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The History of Interconnected Evolution of *Orobanche cumana* Wallr. and Sunflower in the Russian Federation and Kazakhstan

Abstract: Currently, *Orobanche cumana* in the Russian Federation overcomes the influence of dominant genes of resistance in sunflower *Or4*, *Or5*, *Or6*, *Or7*, already known in European countries, and the combined impact of the two recessive genes *or6or7*. The most virulent biotypes of parasite G and H are found in many regions of sunflower cultivation: the Rostov, Voronezh, Volgograd, Saratov, Orenburg, Stavropol and Krasnodar regions. The situation is especially unfavorable in the Rostov region where the race G became predominant in many populations of *O. cumana*. In the Krasnodar region broomrape began to spread in recent years (after a long absence) mainly in the northern regions (and adjacent), bordering with the Rostov region. Here the populations of *O. cumana* are a mixture of races of different virulence. The races D and E still often dominate. However, there are already the parasite populations, where the race G is predominant, and somewhere biotype H is already present. In a sample of seeds from Kazakhstan is dominating the low virulent race C, among which there is a small admixture of biotype G. This combination of low virulent race with a small amount of highly virulent specimens of biotype G indicates the natural origin of the latter, regardless of the influence of the breeding process of sunflower. Some deviations from the basic model of development of *O. cumana* in ontogenesis that contribute to increase and acceleration of seed preproduction of parasite's specimens are described.

Keywords: broomrape, *O. cumana*, populations, virulence, races, sunflower

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The history of *Orobanche cumana* Wallr. parasitism on sunflower in Russia is about 200 years old. In the late seventeenth century, sunflower in Russia could only be found in the homestead gardens. Its sowing as a field crop began in the first half of the nineteenth century, first in Saratov and Voronezh provinces. And here it encountered *O. cumana* that parasitized on sea and Austrian wormwoods (*Artemisia maritima incana* Schm. and *A. austriaca* Jacq.) (Beilin, 1947). Sunflower proved to be a more appropriate host for broomrape than wormwood. From here began its spreading to the new areas of sunflower sowing (Sukachyov, 1899). The first report on mass infestation of sunflower with broomrape in Russia appeared in Voronezh in 1866 (A. Oldamov, Voronezh provincial gazette, 62).

By the late nineteenth and early twentieth centuries, the spreading zone of *O. cumana* expanded so much that this parasite has become a serious threat to sunflower crops (Karzin, 1898; Shreiner, 1894, 1904; Maltsev, 1913; Plachek, 1913). Already then Russian breeder A.I. Stebut wrote: “The sunflower crops were even abandoned in some areas, as there was no sure way of broomrape control” (Stebut, 1913). By 1920, the efforts of breeders at the Saratov experimental station helped to create varieties resistant to broomrape in this area. However, when these varieties were sown for trials on the fields of the Don experimental station in the Rostov region, they were highly affected by the broomrape prevalent in that region. Hence appeared the terminology: “good” broomrape is in the Saratov region and “evil” broomrape is in the Rostov region. They were called races A and B according to the alphabet. They differed greatly in areas of spreading. Race A was more common in the Saratov and Voronezh regions and race B – in the Rostov and Krasnodar regions, Moldova and south of Ukraine (Zhdanov, 1930; Scherbina, 1931; Pustovoit, 1937).

Later, it was discovered that race B is heterogeneous in its composition, so it was called a complex of races B. The immunity to race A consisted in formation of thickening of root tissues around the broomrape seedling that penetrated into it and died there. Sunflower immunity to the complex of races B did not manifest itself outwardly.

In the mid-twentieth century *O. cumana* became a mass phenomenon in all areas of Minor and Central Asia, Ukraine, Moldova, the Caucasus, the Volga region, and in some areas of the Western and Eastern Siberia (Beilin, 1968).

Over the course of history of sunflower cultivation in Soviet Russia (the Soviet Union), there were three periods when severe infestation of crops with broomrape has put the culture under threat of extinction. The interconnected evolution of parasite and host led to the emerging of new races of parasite that were able to overcome the immunity of resistant varieties and hybrids. The last epiphytotic situation developed in the USSR in the early 1970s of the twentieth century, when the broomrape biotype that first appeared in Moldova and was named the Moldovan race (or race C) began affecting previously resistant varieties and spread rapidly in all regions of sunflower cultivation, especially in the North Caucasus. Successful breeding of new sunflower varieties resistant to the Moldovan race during the next dozen of years helped to solve the problem. The immunity of resistant assortment to the Moldovan broomrape biotype was expressed in lignification of vascular walls of root's xylem, isolating them from the haustorial cells of parasite.

The isolation from water-mineral substances led to the destruction of haustorial cells (Antonova, 1978). Since the late 1970s, the widespread cultivation of resistant varieties with this type of immunity in the Soviet Union caused germination of broomrape seeds and gradually led to the elimination of their main resources in soil (Pustovoit *et al.*, 1983).

Approximately until the late 1990s there were no problems with broomrape on sunflower in Russia. It was difficult even to find broomrape somewhere to collect the seeds for testing the resistance of breeding material. However, during the last 20 years the country experiences the increased intensification of cultivation of sunflower as a high-yielding crop: the expansion of planting acreage and non-observance of scientifically based crop rotation. Along with attraction to the country in the 1990s of the sunflower hybrids of foreign breeding as a seed grain, which were susceptible to the local broomrape, it contributed to replenishment of resources of parasite seed in soil and accelerated the race formation. Currently, *O. cumana* that has spread in the southern regions of the Russian Federation affects all domestic and foreign assortment of sunflower.

The question of necessity of the race identification of *O. cumana* on sunflower in Russia and establishment of unified international nomenclature of the races of this parasite is essential.

Since 2006, the workers of the laboratory of immunity and electrophoresis of VNIIMK are systematically carrying out the identification of racial structure of *O. cumana* populations in the southern regions of the Russian Federation: Rostov, Volgograd, Stavropol and Krasnodar. For this purpose the known differentiators of resistance to the races D, E, F created in Romania (LC 1002, LC 1003, LC 1093) are used as well as the line P 96 resistant to the race F in Spain and the line IR7 of VNIIMK breeding possessing the immunity to the races A–G. Sunflower variety VNIIMK 8883, which have never been bred for resistance to broomrape, is used as a control variant.

The accumulated data show that at the beginning of research most populations of parasite had the complete range of biotypes: D, E, F, G with a predominance of less virulent D and E. But by now, the highly virulent race G with an admixture of the even more virulent biotype H is beginning to dominate in many populations. Thus, currently *O. cumana* in Russia overcomes the influence of dominant genes of resistance in sunflower *Or4, Or5, Or6, Or7,* already known in European countries, and the combined impact of the two recessive genes *or6or7*. The problem of finding new resistance genes is especially acute.

The most frustrating soil condition in terms of infestation with seeds of the highly virulent biotypes of broomrape is observed in the most areas of the Rostov region (Antonova *et al.*, 2013) (Table 1). Everywhere the land users here are returning the sunflower to the previous field after 1–3 years, therefore occurs rapid equalization of structure of broomrape populations toward the predominance of the most virulent biotype. Besides the prevalence of the race G in many populations, the most virulent today biotype H is frequently encountered here. The situation in the Rostov region reached its pinnacle, in some places the broomrape infestation makes it impossible to receive yield. It became unprofitable economically to cultivate sunflower, and land users are already trying to find the substitute for this crop.

Table 1: The degree of infestation* of resistance differentiators of sunflower with broomrape populations of the Rostov and Volgograd regions of the Russian Federation, 2013

Area	VNIIMK 8883 susceptible control	Resistance differentiator of sunflower:					The predominant races in population	The presence of other races in population
		LC 1002	LC 1003	LC 1093	P 96	VT 62		
		D *Or4*	E *Or5*	F *Or6*	F *or6or7*	G *Or7*		
The Rostov region								
Bokovsky	58	52	67	57	26	0	G	F
Morozovsky	98	73	55	22	16	11	F	D, E, G, H
Zernogradsky	80	61	39	44	50	5	G	D, F, H
Kagalnitsky	44	38	40	53	17	0	G	E, F, G
Milyutinsky	68	51	41	43	13	8	G	D, E, H
The Volgograd region								
Gorodishchensky	24	20	5	7	2	11	E	D, G, H
Elansky	78	72	46	25	6	0	F	D, E, G,
Novoanninsky	115	28	57	12	2	0	D	F, G
Surovikinsky	84	10	0	0	1	3	D	F, H

Notes: * – The degree of infestation – the number of broomrape tubercles per one affected sunflower plant; Text in italics represents the resistance genes to the specified race in sunflower differentiator.

The situation is slightly better in the Volgograd region, where in some populations are often dominated by the less virulent races D and E (Table 1).

The situation is much better in the Krasnodar region. Here broomrape began to spread in recent years, mainly in the northern (and adjacent) areas, bordering with the Rostov region. It should be noted that according to the local laws, in the Krasnodar region sunflower cannot be return to its previous place earlier than after 5 years. The data in Table 2 show that currently in the Krasnodar region the broomrape populations are a mixture of races of different virulence. This testifies to the recent renewal of seed resources of parasite in soil. Still, the races D or E quite often predominate. At the same time in all studied populations there is an admixture of one or several highly virulent biotypes that overcome the influence of resistance genes *Or4, Or5, Or6, Or7, or6or7* in sunflower. In some areas there are populations of parasite in which the race G is predominant and in some places the biotype H is already present.

Table 2: The degree of infestation* of resistance differentiators of sunflower with broomrape populations of the Krasnodar and Stavropol regions of the Russian Federation, 2013

Area	VNIIMK 8883 susceptible control	Resistance differentiator of sunflower:					The predominant races in population	The presence of other races in population
		LC 1002	LC 1003	LC 1093	P 96	VT 62		
		D *Or4*	E *Or5*	F *Or6*	F *Or6Or7*	G *Or7*		
The Krasnodar region								
Yeysky	94	78	33	5	0	0	E	D, F, G
Brykhovetsky	41	33	26	6	3 (88)	4 (33)	F	D, E, G
Krylovsky	59	56	40	41	20	8	G	E, H
Kushchyovsky	109	62	17	9	9	0	D, E	F, G
Pavlovsky	63	52	39	32	17	5	G	D, E, F, H
The Stavropol region								
Grachevsky	79	42	23	16	3	12	D	E F, G, H
Ipatovsky	92	52	25	31	6	0	D	E, F, G,
Petrovsky	111	86	30	15	2	0	E	D, F, G,
Trunovsky	26	19	25	20	20	4	G	F, H

Notes: Text in italics represents the resistance genes to the specified race in sunflower differentiator; The percentage of affected plants is shown in brackets. *The degree of infestation – the number of broomrape tubercles per one affected sunflower plant.

The data shown in Table 2 indicate that in the Krasnodar region takes place a rapid spread of the highly virulent broomrape biotypes, resistance to which is absent in domestic assortment of sunflower. At the same time, land users, along with the intensification of crop cultivation, tend to grow foreign hybrids resistant to the races E, F, G. Since the seeds of all broomrape biotypes germinate in the presence of roots of resistant hybrids but their seedlings cannot grow in their roots, and because of it the non-virulent races are gradually eliminated from the population. Therefore, in the short term the further equalization of virulent structure of parasite populations toward the predominance of biotypes G and H should be expected. There is the acute problem of finding genes of resistance to these two races specifically.

The situation in the Stavropol region is developing in similar way. The biotypes F, G, H are already detected against the background of the prevalence of the low virulent races D and E. The race G dominates already in some populations (Table 2).

The data in Table 3 and Figure 1 show how widely the race G spread in the Russian Federation. It already prevails in some populations of the Voronezh, Saratov and Orenburg regions.

At the same time, the sample of broomrape seeds, collected in 2012 in the Shemonaikhinsky district of Kazakhstan near Ust-Kamenogorsk, almost entirely consists of low virulent specimens, not able to overcome resistance gene of sunflower *Or4*, i.e. of the race C. However, this sample of seeds already has a small percentage of specimens that overcome the influence of dominant resistance gene of sunflower *Or6* (Table 3). It should be noted that the degree of infestation, equal to one, was observed in 100% of plants of used differentiator line having gene *Or6*. This eliminates the randomness connected with the detection of a single nodule and indicates the presence of a small number of specimens in parasite population that are able to overcome the influence of gene *Or6*.

The presence of a small amount of biotype G among the predominant in a population of race C, non-virulent to the modern assortment of sunflower, may indicate that such instances of highly virulent broomrape have a genuine, natural origin, not related to the “pressure” of the breeding process of sunflower.

The genuine, natural occurrence of specimens with high virulence in low virulent populations of *O. cumana* on sunflower was previously observed in Spain (Molinero-Ruiz *et al.*, 2008). The authors concluded that the cause of it is not the creation of resistant sunflower hybrids, and they are of natural origin. And it proves the necessity of continuous sunflower breeding for immunity to broomrape, refuting the opponents’ opinion that breeding will not have time to

Table 3: The degree of infestation* of resistance differentiators of sunflower with broomrape populations of the Voronezh, Saratov and Orenburg regions of the Russian Federation and the Shemonaikhinsky district of Kazakhstan, 2013

Area	VNIIMK 8883 susceptible control	Resistance differentiator of sunflower:					The predominant races in population	The presence of other races in population
		LC 1002	LC 1003	LC 1093	P 96	VT 62		
		D *Or4*	E *Or5*	F *Or6*	F *Or6Or7*	G *Or7*		
The Voronezh region								
Pavlovsk	73	36	60	43	12	5	G	E, F, H
The Saratov region								
Sovetsky district	58	21	22	42	3	3	G	E, F, H
The Orenburg region								
Alexandrovsky district	34	18	20	21	0	0	G	D
Kazakhstan								
Shemonaikhinsky district, Ust-Kamenogorsk	117	0	0	1***	0	0	C	G

Notes: Text in italics represents the resistance genes to the specified race in sunflower differentiator. *The degree of infestation – the number of broomrape tubercles per one affected sunflower plant; ***All 100% of plants of differentiator line are affected with degree 1.

produce resistant material if it is the reason of generation of highly virulent forms of the parasite. It is obvious that the cause of more rapid spreading of such forms is non-observance of crop rotation, intensification of sunflower cultivation as an economically highly profitable crop.

It should be noted that during the Soviet period of the last century there was not broomrape on sunflower in Kazakhstan. The sunflower there was cultivated in three regions: East Kazakhstan (75% of the total area planted with this crop in Kazakhstan), Semey (The former Semipalatinsk) and Pavlodar (Figure 1), occupying a relatively small area of 100–200 thousand ha. Other areas of Kazakhstan are very droughty and are unsuitable for sunflower cultivation. Today, the Semey region is combined with the East Kazakhstan. At that time, the experimental breeding station of VNIIMK was in the East Kazakhstan region. Its scientific recommendations on crop cultivation with observance of eight-field crop rotation were strictly followed.

Currently, the planting acreage of sunflower in these regions of Kazakhstan increased considerably and is about 700–800 thousand ha. There also takes

Figure 1: The map of spreading of the highly virulent biotypes G and H of broomrape (*O. cumana*), parasitizing on sunflower on the territory of the Russian Federation and Kazakhstan: C, G, H – names of biotypes (races) of the parasite.

place the enhanced intensification of cultivation of this highly profitable crop. The broomrape appeared as a consequence of it. Therefore, it should be expected that in the next few years at least an already existing biotype G will reproduce and spread here.

The analysis of the virulent structure of *O. cumana* populations from different regions of the Russian Federation shows that everywhere the process of their changing develops in a similar manner: there is a rapid equalization of virulence toward the predominance of the most virulent biotype. Today, it is the race G. We can assume that in the East Kazakhstan region under the conditions of immoderate cultivation of sunflower the situation will evolve in a similar manner, and in a few years this race will also become predominant there.

Our observations of highly virulent parasite populations in the Rostov region showed that here *O. cumana* has a high potential of reproductive function, wide possibilities of adaptation to the conditions of permanent destruction. We would like to draw attention to the fact that at the present stage in the ontogenesis of *O. cumana* there are frequent deviations from the model of development when one shoot, or rarely two shoots from one tubercle are dominating. The changes have occurred in the development of a tubercle. This structure has become multipolar, capable of setting multiple meristematic zones and development of adventive shoots from them. There is a tendency to the accelerated development of adventive shoots. The apexes of rudimentary roots of tubercle acquired the ability to grow directly into the stem, skipping the stages of penetration into the root adjacent to the parent tuber and the formation of a new tubercle followed by setting in it an apex of additional shoot (Antonova *et al.*, 2012). This hastens a lot the formation of additional seeds from one specimen of parasite and is one of the proofs of its evolution toward increase of the potential of the reproductive function.

Currently, the surprising phenomenon is the ability of *O. cumana* to form flowers, their self-pollination and ripening of seeds in them on certain adventive shoots, hidden in soil due to their underdevelopment and inability to reach the surface (Antonova *et al.*, 2012). The accelerated formation of seeds and increase of seed reproduction by any means are the characteristic features of highly virulent biotypes of *O. cumana* on sunflower in the Rostov region.

In our opinion, there is an acute problem of finding new genes of resistance to *O. cumana* in sunflower. Moreover, it is also necessary to study cause and effect relationship of immunity to highly virulent broomrape in wild species of sunflower, which have been identified by several authors (Ruso *et al.*, 1996; Sukno *et al.*, 1998; Fernandez-Martinez *et al.*, 2000; Terzič *et al.*, 2010; Christov, 2012; Antonova *et al.*, 2011) as unaffected. This huge stratum of routine research work should be done in order to understand what exactly is brought from wild species into cultivated sunflower controlled by resistance genes to *O. cumana* and what, causing the immunity of roots, it loses in the process of breeding of productive and other agronomic characters. It is necessary to conduct a thorough comparative study of physiological-and-biochemical and anatomical-and-morphological characteristics of roots of different genotypes of wild and cultivated sunflower in order to identify acceptable for breeding factors of incompatibilities with the modern most virulent biotypes of *O. cumana*.

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