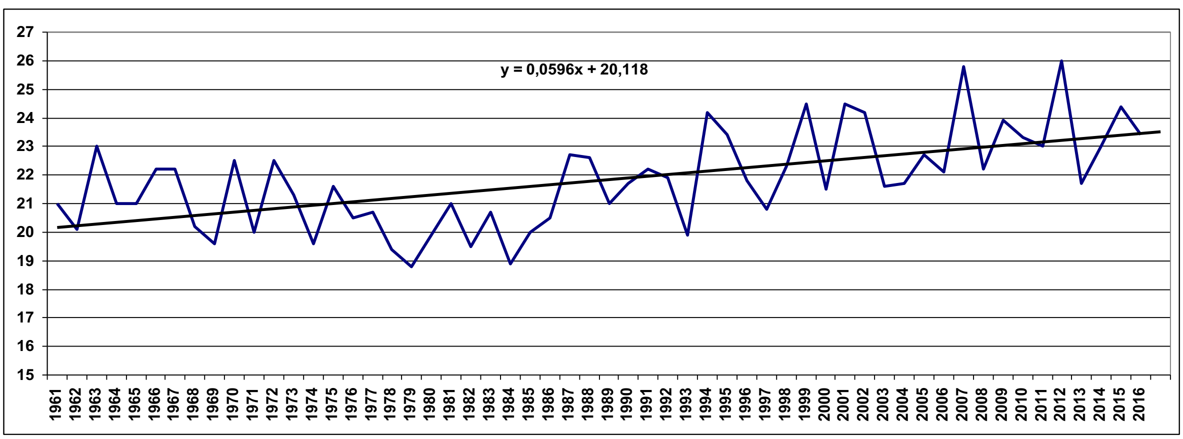
To assess current and future risks of broomrape expansion, we analyzed multiannual data (1961–2016) and highlighted trends specific to recent years, characterized by an increase in temperature (Fig. 6) and a decrease of relative humidity (Fig. 7).

Considering the increase in average temperatures in July in the Republic of Moldova during 1961–2014 (Fig. 6), we can conclude that favorable conditions for spreading of this parasite will be maintained in the near future. Although in the north part of the country (Fig. 6A) the multiannual average temperature in July is 19.6 °C, *i.e.* 2.20 °C and 2.30 °C less than in the central (Fig. 6B) and south part (Fig. 6C), the temperature here increases faster compared to the rest of the territory (0.0607 °C / year). This could mean that the favorable temperature for the spread of broomrape could be reached faster.

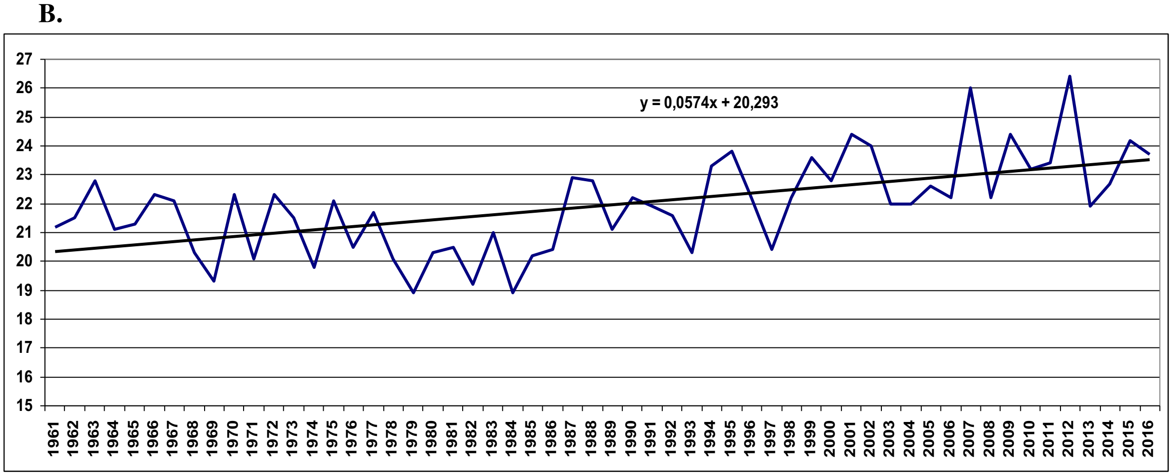
The relative air humidity in July shows a downward trend during 1961–2016. Although the multiannual average values in the north are greater, *i.e.* 70.4%, compared to the rest of the country, where the multiannual average varies between 62.2% (center) and 63.2% (south); the humidity in the northern part of the country is decreasing significantly by about 0.0363% per year (Fig. 7A–C).



A.



B.



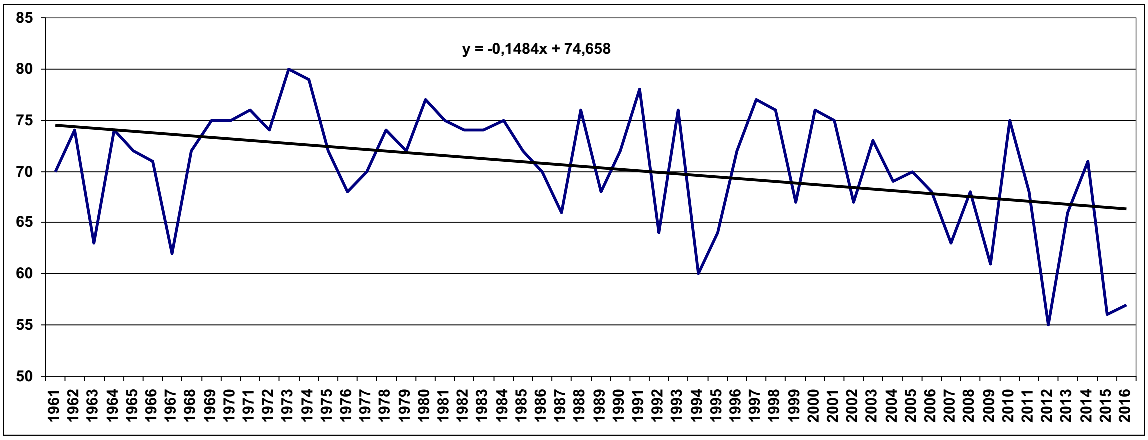
C.

Fig. 6. Dynamics of monthly (July) average temperatures on the territory of the Republic of Moldova (A – North, B – Center, C – South) between 1961 and 2016.

3 Conclusions

This study indicates that the persistence climate change could potentially create favorable conditions for infecting the sunflower in all areas, including the expansion of broomrape to the north of Moldova.

We conclude that the prevalence of broomrape infection mainly in the southern and central part of the Republic of Moldova and its sporadic presence in the northern part can be influenced, not only by short rotations and sunflower cultivars, but also by weather conditions such as higher temperatures and lower humidity in the south and center.



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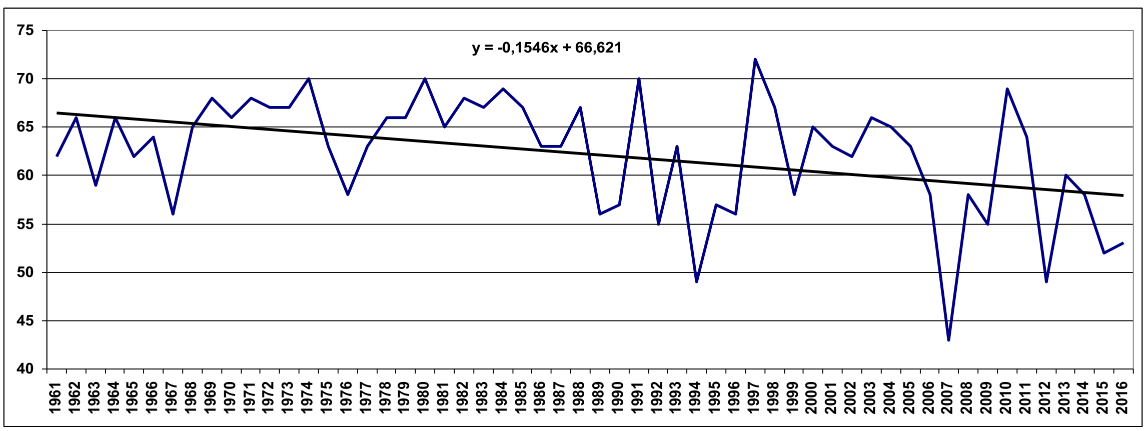

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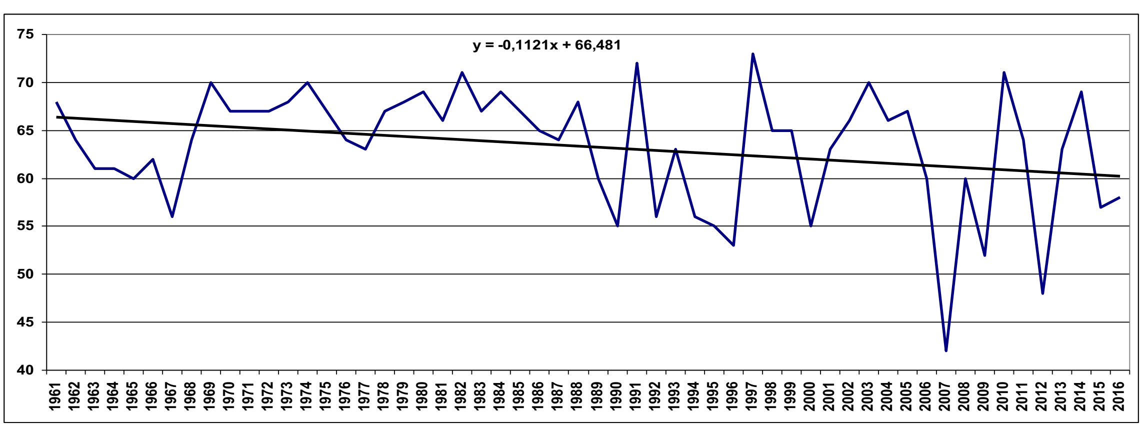

C.

Fig. 7. Dynamics of monthly (July) average relative humidity on the territory of the Republic of Moldova (A – North, B – Center, C – South) between 1961 and 2016.

Thus, over the years, these conditions could have contributed to the development of the parasite in these areas. Based on the multiannual data and the trends observed recently, characterized by the increase of the temperature and the decrease of the relative humidity, we can conclude that climate change will create favorable conditions for infecting sunflower plants in all cropping areas, including the expansion of broomrape to the north of Moldova.

Since early (winter) sowing of sunflower hybrids susceptible to the holoparasite leads to a significant reduction in *Orobanche* infection (Castejon-Munoz *et al.*, 1993), and temperatures exceeding 40 °C, with increased moisture content, are lethal for broomrape seeds (Habimana *et al.*, 2013), these conditions could be exploited as an effective parasite control technique (Mauromicale *et al.*, 2001). Our data on the influence of environmental conditions could be used in establishing the optimum sunflower sowing period, sunflower zonation and diversifying of strategies for pathogen control.

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Conflicts of interest. The authors declare that they have no conflicts of interest in relation to this article.

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