DILEMMAS ABOUT NEW SUNFLOWER BROOMRAPE RACES (*Orobanche cumana* Wallr.)

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The genus *Orobanche*

The genus *Orobanche* contains over 100 species

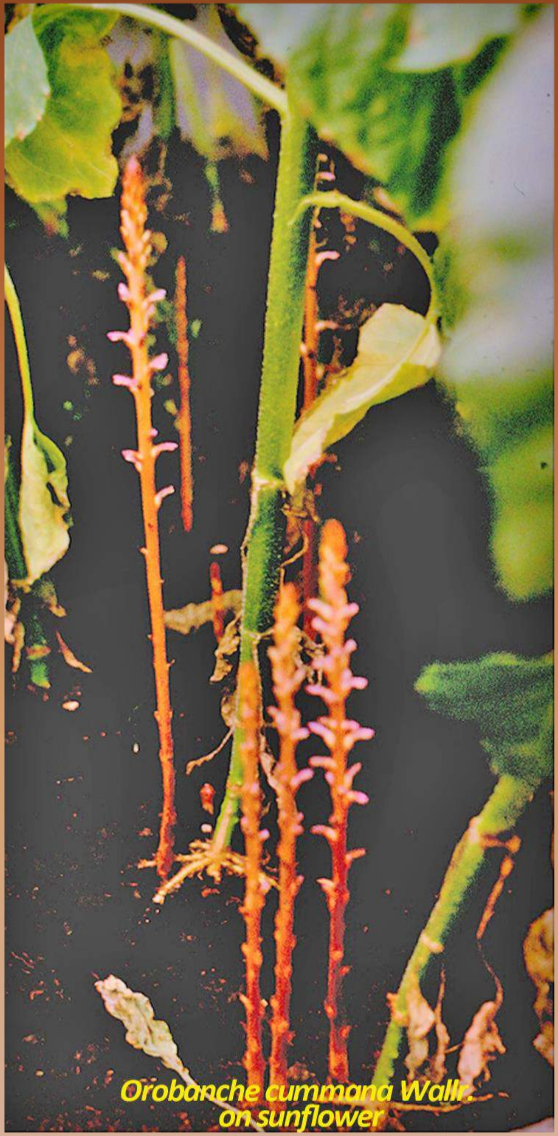
Broomrape species have been reported in a total of 58 countries

Orobanche cumana Wallr = *Orobanche cernua* Loefl.

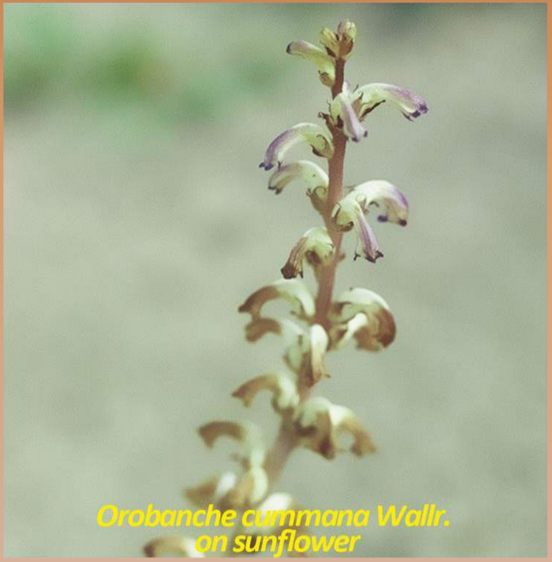
Orobanche on different plant species...

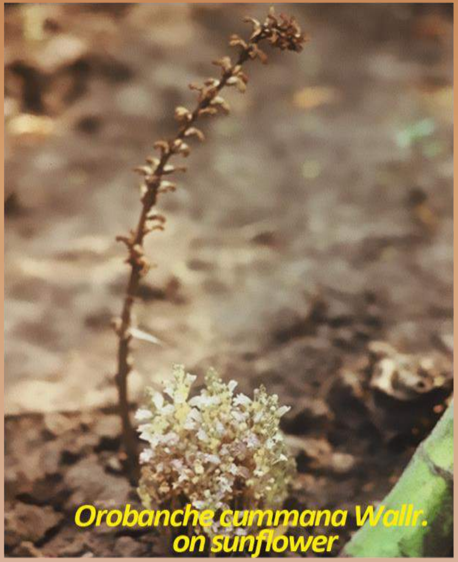
Orobanche on different plant species...

Orobanche on sunflower + ? ! ...

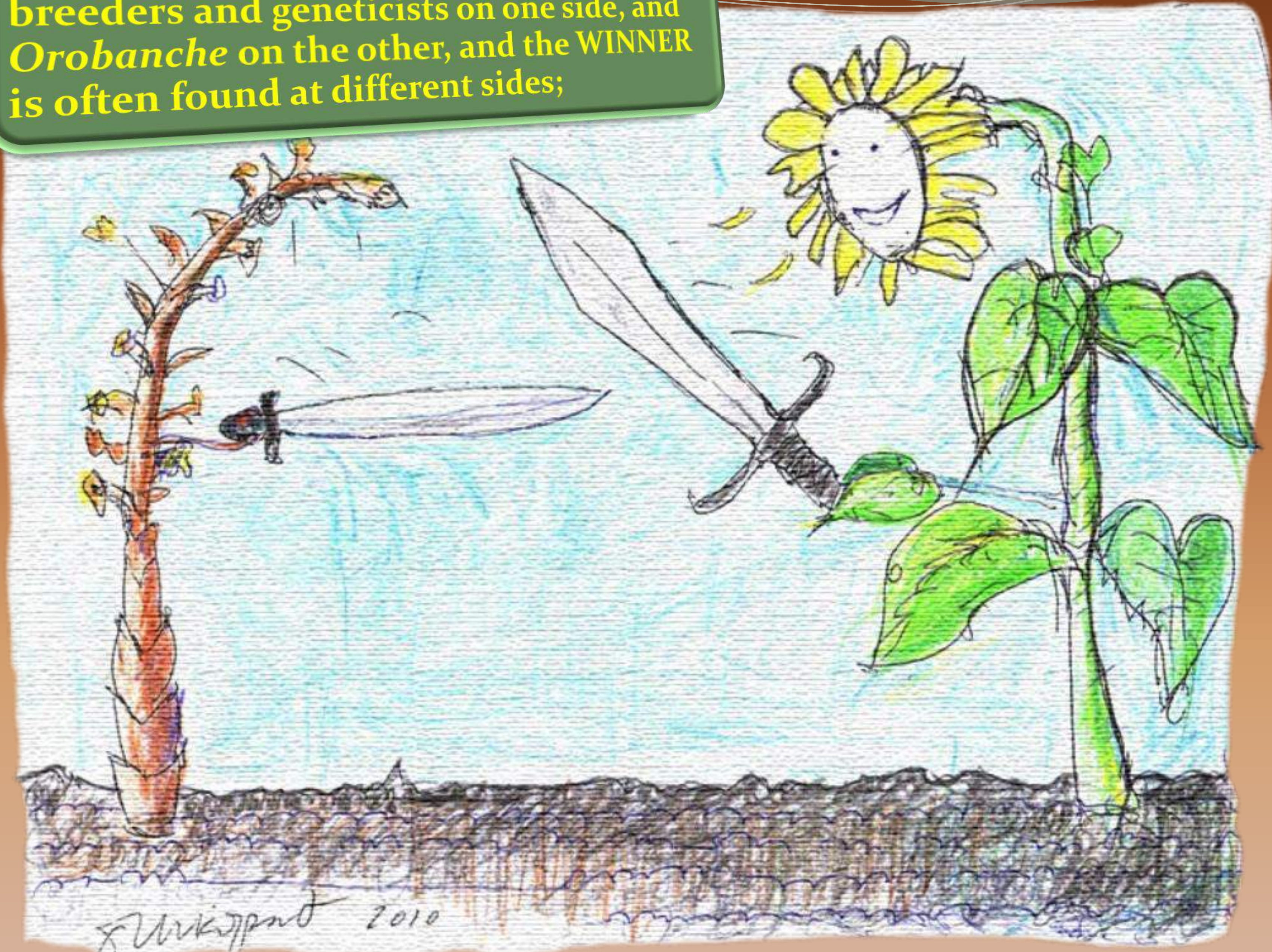


Orobanche on sunflower + ? ! ...





There is permanent combat between breeders and geneticists on one side, and *Orobanche* on the other, and the WINNER is often found at different sides;



SEEDS AND GERMINATION

Seed and germination

The fruit is a capsule, the seeds are brown, 0.2-0.4 мм in size;

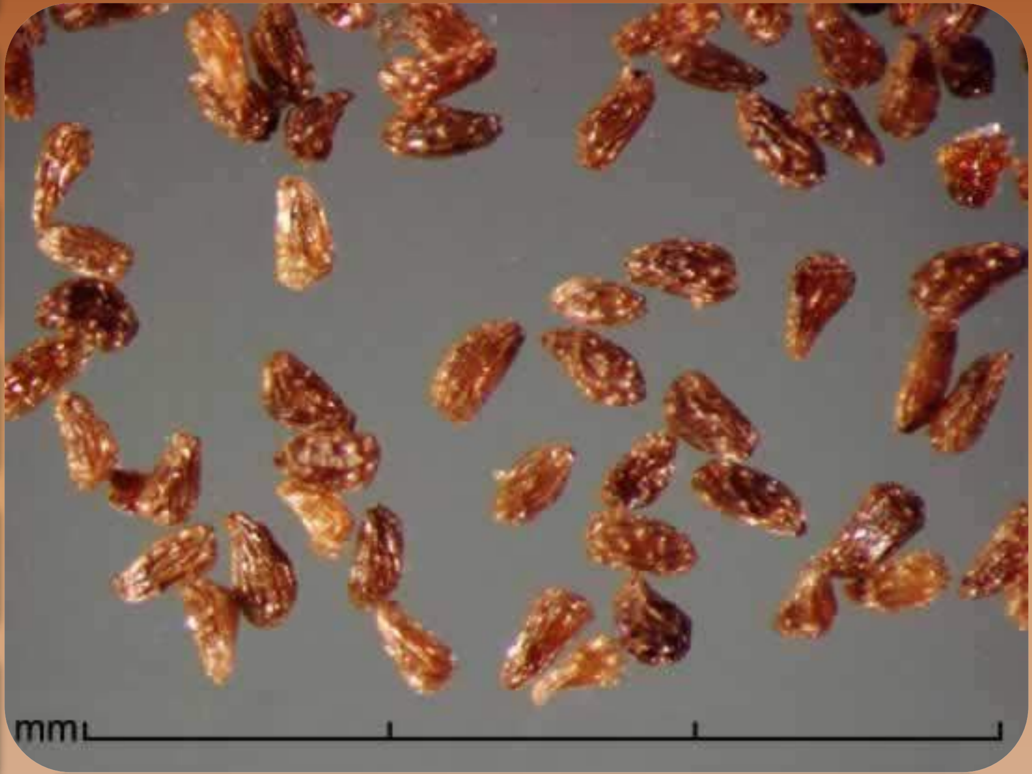
Flower number per plant: 30-67;

Seed number per flower: 2,500-10,500;
Absolute seed weight: 0.001 g;

Seed number per plant: > 87,000;

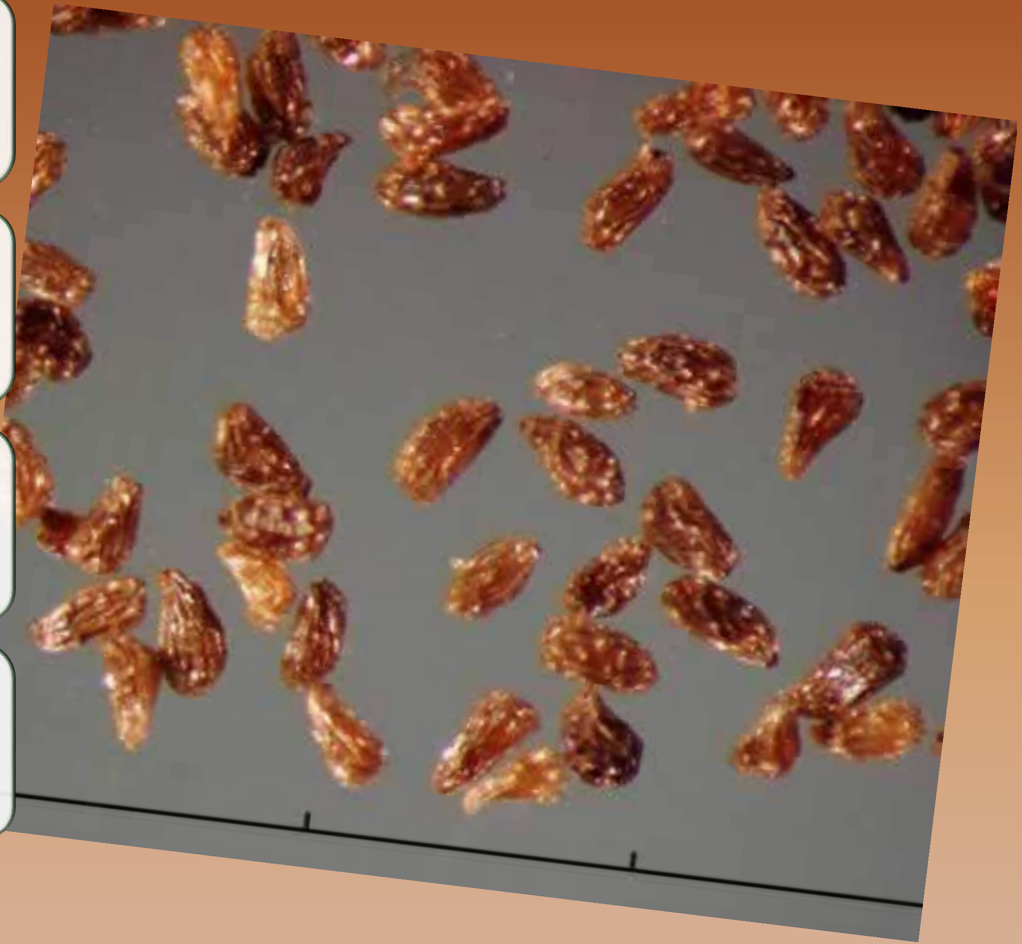
Number of seeds: >44,000/g;

Broomrape is transported by wind and vehicles (combines, tractors, trucks...)



SEEDS AND GERMINATION

- Broomrape seeds may lie dormant in the soil for more than 20 years and still remain viable;
- The seeds germinate in moist soil at a temperature of 20-25°C;
- The seedlings take the form of a thin strand – the growth shows positive gravitropism;
- If the seedling does not come into contact with the root of the host plant, it will die relatively quickly;
- Germinability is affected by the pH of the environment as well as by the root exudates of the host (germination stimulants);



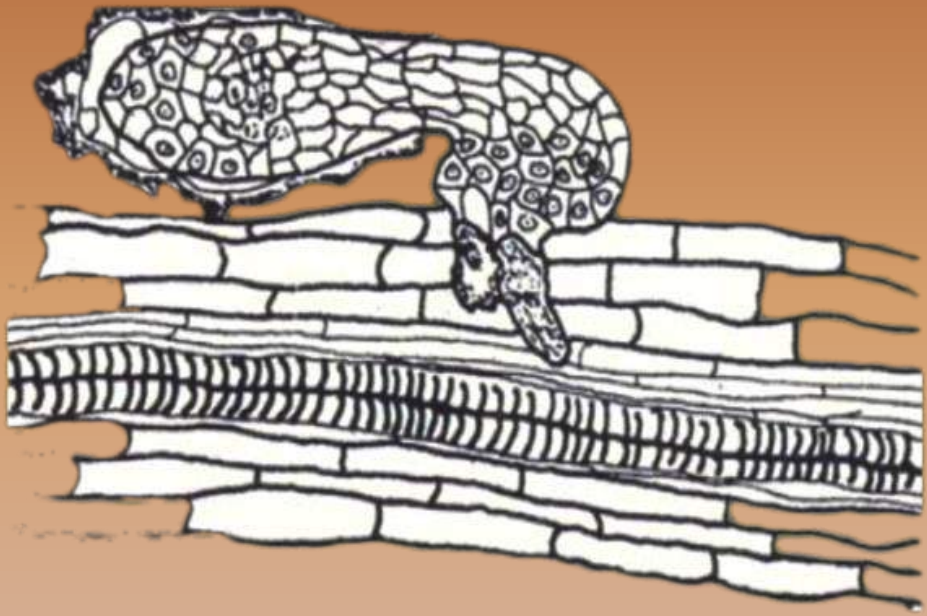
Range of physiological races of *Orobanche cumana Wallr*. at Constanta, Tulcea, and Braila (Romania) (Vranceanu *et al*. (1980), Pacureanu *et al*. (1998))

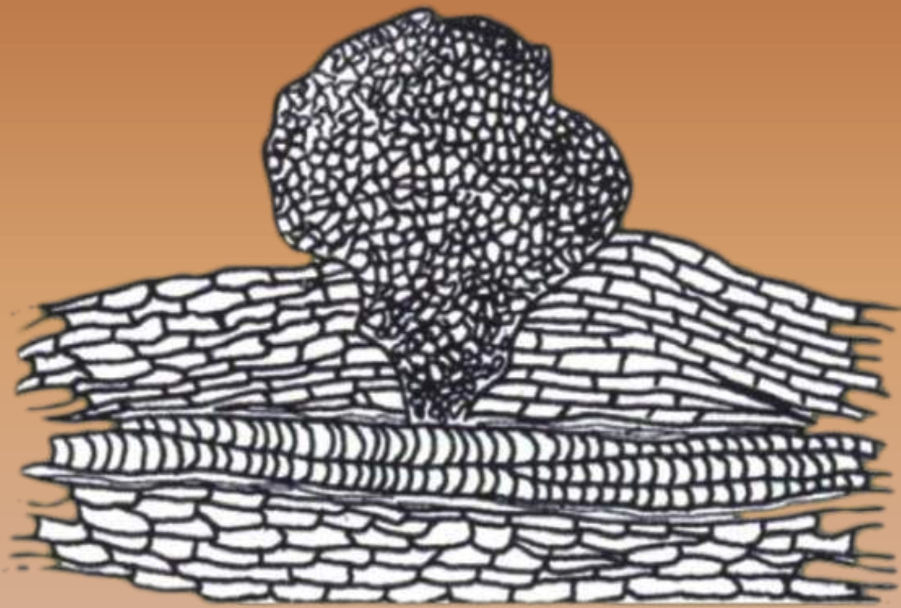
Differential line	Resistance gene	Natural infestation - Constanta, 1997						Inoculation, Fundulea, 1997					
		Sunflower line response to broomrape race						Sunflower line response to broomrape race					
		A	**B**	**C**	**D**	**E**	**F**	**A**	**B**	**C**	**D**	**E**	**F**
LC-1093	*Or6*	R	R	R	R	R	R	R	R	R	R	R	R
P-1380-2	*Or5*	R	R	R	R	R	S	R	R	R	R	R	S
S-1358	*Or4*	R	R	R	R	S	S	R	R	R	R	S	S
Record	*Or3*	R	R	R	S	S	S	R	R	R	S	S	S
Zhdanov	*Or2*	R	R	S	S	S	S	R	R	S	S	S	S
Kruglik A-41	*Or1*	R	S	S	S	S	S	R	S	S	S	S	S
AD-66		S	S	S	S	S	S	S	S	S	S	S	S

R - resistant
S - susceptible

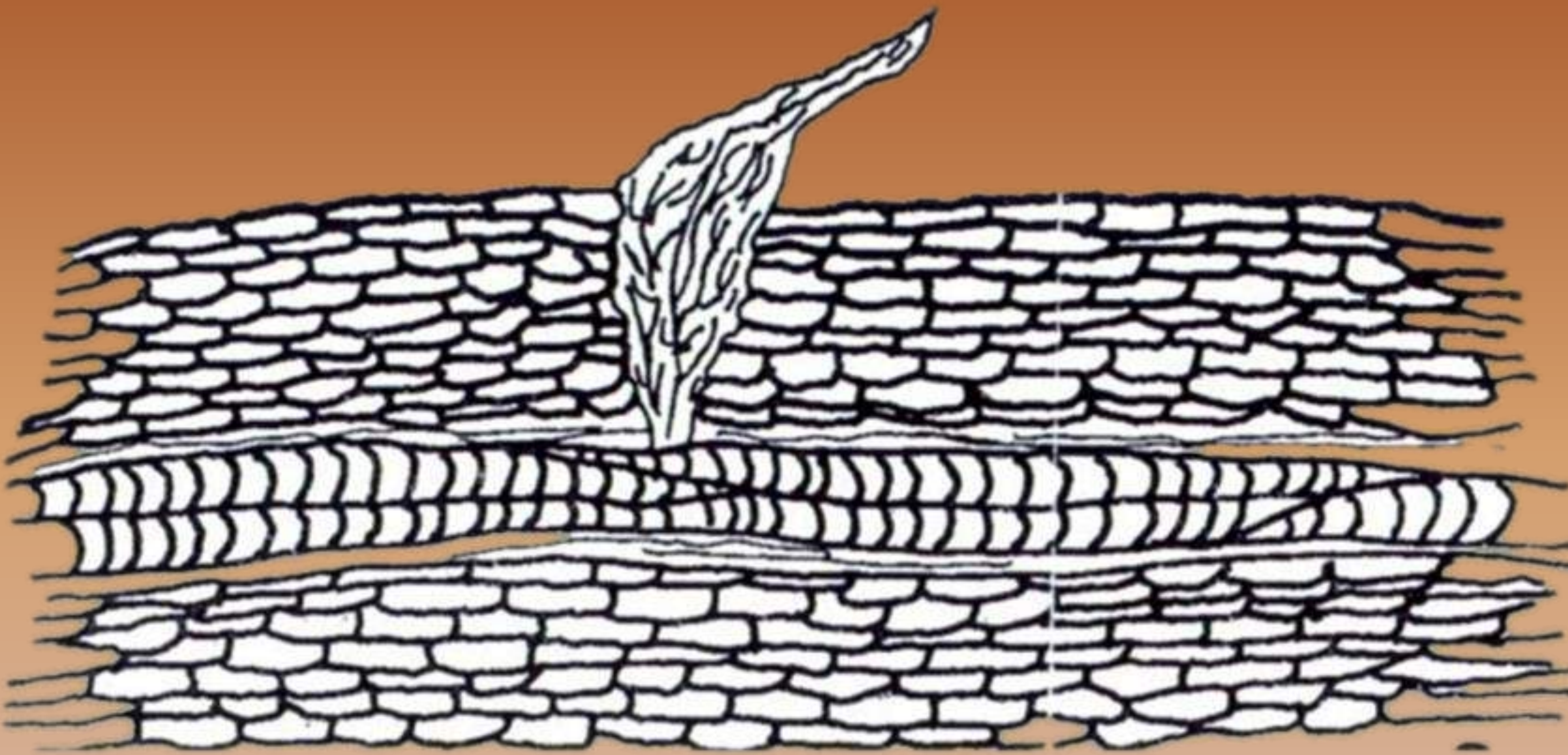
1. Germination of broomrape seeds, the penetration of the root, and the formation of a primary haustorium during the infection of resistant and susceptible forms of sunflower (8 days after germination)

2. The haustorium penetrating the central cylinder of the radicle (xylem) in a susceptible sunflower genotype (14 days after germination)



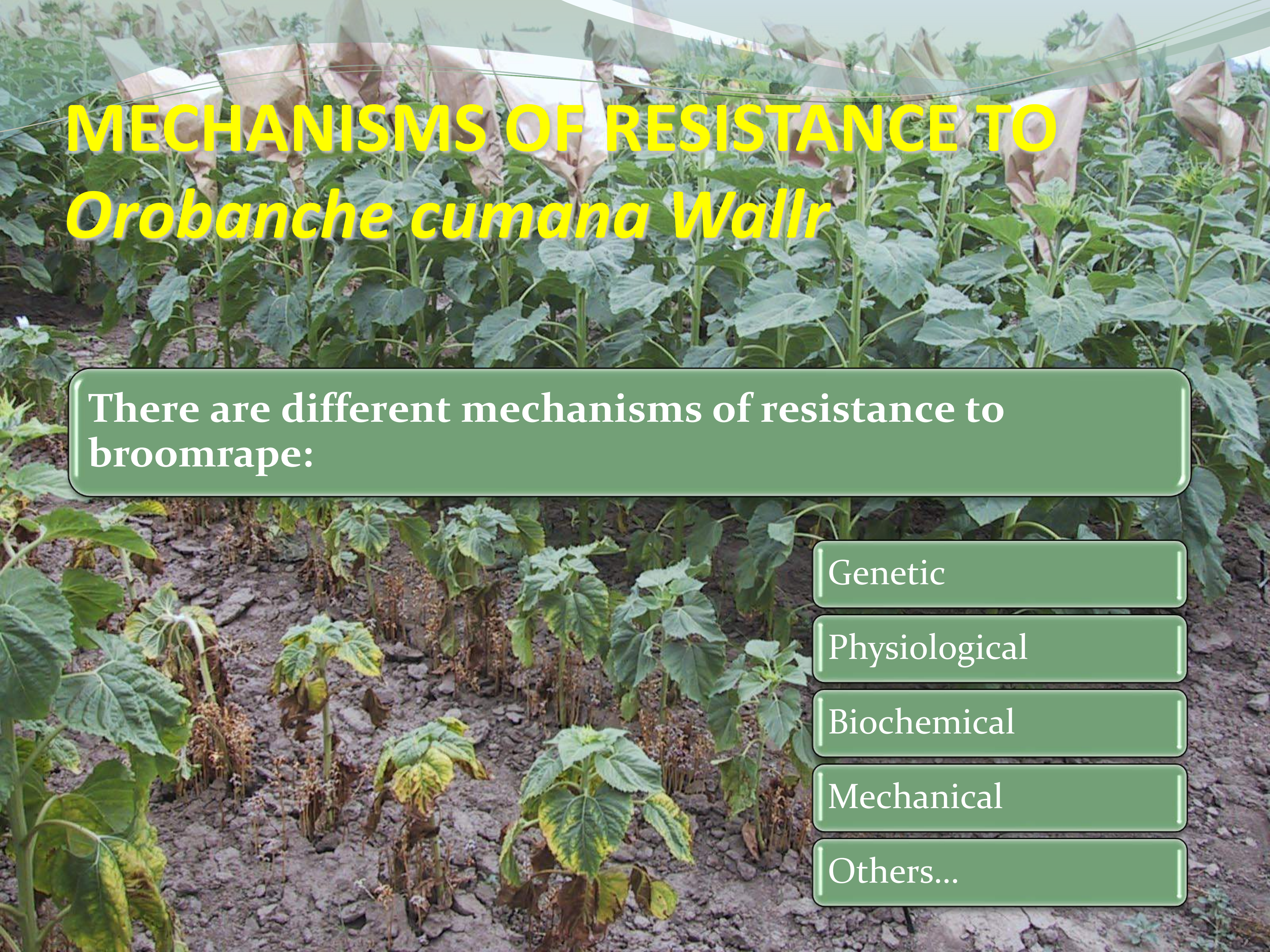


3. Broomrape germ dying in a resistant sunflower genotype



Panchenko, 1975

MECHANISMS OF RESISTANCE TO *Orobanche cumana Wallr*



There are different mechanisms of resistance to broomrape:

- Genetic
- Physiological
- Biochemical
- Mechanical
- Others...

SOME OF THE PARAMETERS OF SUNFLOWER RESISTANCE TO *Orobanche cumana Wallr.*

According to Richter (1924) (as cited by Morozov, 1947):

- Susceptible sunflower plants have low pH values of the root system (acidity);
- Two physiological thresholds: 1. In acid soils, broomrape seeds germinate easily; 2. In alkaline ones, susceptible sunflower genotypes become "resistant" and no broomrape infestation occurs.

SOME OF THE PARAMETERS OF SUNFLOWER RESISTANCE TO *Orobanche cumana Wallr.*

According to Suhorukov (1930) (as cited by Morozov (1947):

- Increased soil acidity leads to an increase in peroxidase activity, resulting in sunflower nonresistance to the pathogen;

Ukrajinski (1947) notes that broomrape resistance is determined by the specific responses of the sunflower root system;

- The author proved that resistant sunflower genotypes have a low pH at the start of the season and a high pH at the season's end.

SCREENING METHODS

Planting the breeding material on naturally infested plots

Evaluation of field resistance - introduction of broomrape seeds at sunflower planting

Resistance evaluation in the greenhouse – planting in pots (sunflower seeds + broomrape seeds)

Rapid evaluation of resistance in the greenhouse – planting on tables (soil + broomrape seeds + sunflower seeds)

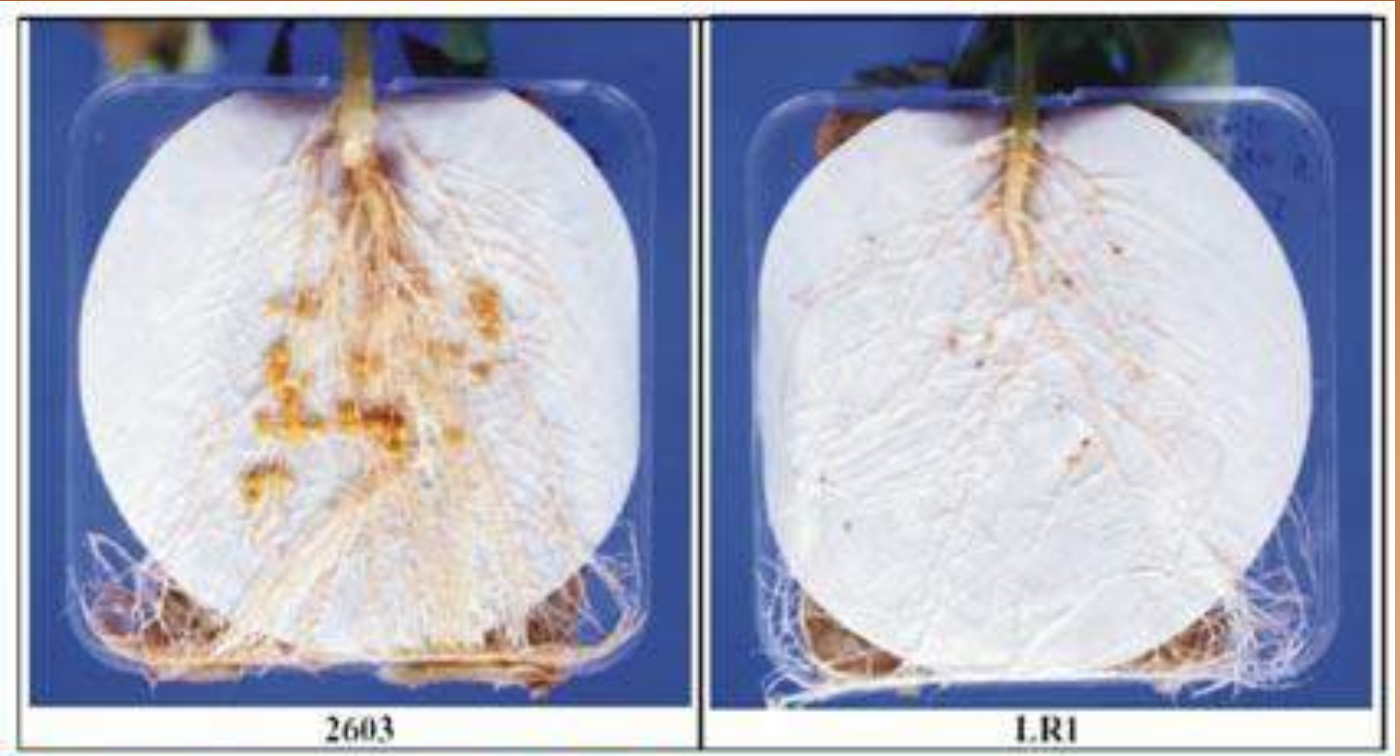
According to Panchenko

Rapid evaluation of resistance in the greenhouse - planting in test tubes

substrate
+
broomrape seeds
+
sunflower seeds

(Grezes-Besset)
Improved by Mihaljcevic

Hydroponic co-culture of susceptible (2603) and resistant (LR1) sunflower parasitized by broomrape, 21 days after infestation.



Patricia Labrousse *et al.* 2004.

Physiological features of possible regulation of sunflower resistance to broomrape (*Orobanche cumana* Wallr.)

Kyrychenko *et al.* (2018)

- 1. Physiological aspects of sunflower resistance to broomrape (*Orobanche cumana* Wallr.)
- 2. Influence of broomrape affection on morphological and physiological parameters of sunflower plants
  - 2.1. Total content of phenolic compounds in broomrape (*O. cumana* Wallr)-infected sunflower plants.
  - 2.2. Activity of oxidizing/reducing enzymes in different genotypes of sunflower infected by angiosperm parasite.

Physiological features of possible regulation of sunflower resistance to broomrape (*Orobanche cumana* Wallr.)

Kyrychenko *et al.* (2018)

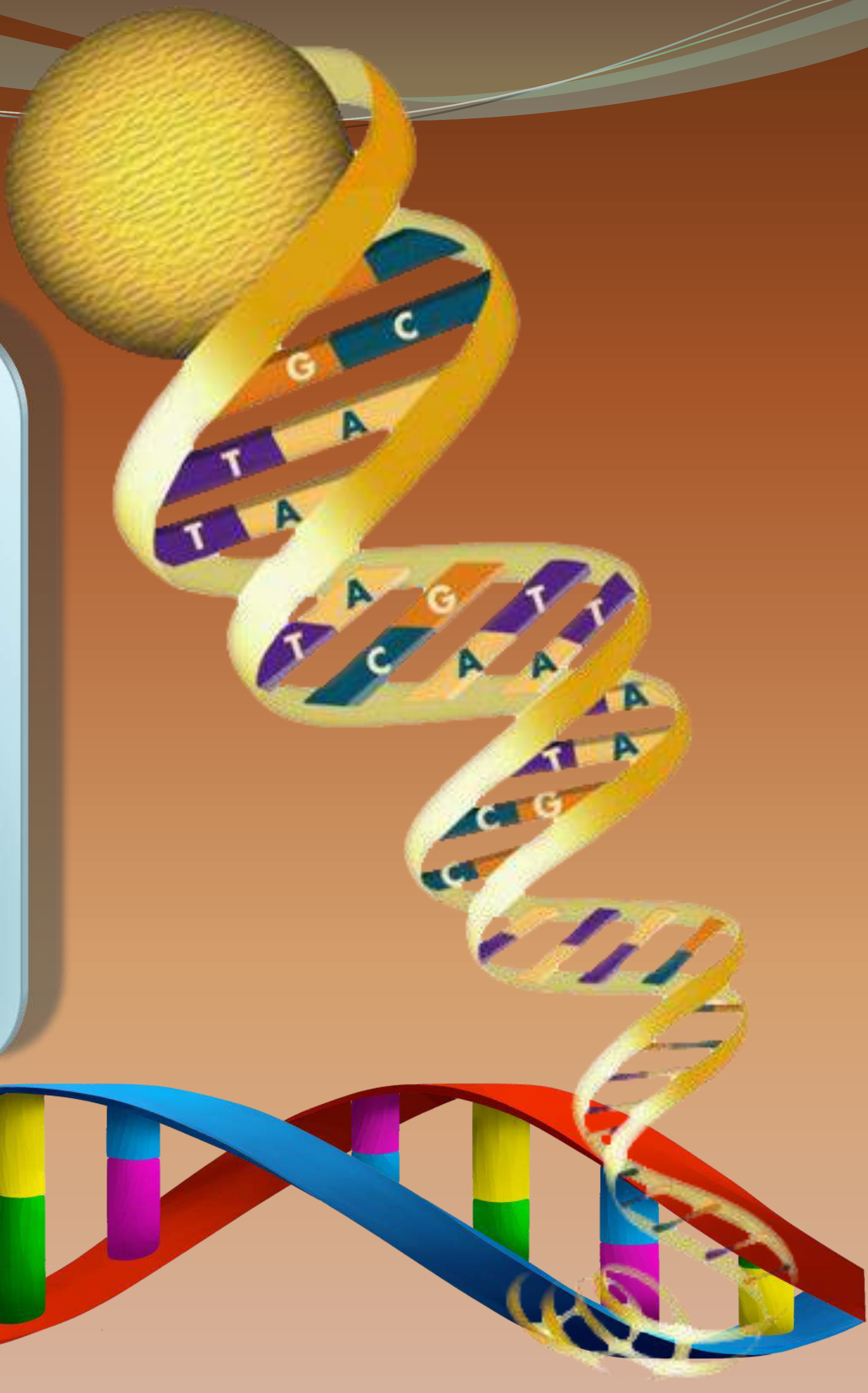
- Broomrape infection inhibits the growth of susceptible sunflower plants, however, it hardly affects morphological parameters of resistant sunflower genotypes. It was revealed that resistant and susceptible genotypes differed by phenol levels in plants under the influence of *Orobanche cumana* Wallr. The values of resistant accessions were higher than those of susceptible ones. It was determined that infection sunflower plants by angiosperm parasite considerably affected enzyme activities.

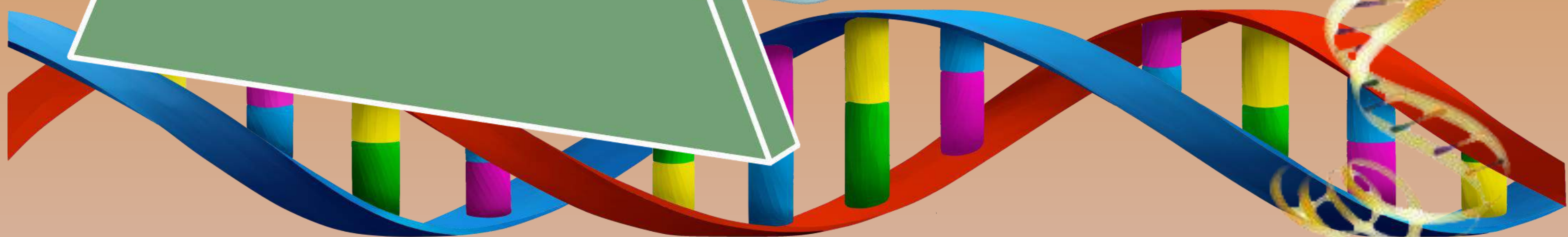
Physiological features of possible regulation of sunflower resistance to broomrape (*Orobanche cumana* Wallr.)

Kyrychenko *et al.* (2018)

- The most distinct patterns were observed on day 14 after infection, which indicates the feasibility of conducting research during this period. It was shown that the activities of enzymes (polyphenol oxidase, peroxidase and catalase) significantly increased, except those in the line-susceptibility standard, the values of which remained almost unchanged or substantially reduced after broomrape infection.
- Basing on our data, we developed a biochemical express-method for assessing sunflower genotypes for resistance to *Orobanche cumana* Wallr. The method consists in determination of catalase activity in green plant material.

Molecular markers





BREEDING METHODS:

- Conventional methods
- Induced methods
- MAS (Marker-Assisted Selection)
- Somatic hybridization
- Genetic transformation (GMO)

SOURCES OF RESISTANCE:

- Varietal populations
- Landraces
- Inbred lines
- Hybrids resistant to broomrape
- Interspecific hybrids (cultivated x wild)
- Wild species of the genus *Helianthus*

Wild sunflower species containing genes for resistance to *Orobanche cumana* Wallr.

- *Helianthus tuberosus;*
- *H.smithii;*
- *H.pauciflorus;*
- *H.nuttallii ssp. nuttallii;*
- *H.pumilus;*
- *H.maximiliani;*
- *H.hirsutus;*
- *H.eggertii;*
- *H.divaricatus;*
- *H.decapetalus*
- *H.ciliaris;*
- *H.resinosus;*
- *H.laevigatus;*
- *H.gigantheus;*
- *H.grosseserratus;*
- *H.agrestis;*
- *H.anomalus;*
- *H.debilis ssp.cucumerifolius;*
- *H.exilis;*
- *H.rigidus*

Table 1: The degree of broomrape (*Orobanche cumana* Wallr.) resistance in different sunflower genotypes at various Romanian localities in 2014 (Number of broomrape plants/main plot)

No.	Genotype	Locality			
		Cuza Voda	Crucea-Stupina	Valea Canepii	Tulcea-Agighiol
1	LC-1093 (rase F)	542	154	1.136	267
2	Performer	1.532	437	3.397	432
3	LG-5661	48	28	40	10
4	PR64LE20	69	58	129	43
5	No. 97	0	46	70	18
6	No. 144	0	72	4	9
7	No. 185	0	3	1	4
8	No. 194	0	32	130	25
9	No. 242	0	64	17	11
10	No. 341	0	30	123	35
11	No. 378	0	2	1	0
12	No. 388	0	110	63	10
13	No. 367	38	0	1	2
14	No. 143	-	38	0	14
15	No. 9	60	100	246	0
16	No. 188	20	8	1	0
17	No. 190	2	36	1	0
18	No. 191	3	33	5	0
19	No. 219	150	53	280	0
20	No. 274	40	40	28	0
21	No. 302	50	7	45	0
22	No. 348	1	9	3	0
23	No. 365	3	18	15	0
24	No. 381	8	23	4	0
25	No. 383	1	18	1	0
26	No. 384	2	7	2	0
27	No. 386	0	12	15	0

Table 2: The degree of broomrape (*Orobanche cumana* Wallr.) resistance in different sunflower genotypes at various Romanian localities in 2015 (Number of broomrape plants/plot)

No.	Genotype	Localities									
		Ciresu-Braila		Iazu-Ialomita		Stupina-Constanta		Topolog-Tulcea		Viziru-Braila	
		Sunflower plants/plot	Broomrape plants/plot	Sunflower plants /plot	Broomrape plants /plot	Sunflower plants /plot	Broomrape plants /plot	Sunflower plants /plot	Broomrape plants /plot	Sunflower plants /plot	Broomrape plants /plot
1	Performer	89	679	48	97	39	102	46	169	45	204
2	LC-1093	47	41	43	8	32	41	43	51	46	19
3	LG-5542	45	0	-	-	38	5	46	6	46	0
4	LG-5631	46	12	-	-	-	-	-	-	-	-
5	PR64LE20	48	16	44	0	41	18	44	26	44	0
6	Hy-1	128	887	95	136	47	68	95	113	105	38
7	Hy-2	120	798	105	87	55	95	95	233	94	98
8	Hy-7	126	0	95	0	44	0	96	0	141	0
9	Hy-9	124	0	106	1	55	11	82	18	100	8
10	Hy-10	132	2	94	0	46	16	93	59	95	30

Table 3: Evaluation of broomrape (*Orobanche cumana* Wallr.) resistance in different sunflower genotypes at the infested plot: VNIIMK – Don trial station, Rostov on Don in 2017

No.	Genotype	Percentage of infested sunfloer plants/main plot	Degree of infestation
1	Donskoy-22	100	
2	Bella	30	3,0
3	LG-5580	23	1.0 – 1.2
4	PR66LE25	19.4	2.6
5	Hy-7 (F_1)	17.1	1.1
6	F_2 (Hy-7)	35.1	2.0

Broomrape races…

- However, speaking of the new races G and H, we might not be completely sure. The question is whether the same mutation can occur in broomrape populations, in various countries during the same year? Certain differences which have not yet been studied in detail surely exist.

Broomrape races…

- The question regarding the degree of virulence in the new broomrape populations remains unanswered. Significant differences have best been illustrated by Pacureanu-Joita *et al.* (2012), stating that the most aggressive populations were found in Moldova, Romania, Russia, and Turkey.

Broomrape races…

- New studies worth mentioning are the ones carried out by Duca *et al.* (2017), who performed molecular characterization of 39 broomrape populations from Moldova using SSR markers.

- Based on the obtained results, the authors concluded that some populations from the Southern region (especially Carabetovca, Alexanderfeld, Stefan-Voda, and Slobozia Mare) have shown major differences in the obtained profiles and presented a high degree of genetic variability. The study revealed genetic diversity of *O. cumana* populations and contributed to research by providing useful information on this economically important pathogen.

Broomrape races...

- In conclusion, the results obtained in this research partly differ from the results obtained by Rișnoveanu *et al.* (2016), which was to be expected since the trials were carried out at various localities (plots). However, the results concur with authors' conclusion, confirming identification of new races of *Orobanche* previously undetected at the trial localities.

BROOMRAPE CONTROL BY HERBICIDES

Herbicide group:

- imidazolinones (imazethapyr, imazamax, imazapyr)

Sources of resistance:

- Wild *Helianthus annuus* L.
- (discovered by Al- Khatib *et al*., 1998 – a crop of soybean)
- Genes incorporated into elite lines of cultivated sunflower by backcrossing (BC) + screening for resistance
- Resistance controlled by two partially dominant genes (*Imr1; Imr2*), *Imr2* – modifier gene.

- Novi Sad sunflower team was among the first to develop commercial IMI–resistant hybrids
- Sala et al. (2008) identified a new source of resistance using induced mutations
- (EMS – ethyl methanesulfanate, 0.25% solution)
- New IMI–resistance gene: CLHA – PLUS (partial dominance)
- CLHA–PLUS differs from *Imr1*
- Excellent weed control + control of *Orobanche*!

Resistance in wild sunflower…

- At the beginning of the 21st century, public resistance sources were found in the wild sunflower *Helianthus annuus* L. discovered in Kansas (USA). Resistance source named CHLA- PLUS, obtained by Sala (2008) through induced mutations, has lately been used by the company BASF.
- It has been shown experimentally that the gene CHLA-PLUS a higher degree of IMI-resistance than the gene Imr_1, Imr_2. Breeding centers wishing to use the CHLA-PLUS gene for breeding purposes have to sign a contract on its use with the company BASF. At the same time, BASF provides a protocol for screening for resistance at the molecular level (CLEARFIELD® Protocol SF30).

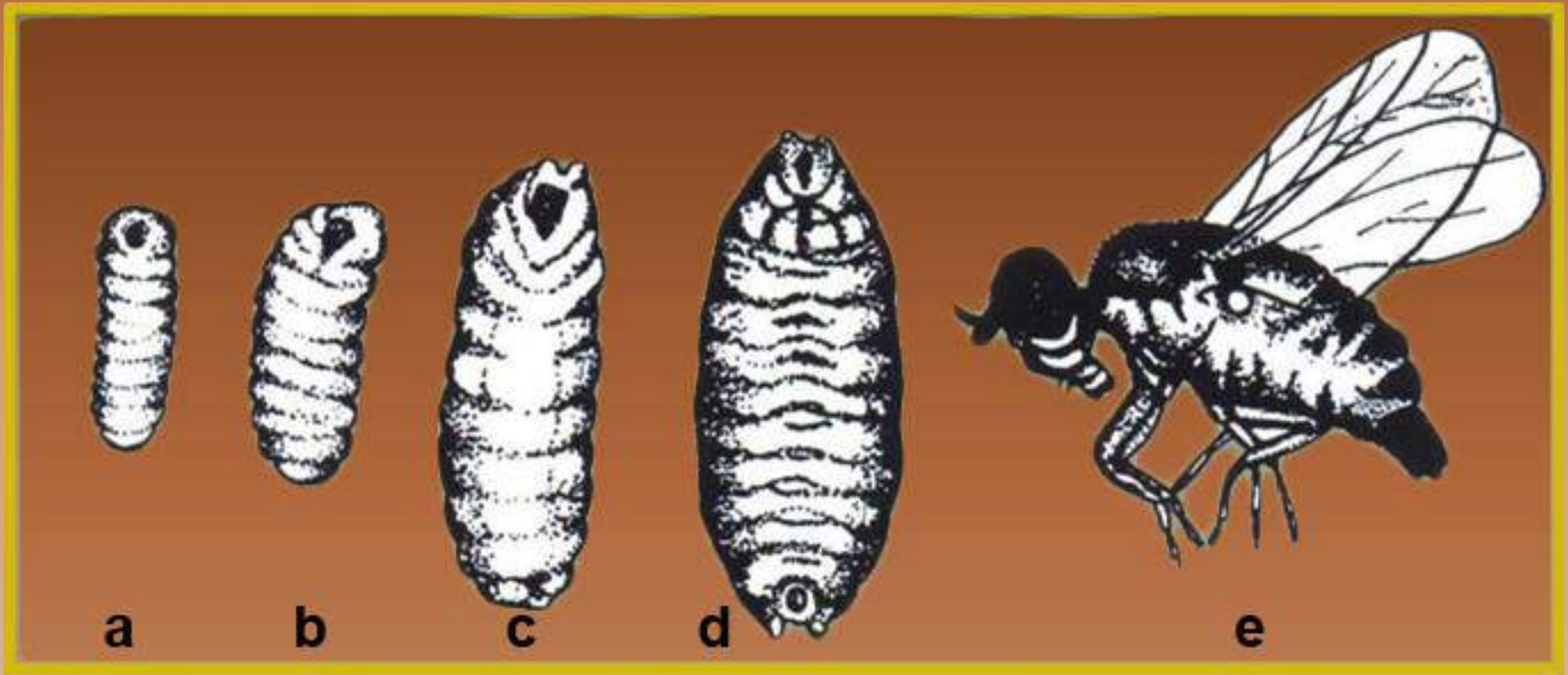
Resistance to broomrape...

- The results of Gorbachenko *et al.* (2018) suggest the possibility of developing new cultivars of sunflower resistant to the new variability of broomrape populations.
- This method of testing broomrape resistance has its drawbacks due to the differences in broomrape distribution at the testing plots. Also, broomrape expression depends on several factors, such as soil type, distribution and amount of rainfall during the vegetation period, temperature, pH value, and so forth.

- The results of Gorbachenko *et al.* (2018) offer the possibility of developing sunflower hybrids resistant to the new variability of broomrape populations which cannot be controlled by genes for race H.

Biological control of *Orobanche*

INSECT:
Phytomyza orobanchia Kalt.



WHAT SHOULD BE DONE NEXT?

- An international project of global magnitude needs to be established;
- Because of quarantine limitations, an organization should be designated to store seeds of *Orobanche cumana* Wallr. from all over the world and to make them available for use on global level;
- An organization should also be identified that would be willing to investigate in detail the genetic variability of broomrape on the molecular level;
- Genotypes of cultivated sunflower and wild *Helianthus* should be tested using the total variability of *Orobanche;*

WHAT SHOULD BE DONE NEXT?

- Wild sunflower species possessing complete resistance to total *Orobanche* variability should be used for incorporating the resistance genes into elite lines of cultivated sunflower;
- It should be established which organizations in the world would be interested in taking part in such a project (public + private sector);
- Issues of intellectual property rights and the right to use the new genetic variability should be settled in advance;
- Efforts should be made to identify those in whose interest it would be to provide funding for a global project of this kind;

Conclusions

Based on the studies in sunflower genetics and breeding for broomrape resistance in the Black Sea area, especially in Romania and Russia, the following conclusions can be made:

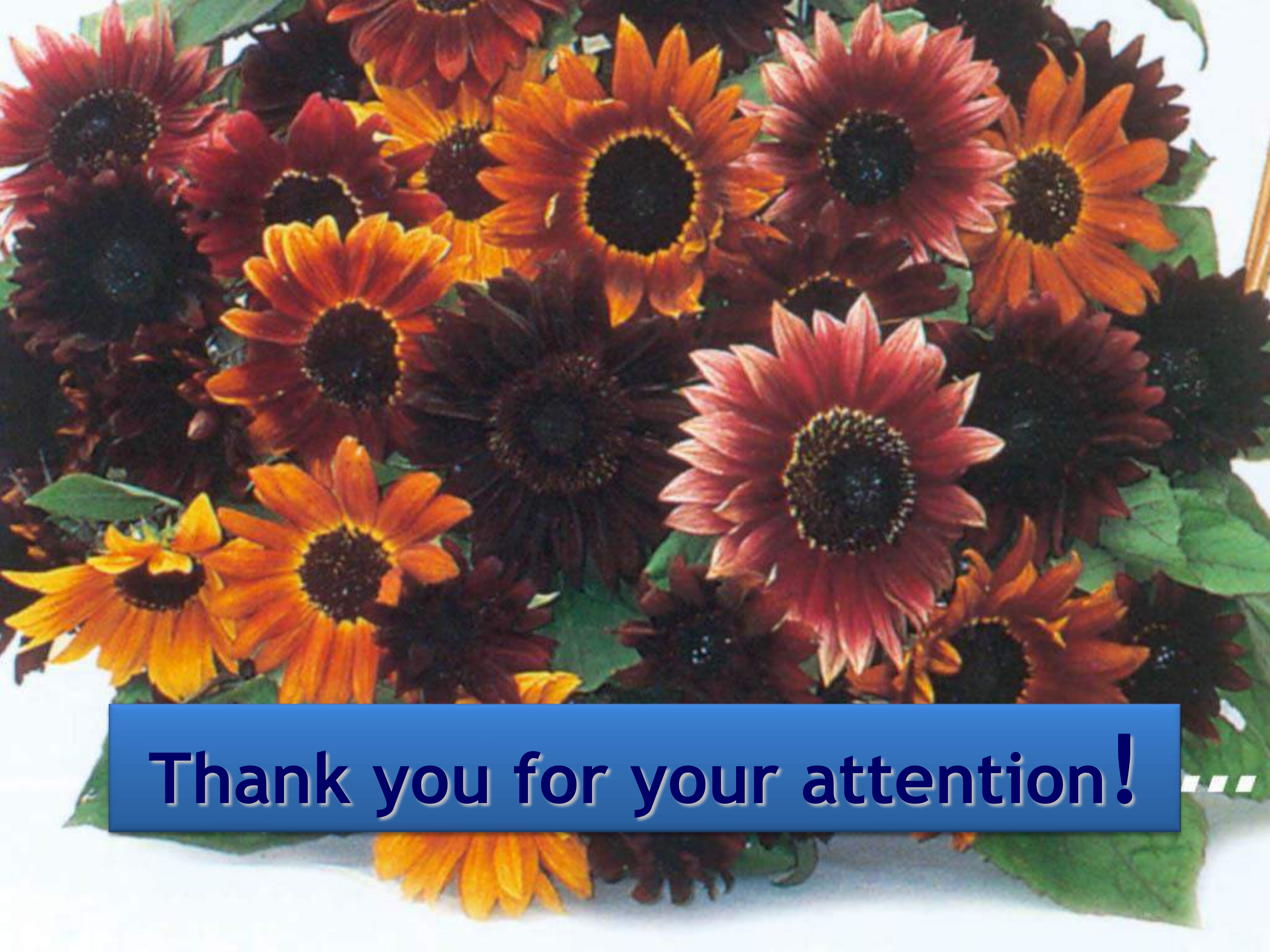
- Methods have been developed for the evaluation of resistance to *Orobanche*;
- Conventional methods and MAS (Marker Assisted Selection) have been developed in breeding for resistance to *Orobanche*;

Conclusions

- In Romania, new virulent population (races) of broomrape have emerged, especially in the regions of Braila, Tulcea, and Constanta. At certain localities, new variability of broomrape populations has been observed, which cannot be controlled by genes for race H;
- Likewise, at the trial station of VNIIMK – Don Trial Station in Russia, a new virulent broomrape population emerged, which cannot be controlled by genes for race H. Luckily, sunflower breeders have developed new genotypes resistant to the new variability of broomrape populations;

Conclusions

- There is permanent change in populations of *Orobanche cumana* Wallr., thus hindering the success of breeding for resistance;
- Sources of broomrape resistance genes are found in certain wild species of *Helianthus*, which are transferred after detection to the elite lines of cultivated sunflower using interspecies hybridization;
- Besides control through genes for broomrape resistance, *Orobanche* can successfully be controlled by the development of IMI-resistance hybrids.


Thank you for your attention!...