RACE COMPOSITION AND PHENOLOGY OF SUNFLOWER BROOMRAPE (*OROBANCHE CUMANA* WALLR.) IN UKRAINE

Ekaterina MAKLIAK[1*], Viktor V. KYRYCHENKO[1], Maria JOIŢA-PĂCUREANU[2]

[1]*Laboratory of Sunflower Breeding and Genetics, The Plant Production Institute nd. a. V. Ya. Yuriev of NAAS, Kharkiv, Ukraine*
[2]*National Agricultural Research and Development Institute, Fundulea, Romania*
[*]*Corresponding author: emaklyak@gmail.com*

SUMMARY

Sunflower broomrape (*Orobanche cumana* Wallr.) is widely common in Ukraine. There is an increase in the number and harmfulness of broomrape in the crops due to violations of the farming system and over-saturation of crop rotations with sunflower. The study was carried out (i) to investigate broomrape populations, which were collected in areas of mass affection of sunflower, in terms of their race composition; and (ii) to explore the parasite's phenology in the northeast part of Ukraine. Broomrape race composition was determined under controlled conditions in a phytotron and in the experimental fields. There were no parasite biotypes with virulence higher than that of race G in *O. cumana* population collected in the east of Ukraine. The presence of a biotype, which was more virulent than race H, was proved in *O. cumana* population collected in the southeast of Ukraine. Race H was in the population of broomrape from northeast part of Ukraine. Our data attest to rapid spread of more virulent races of the parasite in Ukraine. In the northeast of Ukraine, the onset of broomrape anthesis is within the first 10 days of July. The parasite ripens within the third 10 days of August.

Key words: broomrape races, broomrape phenology, controlled conditions, field conditions, *Helianthus annuus*

INTRODUCTION

Sunflower broomrape (*Orobanche cumana* Wallr.) is widely common in Ukraine. It causes considerable losses in the yield in the east and south of the country, in Odesa, Mykolaiv, Kherson, Zaporizhzhia, Dnipropetrovsk, Donetsk, Luhansk regions as well as in certain districts of Kharkiv, Kirovograd and Cherkasy regions (Kyrychenko, 2005). Saturation of crop rotations with sunflower in these areas can exceed 30% (State Statistics Service of Ukraine, 2017). So, there is an increase in the number and harmfulness of broomrape *Orobanche cumana* Wallr. in the crops due to violations of the farming system and over-saturation of crop rotations with sunflower.

For Ukraine, *Orobanche cumana* Wallr. is a plant-kenophyte. It penetrated into Ukraine after Columbus had discovered America (Ministry of Ecology and Natural Resources of Ukraine, 2014). Presumably, initially it parasitized on *Artemisia* plants; at the end of the 19th century it switched to sunflower (Novopokrovskiy and Tsvelev, 1958).

B. Yenken was the first in Ukraine who started of sunflower breeding for resistance to broomrape at Kharkiv Breeding Station (now the Plant Production Institute nd.a. VYa. Yuriev of National Academy of Agrarian Sciences of Ukraine) in 1909. As a result, variety Zelenka 76, which was resistant to race A, was created (Kyrychenko, 2008). At the end of the 1920s, mass spread of broomrape was observed in Ukraine, which almost brought sunflower cultivation to stop due to a drastic decline in yields. This happened when, in some regions of the east of the country (Donetsk region), new race B (or a group of races) affected 100% of varieties, which were considered as broomrape-resistant (Bukherovich, 1967). The well-known breeder V.S. Pustovoyt used local accessions from Donetsk, Dnipropetrovsk, Poltava and Zaporizhzhia regions of Ukraine in breeding for resistance to the group of races B (Pustovoyt, 1975).

In 1963, in Kaushany district of Moldova, near the border with Ukraine (Odesa region), every single variety was infected by a new race of broomrape called "Moldavian" (Bukherovich, 1967). At the end of the 1960s, in Donetsk, Dnipropetrovsk, Odesa regions, sunflower varieties, which were resistant to the group of races B, got affected by broomrape. It was found that the virulences of the Odesa and Moldovian populations of broomrape were identical (Burlov, 1976).

In the early 1980s, studying various geographic isolates of broomrape in relation to a collection of sunflower accessions with various resistance, Burlov and Artemenko (1983) revealed that the Odesa population of broomrape comprised least five groups of physiological races (A, B, C, D, E). It was identical to the broomrape populations, which were common in other Ukrainian regions growing sunflower, in particular in Donetsk region. It was emphasized that resistance controlled by the Or_5 gene was universal.

In the early 2000s, another change in the broomrape race composition occurred in Ukraine. Studying seeds form the broomrape population collected in the south of Odesa region, Burlov and Burlov (2010) found race G determined the population. Race G was reported in the northern steppe of Ukraine (Luhansk region) in 2013 (Khablak, 2013).

Studies of researchers of the Plant Production Institute nd.a. VYa Yuriev of NAAS, which are aimed at search and investigation of broomrape-resistant sunflower biotypes, were initiated in the 1960's. At that time, there were no sunflower varieties with resistance to new broomrape races, and this fact urged to search for sources of resistance among wild species of the genus *Helianthus*. Due to crossing sunflower with annual and perennial wild species, sources of resistance to major pathogens and broomrape were identified (Sytnik, 1972). Broomrape-resistant forms with a variety of morphological and economic traits were derived from the perennial hexaploid species *Helianthus tuberosus* L. via pair and saturating crossings with individual selection on severe infection (Sytnik and Gumeniuk, 1983; Kyrychenko and Sytnik, 1985).

In the 1980s, effects of the infection load, sunflower plant number per vessel, storage period of broomrape seeds and illumination length on damage to plants were studied under phytotron conditions (Kyrychenko *et al.*, 1987, Petrenkova *et al.*, 2012). Due to the controlled climate, new breeding material of female and male sunflower that was resistant to widespread (in that period) races of broomrape was created (Aladiina, 1991). Lines Kh1002B and Kh711V were recommended for breeding practice, and commercial sunflower hybrids were developed on their basis: Kharkivskyi 49, Kharkivskyi 58, Svitoch, Oskil, Kovcheh, Yason. These hybrids occupied large sown crops in Ukraine for a long time.

The study was carried out (i) to investigate broomrape populations, which were collected in areas of mass affection of sunflower, in terms of their race composition; and (ii) to explore the parasite’s phenology in the northeast part of Ukraine.

MATERIAL AND METHODS

Controlled conditions

Broomrape race composition under controlled conditions in a phytotron was determined during two winters: 2010/2011 and 2016/2017. Broomrape seeds for the 2010/2011 assessment were harvested from a landrace in the east of Ukraine (Amvrosiivska district, Donetsk region) in 2010. Broomrape seeds for the 2016/2017 assessment were harvested from collection sunflower accessions sown in a 4-field crop rotation of the Plant Production Institute nd.a. VYa Yuriev of NAAS (Kharkiv region, Kharkiv region) in 2014 and from experimental sunflower accessions sown as a monocrop at the Institute of Oil Crops of NAAS (Zaporizhzhia district, Zaporizhzhia region) in 2016.

Broomrape seeds were harvested during the stage of full ripeness. Capsules were separated from stems, dried outdoors in the shade and stored in paper bags in a dark dry place at room temperature. Immediately before using, broomrape capsules were ground and passed through a laboratory sieve.

Seeds of sunflower accessions - differentiators of broomrape races were received under the collection exchange program from Maria Pacureanu-Joita.

The differentiators used in 2010/2011 were as follows (names are given according to the submitted list): line LC1002V - to race D, line LC1003V and hybrid F_1 – to race E, line LC1093A – to race F, hybrid F_1 – to race G, hybrid F_1 – to race H (?) and line AD-66 that is susceptible to all races.

The differentiators used in 2016/2017 were as follows: LC1002B – to race D**,** LC1003B – to race E, LC1093B – to race F, H_1 – to races E–F, H_3 – to races G–H, H_4 – to race H.

To artificially infest sunflower with broomrape and to detect resistant lines, we used a controlled climate (phytotron). Sunflower seeds were sown in 5-liter vessels filled with infected soil mixture, at the rate of 2 g of broomrape seeds per 5 kg of soil. Ten plants were left in the vessels after sunflower germination. The controlled parameters were: air temperature + 24 ... + 28°C, relative air humidity 70 ... 80%, illumination length – 16 hours/day.

Broomrape-induced damage to sunflower plants was evaluated 40 days after seeding differentiator seeds.

Field conditions

The field studies were conducted in the experimental fields of the Plant Production Institute named after VYa Yuriev of NAAS (49°59'36"S, 36°27'17"W) in 2010-2011. The study area was forest-steppe. The soil is mainly powerful weakly-leached heavy-loamy silt chernozem on silt-loamy carbonate loess. The total soil depth is more than 75 cm with good humus content (humus content in the plowing horizon is 5.46-7.28%). The climate of the zone is moderately continental. The sum of active temperatures above 10°C amounts to 3,000°C and satisfies the sunflower's need for heat.

The conditions of 2010 were unfavorable for sunflower due to abnormally high air temperatures (up to 39.8°C) during anthesis and seed filling (July-August) on severe soil and air drought. However, in general, the rainfall exceeded the norm (average for 1960-1991) by 22% during the vegetation period of 2010 (May-September), due to precipitation in May and September. A record-breaking high temperature for August (39.8°C) was documented that year. In 2011, the precipitation amount exceeded the norm by 16.1%. The precipitation amount in the 3rd 10 days of June (121.0 mm) and the 1st 10 days of July (89.3 mm) provided enough moisture in soil before anthesis. The temperature was higher than the norm in May (+2.2°C), June (+2.2°C), July (+3.7°C), August (+1.8°C), and September (+1.6°C). In general, the conditions of 2011 were more favorable for the growth and development of sunflower compared to the 2010 conditions.

The infection background was created by seeding broomrape seeds together with sunflower seeds in each seedhole with manual planters. To facilitate the inoculation, broomrape seeds were mixed with soil at a ratio of 2 g of broomrape seeds to 2 kg of soil. Sunflower plants were seeded at a distance of 70 cm between the rows and 70 cm between the plants in a row. Broomrape affection of sunflower plants on artificial infection in the field was measured in the phase of physiological ripeness of differentiator plants.

The phenologies of sunflower and broomrape were monitored. In sunflower line Skh2111A, the development phases were determined according to the scale of Shneiter and Miller (1981): VE; R1; R5; R8; R9. The following broomrape development phases were distinguished: emergence of floriferous shoots over the ground, onset of anthesis, ripening onset.

Date analyses

Under the controlled conditions, the number of affected plants (N1) and the number of parasite tubers (B) were counted on roots of each differentiator. In the field, the number of affected plants (N1) and the number of the parasite floriferous shoots (V) were counted in the differentiator plots. In the both variants, 20 plants of each sunflower accession (N) were taken in one replicate. The percentage of affected plants (P) was calculated according to the formula: P = N1 × 100 / N. The affection degree (I) was determined by the parasite tuber number (phytotron) or the parasite floriferous shoot number (field) per one affected sunflower plant according to the formula: I = V/N1.

RESULTS AND DISCUSSION

Affection of sunflower by an *O. cumana* population of from the east of Ukraine (Donetsk region)

Analysis of the affection of accessions – race differentiator proved high virulence of an *O. cumana* population collected in the east of Ukraine (Donetsk region) in 2010 (Table 1). The contamination of surveyed sunflower crops with broomrape exceeded the economic threshold of the parasite harmfulness (3-5 broomrape floriferous shoots per sunflower plant). Both the phytotron and field data showed that the broomrape affection degree of F_1 hybrids - differentiators of races G and H was 0.0 floriferous shoots or parasite tubers, that is, there were no parasite biotypes with virulence higher than that of race G in the population.

Table 1: Affection of Accessions - Differentiators by *O. cumana* from the East of Ukraine (Donetsk Region)

Accession - differentiator	To race	Field conditions		Controlled conditions	
		% of affected plants	Affection degree	% of affected plants	Affection degree
LC1002B	D	60.0	5.7	100.0	5.2
LC1003B	E	20.0	1.0	100.0	8.4
F_1	E	80.0	5.9	100.0	8.2
LC1093A	F	40.0	5.3	70.0	2.0
F_1	G	0.0	0.0	0.0	0.0
F_1	H (?)	0.0	0.0	0.0	0.0
AD-66	susceptible	100.0	12.0	100.0	13.6

The affection degree of differentiators of races D (LC1002B) and E (LC1003B, F_1 hybrid) in the phytotron was within 5.2 - 8.4 tubers per plant, and there were no resistant plants (100% of affected plants). In the field, the percentage of affected plants in these differentiators varied from 20 to 80%. We found no resistant plants in line AD-66, which does not have genes of resistance to broomrape, under any conditions.

The affection degree of differentiator of race F (LC1093A) in the phytotron was 2 tubers per plant, and the percentage of affected plants was 70%. The difference in the broomrape affection degree between different differentiators was small (from 2.0 to 8.4 tubers per affected plant). However, we hypothesized that race F predominated in the population (the affection degree of differentiators to race E was 8.2-8.4). According to the affection of line LC1093A, race G accounted for the lowest percentage (70.0% of affected plants, 2.0 tubers per affected plant under the controlled conditions) in the studied population

Affection of sunflower by *O. cumana* populations from the southeast (Zaporizhzhia region) and the northeast (Kharkiv region) of Ukraine

Analysis of the affection of accessions – race differentiators in the phytotron proved high virulence of *O. cumana* populations collected in the southeast of Ukraine (Zaporizhzhia region) in 2016 and in the northeast of Ukraine (Kharkiv region) in 2014 (Table 2). The contamination of surveyed sunflower crops with broomrape exceeded the economic threshold of the parasite harmfulness of (3-5 broomrape floriferous shoots per sunflower plant).

Table 2: Affection of Accessions - Differentiators by *O. cumana* from the Northeast and Southeast of Ukraine

Accession - differentiator	To race	Controlled conditions[1]		Controlled conditions[2]	
		% of affected plants	Affection degree	% of affected plants	Affection degree
LC1002B	D	100.0	2.2	25.0	2.0
LC1003B	E	100.0	5.5	100.0	1.8
LC1093A	F	100.0	4.7	100.0	3.9
H1	E-F	100.0	3.4	100.0	5.0
H3	G-H	100.0	3.3	44.4	4.5
H4	H	100.0	5.7	0.0	0.0

[1] – Broomrape seeds collected in the southeast of Ukraine (Zaporizhzhia region)
[2] – Broomrape seeds collected in the northeast of Ukraine (Kharkiv region)

Using this set of differentiators, from 100% affection of plants of all accessions by a broomrape population from the southeast of Ukraine we proved the presence of a biotype, which was more virulent than race H.

The virulence of broomrape from the northeast was weaker. There were no affected plants in the differentiator of race H (H4), suggesting absence of a biotype that is more virulent than race H in the population. 44.4% of affected plants of the differentiator to races G-H (H3) indicate that there was race H in the population.

O. cumana phenology in the northeast of Ukraine

Phenological monitoring of the development of sunflower line of Skh2111A and *O. cumana* in the northeast of Ukraine (Kharkiv region) (field infection) allowed establishing the following regularities (Table 3). Upon the optimal period of sowing in the study area, when the soil temperature at the seeding depth reaches 12°C, sunflower shoots emerged on May 15, 2010 and on May 11, 2011. Broomrape appearance over the soil surface coincided with the sunflower development phases R3-R4. In 2011, the weather conditions were more favorable for sunflower than those in 2010, broomrape anthesis preceded sunflower anthesis, and broomrape ripened during the phase R8 of sunflower. In 2010, the onset of broomrape anthesis coincided with the onset of sunflower anthesis, and broomrape ripening - with the phase R9.

Table 3: Main Phenological Phases of the Development of Sunflower Line Skh 2111 A and *O. cumana*, the northeast of Ukraine (Kharkiv region), artificial infection in the field

Year	Date	Developmental phase	
		Skh 2111 A	*O. cumana*
2010[1]	May 15	VE	–
	June 17	R1	–
	July 02	R4	Floriferous shoots emerged over the soil surface
	July 07	R5	10 % floriferous shoots flowered
	August 26	R9	Ripening
2011[2]	May 11	VE	–
	June 25	R1	–
	June 30	R3	Floriferous shoots emerged over the soil surface
	July 14	R5	90 % floriferous shoots flowered
	August 25	R8	Ripening

[1] – sowing date May 05
[2] – sowing date May 03

CONCLUSIONS

The herbaceous parasite *O. cumana* is a threat to sunflower crops in Ukraine. Affection of commercial crops by broomrape in the main regions of sunflower cultivation exceeds the economic threshold of harmfulness. Evaluation of the virulence of the broomrape populations from the east (Donetsk region), northeast (Kharkiv region) and southeast (Zaporizhia region) of Ukraine related to European accessions – race differentiators demonstrated race heterogeneity of these populations. Of the studied populations, the broomrape population from the southeast of Ukraine was the most virulent. Using the test set of differentiators, we proved the presence of a biotype, which was more virulent than race **H,** in this population. Our data attest to rapid spread of more virulent races of the parasite in Ukraine.

In the northeast of Ukraine, the onset of broomrape anthesis is within the first 10 days of July. The parasite ripens within the third 10 days of August.

REFERENCES

Aladiina, Z.K., 1991. Creation of starting material of broomrape-resistant sunflower in the eastern forest-steppe of the Ukrainian SSR, Author's abstract of the thesis for the Candidate Degree in Agricultural Sciences, 06.08.05. Ukranian Research Institute of Plant Production, Breeding and Genetics nd.a. VYa Yuryev, Kharkiv, pp.1-16.

Petrenkova, V.V., Borovska, I.Yu. and Kyrychenko, V.V., 2012. Sunflower major diseases, *In:* Basics of field crop breeding for resistance to harmful organisms: manual; Kyrychenko, V.V. and Petrenkova, V.P. (Eds.), NAAS, Plant Production Institute nd.a. VYa Yuryev, Kharkiv, pp. 270-295.

Bukherovich, V., 1967. Determination of race composition of sunflower broomrape of Moldovian origin, *In*: Collection of works on oil crops, issue 2, Krasnodar Publishing House, pp. 6.

Burlov, V.V., 1976. Inheritance of resistance to a local race of broomrape (*Orobanche cumana* Wallr.) in sunflower. Genetika XII (2): 44-51.

Burlov, V.V. and Artemenko, Yu., 1983. Gene pool of resistance and virulence in the evolutionary linked couple of sunflower (*Helianthus annuus* L.) – broomrape (*Orobanche cumana* Wallr.). Genetika XIX (4): 629-664.

Burlov, V.V. and Burlov, V.V., 2010. Effectiveness of the genes *Or* for sunflower resistance to new races of broomrape (*Orobanche cumana* Wallr.). Selektsiia i Nasinnytstvo 98: 28-37.

Khablak, S.H. and Abdulaieva, Ya.A., 2013. Race composition of broomrape (*Orobanche cumana* Wallr.) on sunflower in the northern steppe of Ukraine. Visnyk Ahrarnoi Nauky Prychornomoria 3(73): 116-121.

Kyrychenko, V.V. and Sytnik, M.S., 1985. Interspecies hybrids as starting material for heterosis sunflower breeding . Selskokhozyaystvennaya Biologiya 10:12-14.

Kyrychenko, V.V., Gumeniuk, A.D., Dolgova, El.M. and Aladiina, Z.K., 1987. Sunflower breeding for resistance to broomrape and improvement of an express diagnostic method in the phytotron conditions. *In:* Selektsiya i Semenovodstvo, Republican interdepartmental thematic scientific collection, Kyiv, issue 63, pp. 44-46.

Kyrychenko, V.V., Gumeniuk, A.D. and Ozhereleva, V.I., 2008. Yenken Boris Karlovich, *In:* Yuriev's school of plant producers, Scientific edition, Plant Production Institute nd.a. VYa Yuryev of UAAS. Kharkiv, Mahda LTD, pp. 76.

Kyrychenko, V.V., 2005. Breeding and seed production of sunflower (*Helianthus annuus* L.), Kharkiv, pp. 33-42.

Ministry of Ecology and Natural Resources of Ukraine, 2014. Web-site: http://kharkivoda.gov.ua/content/documents/891/89034/Attaches/dopovid_ pro_stan_nps_harkivska_oblast_2014.pdf.

Novopokrovskiy, I.V. and Tsvelev, N.N., 1958. Family *Orobanchaceae*, *In*: Flora of the USSR, The USSR AS, vol. XXIII, pp. 19-115.
Pustovoyt, V.S., 1975. Main streams in breeding. *In:* Pustovoyt, V.S. (Eds.), Sunflower, Moscow, Kolos, pp. 153-163.
Shneiter A.A. and Miller, J.F., 1981. Description of sunflower growth stages. Crop Science 20: 901-903.
State Statistics Service of Ukraine, 2017. Web-address: http://www.ukrstat.gov.ua/druk/publicat/kat_u/publ7_u.htm.
Sytnik, M.S., 1972. Use of interspecies hybridization in sunflower breeding for the forest-steppe of the Ukrainian SSR. Author’s abstract of the thesis for the Candidate Degree in Agricultural Sciences, 06.01.05. Ukranian Research Institute of Plant Production, Breeding and Genetics nd.a. VYa Yuryev, Kharkiv, pp. 1-23.
Sytnik, M.S. and Gumeniuk, A.D., 1983. Development of initial forms of sunflower by remote hybridization, *In:* Selektsiya i Semenovodstvo, Republican interdepartmental thematic scientific collection, Kyiv, issue 55, pp. 21-24.