IV. HERBICIDE TOLERANCE AND OTHER CONTROL METHODS

4th International Symposium on Broomrape in Sunflower

SUSTAINABLE SUNFLOWER BROOMRAPE CONTROL WITH A DUAL MODE OF ACTION APPROACH

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Abstract

Effective broomrape control is one of the main cornerstones for a viable sunflower production in Europe. Many different strategies to reduce the impact of broomrape on sunflower have been tested. Today only breeding for genetic tolerance against broomrape and the chemical control of the parasite in herbicide tolerant sunflowers remain.

Conventional weed control systems developed for non-parasitic weeds are not suitable for broomrape control due to the underground development of the host attached parasite. With the introduction of the herbicide tolerant Clearfield® Production System for sunflower, the first effective chemical tool against *Orobanche cumana* has become available. It was quickly adopted by sunflower farmers which are faced with difficult to control weeds and multiple, quickly changing races of broomrape. The Clearfield Production System, as well as the newly developed Clearfield® Plus Production System, are both based on altered target-site acetohyrdoxyacid synthase (AHAS) genes, natural and induced mutations which allow the utilization of customized imidazolinone herbicides in sunflower. Target-site herbicide tolerance of the host crop is important for *O. cumana* control, as it permits a longer duration of herbicide activity in the host plant, compared to a metabolism based tolerance with an accelerated degradation of the active ingredient. Effective control of broomrape requires herbicides which are systemic – to be translocated from the leaves to the roots and the host-attached parasite. An additional advantage for a lasting broomrape control is a certain residuality of the herbicide in the soil, which allows recharging of the active ingredient via the root system during the vegetation period. Herbicide concentration in the host plant determines the duration of efficacy and is strongly depending on the initial uptake rate of the herbicide. Therefore, herbicide formulation, dose rate, application timing and environmental conditions have substantial influence on the *O. cumana* efficacy. However, weed and broomrape management strategies based on a single mode of action with only ALS-inhibiting herbicides are not sustainable mid or long term and might result in resistance problems. Broomrape resistance breeding was and still is the foremost non-chemical strategy to be employed by most sunflower breeders. The resistance is mainly based on dominant, single race specific genes obtained from wild sunflower sources allowing single-cross hybrid breeding. The sole reliance on the single gene resistance has possibly accelerated the quick evolution of *O. cumana* populations to more virulent races, which requires a continuous search for new resistance sources. This problem increases the complexity of resistance breeding as it requires the combination of a strong genetic background to complement the dominant single gene specifically with the latest races F, G and more. Today, in specific areas with a high pressure of *Orobanche* and the presence of very virulent races, even this combination is not providing complete efficacy.

To avoid or delay the rapid development of new pathotypes of *O. cumana* and/or elude the evolution of herbicide resistance in broomrape, Clearfield and Clearfield Plus sunflowers should be, if not already, combined with the latest genetic broomrape resistance. This approach to the broomrape problematic is the current golden standard and allows for sunflower breeders to keep their hybrids longer in the market and frees up time for new developments in genetic tolerance. On the other hand, the genetic tolerance complements the herbicide activity and adds a second mode of action to prevent or at least to delay as long as possible the occurrence of broomrape herbicide resistance. This approach provides the most sustainable method of broomrape control and the proper utilization of these technologies allows excellent weed control, including an effective control of *O. cumana* in sunflower.

Keywords: sunflower, broomrape, genetic tolerance, Clearfield

BROOMRAPE EPIDEMIOLOGY AND INTEGRATED CONTROL

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Abstract

Sunflower broomrape caused by *Orobanche cumana* Wallr, as with other broomrape species, is very difficult to diagnose and control because this parasite has a long underground development stage. Despite several resistance genes being introduced into commercial varieties and hybrids, new virulent races continue to appear every time a new resistance gene is released. In developing and implementing an integrated control program we have encountered some difficulties, mainly coming from a lack of understanding of broomrape epidemiology and the prevalence of some myths well implanted in farmers and scientist mindsets. In this document some of these myths will be discussed and the most frequent patterns of field infection presented. A mathematical model corroborates that from the first infection in a field, to a very high infection stage, only three or four generations of sunflowers are needed to be grown in the same field. The infection pattern that can be observed in the fields each subsequent sunflower crop is the same irrespective if the crop rotation is two or nine years. The Syngenta sunflower integrated broomrape control program, now under the brand name SOLGUARD™, was presented at the last sunflower broomrape conference in Cordoba 2014. This included the use of three pillars: Genetic control; crop management and chemical control. The SOLGUARD™ program, through field race diagnosis, provides farmers with personalized plot by plot advice for the sustainable management of this parasitic weed. The reduction of primary infections, as well keeping the broomrape seed bank as low as possible, seems to be essential towards the eradication of this parasitic weed.

1. Introduction

Six broomrape species are regarded as either widespread or acute agricultural problems. ***O. crenata* Forsk.** Including *O. speciosa* D.C., ***O. cumana* Wallr.**; ***O. cernua* Loefl.; *P. ramosa* (L.) Pomel,** (Syn *O. ramosa* L., including *O. nana* Noe and *O. mutely* Schultz); ***P. aegyptiaca* (Pers.) Pomel**. (Syn. *O aegyptiaca* Pers.); and ***O. minor* Sm.** They are difficult to diagnose and control because these parasites have a long underground development stage, and by the time they emerge much of the damage has already been done. The long term impact of broomrape can be very serious: their seed may persist in soil for decades and their spread is facilitated by man, agricultural tools, planting seeds and animals, leading to an accelerated increase in the infested areas. In some countries there have been programs for the eradication or control of broomrape, such as the "Emergency plan for broomrape control in Israel" and "The branched broomrape eradication program in Australia" (Joel M., and Manor H. 2005; Warren Philip, 2005). Despite the failure of the Australian branched broomrape eradication program, this has provided a series of principles to manage the risks and to minimize the impact of broomrape infection applicable to sunflowers.As with many obligate parasites, sunflower broomrape produce numerous small seeds, which can survive in the soil for several years when non host crops are grown in the field, but only germinates when stimulated by the host root presence. The description of *Orobanchaceae* seeds and seedlings, germination stimulation and their germination ecophysiology have been reviewed recently, (See Joel, D.M., *et al*., 2013 chapters 8 to11) and this knowledge is essential to understand the epidemiology of broomrape. However, there have been few recorded stages on how a field is infected and how the broomrape seed bank builds in the soil.After the first observation in 1993, of a sunflower field with severe damage caused of Race F or sunflower broomrape, (Alonso L.C. *et al.,* 1996) we followed its expansion and distribution in Spain over the next 12 years (Fernandez-

Escobar J., *et al.*, 2008). As the crop rotation in Southern Spain is very short, including often wheat after sunflowers and vice versa, these observations allowed us to understand better how new broomrape races are formed and what are the patterns of field infection. The Syngenta integrated sunflower broomrape control program was presented at the last sunflower broomrape conference (Alonso, L.C. 2014) and this included the use of three pillars: Genetic control; chemical control and crop management.
Genetic resistance genes are very difficult to find in most host species infected by any Orobanche specie, being the exception, the sunflower -*O. cumana* interactions as several resistant genes have been found in the wild species of *Helianthus* (Alonso. L.C., 1998).
Chemical control has only been possible in very few cases with the use of systemic herbicides that inhibit protein synthesis, such as the use in herbicide tolerant sunflowers of imidazolinones herbicides (Alonso *et al.*, 1998).
The use of trap crops or false hosts to induce suicide germination of broomrape seeds has been proposed, with different crops mentioned as trap crops of different *Orobanche* species (Goldwasser Y and J. Rodenburg 2013). In the case of *Orobanche cumana*, some trap crops have been recently published in Russia. (Antonova T. S., *et al.*, 2015).
In developing our program for a sustainable control of broomrape in sunflowers, we have found some difficulties mainly coming from lack of broomrape epidemiology understanding and the prevalence of some myths well implanted in farmers and scientists mindsets. In this presentation I will try to break some of these myths and bring an update of Syngenta's integrated broomrape program, now under our brand name SOLGUARD™.

2. Some common myths

2.1 Myth 1. A new sunflower broomrape race appears every 10 years and then expands!

This myth includes some other common thinking, such as the belief that ONLY one race is present in each field, and that broomrape races under the same letter (A, B, C…) are identical across the same country, or even across different countries. *Orobanche cumana* have been demonstrated to produce seeds by cross and self-pollination (Rodriguez-Ojeda, M.I. *et al.,* 2013), and there are indications of apomixis which have been observed (see Joel D.M., 2013 for review). These reproduction mechanisms, when combined with a probable enhanced mutation capacity, as has been observed in many obligate parasites, allow the broomrape to produce seeds of various genetic composition in each generation. Thus, new races evolve from previous races in infected fields, and under the same letter characterization there may be many different parasite mutants having in common their virulence on certain sunflower differential lines.
Some evidence point towards pre-existence of broomrape race F seeds prior to the use of sunflower hybrids resistant to race E. Broomrape seed samples collected in Spain at the end of the 1980s and identified as race E, had seeds of higher virulence. (Molinero-Ruiz *et al.,* 2008). Figure 1 (Alonso L.C. 2014) illustrates how preexisting broomrape mutations may be screened and selectively multiplied by the introduction of sunflower resistant hybrids, to become "new races". In subsequent generations, these new races will become dominant in the soil seed bank of the infected fields as they multiply, whereas the old races germinate and are unable to infect and as a subsequence they die.

2,2 Myth 2. Races with the same letter are identical across each country and from country to country.

The letter denomination of broomrape races indicate only that the broomrape isolates under the same letter are capable to infect certain "differential" sunflower lines. Two broomrape samples from different fields within a country or among countries may be classified as a race with the same letter and be different. In a recent study (Martin-Sanz *et al.*, 2016) it has been shown that broomrape isolates from different countries classified as race G, based on their virulence on race F resistant hybrids, can be further differentiated with the use of new sunflower differential lines, such as the inbred P-96. Whereas this line showed resistant reaction in a laboratory test using four broomrape isolates of race "G" from Spain, it was susceptible to broomrape race "G" from other Eastern Europe countries. Thus, the race "G" of these Spanish broomrape isolates were not introduced from other countries, but emerged locally from previous broomrape races in the fields. Furthermore, if P-96 is used as a new sunflower differential line resistant to race G, the Spanish broomrape isolates used in this study could be classified as race G and the others from East Europe as race H. (Table 1).

2,3 Myth 3. The most common way broomrape seed disperses is by wind

Broomrape seed can disperse by wind as they are very small. The myth is to believe that wind can disperse broomrape seeds uniformly across an entire infected field and to neighboring fields, as with a windborne disease, due to its small size. Flying a long distance from the last successful infection does not seem to be a good strategy for an obligate parasite. *Artemisia spp*., the wild natural host of *Orobanche cumana,* often appears in patches in the field. Most ripe broomrape seeds fall to the ground landing within half a meter of the parent plant (Warren P., 2005). Broomrape seeds have developed some traits to avoid flying-long distances. They are quite electrostatic in order to cling to soil particles as soon as they touch them. Also, they have a seed surface quite wrinkled-alveolated instead of flint resembling a golf ball. The dimpled broomrape seed surface may help it to fall more straight to the soil, pulled by gravity, than if the seed surface were flint.

3. The pattern of broomrape field infection and the number of years required for it.

Observations made in different farmers' fields during the last 30 years in Spain, and in sunflower trials planted in naturally infected broomrape fields, have helped to set a model for the broomrape soil seed bank built. *i.e*., the way a sunflower field evolves from the first infections of a new race to a field in which the broomrape seed bank is so large that it is not possible to grow sunflower without serious yield losses. This pattern has been possible to deduct because in Spain farmers often follow a very short rotation such as wheat/sunflowers or even repeating sunflowers after sunflowers in some years.

Table 2 & Figure 2 includes the 4 infection levels frequently observed in a field after the first infections, or a new race evolving inside an infected field planted with a new resistant sunflower hybrid. The main reasons for this pattern of development are the fact that most broomrape seeds fall and stay in the soil close to the infected plant and disperse with the help of conventional tillage. The progress of broomrape infection under non tillage is clearly much slower than under conventional tillage.

Level 1. Infection only occurs in few individual plants per hectare, not in groups. These individual plants are often distributed at random in the field. This is often observed in a heavily infected field when planting a new broomrape resistant hybrid. Often this level of infection passes unnoticed by farmers and even technical experts and as there is no damage in the crop everyone is happy with the control provided by the new resistance. However, broomrape plays an evil strategy. It makes everyone believe it does not exist until it is too late, as these individual infected plants may be screening and multiplying a new broomrape race.

Level 2. Can be observed**,** next time sunflower is planted in a field that had level 1 infection. Due to traditional tillage, small groups of plants infected are often consecutive in the same row when plowing and planting was done in the same direction. When plowing and planting are made in cross directions, the consecutive infected plants are often in the direction of the plowing. Also in these fields, one can see many individual plants infected with a random distribution. This infection level causes insignificant damage to the crop and farmers often do not see it.

Level 3. Broomrape infection appears in relatively small patches. More broomrape shoots/plant are observed in the center of these patches. The interpretation is that these patches are formed from the cumulative broomrape seed from the previous sunflower crop, with consecutive infected plants in the Level 2 infected field. As in the level 2 infected field, there are also individually infected sunflower plants, also groups of consecutive infected plants, as well as single infected plants observed across the plot in a level 3 infected field. Field damage and farmers awareness depends on the number of patches and their size, but often some damage is observed. However, the sunflower yield may still be good

Level 4. Broomrape infection becomes generalized with large patches of sunflower plants with multiple broomrape shoots. Plants in the center of these patches have shorter height and delayed flowering as they have more broomrape shoots/plant than in the border of the patches. These large patches often overlap giving the impression of generalized field infection. Crop damage is evident, and farmers often call only when they see this high level of infection. Growing sunflower crop in these level 4 infected fields is not possible unless using broomrape chemical control with Clearfield hybrids plus IMI herbicides, or with a new genetic resistant hybrid.

The spread pattern in patches demonstrates that the broomrape seeds don't travel much once they fall to the soil

4. Mathematical model to explain the 4 levels of infection

In order to see the impact of the different things that can influence the broomrape seed bank build up in the soil, the following formula has been used starting from a single infection per ha.

$\mathbf{N(m)} = \mathbf{A(m}\text{-}1) \times \mathbf{Y}^{n} \times \mathbf{Z}$

Where
Nm = Number of broomrape shoots per ha in the "**m**" sunflower crop after 1rst infection
Am = Nº seed produced.
Am = **X** x **Nm**, where the **X** variable = number of seed produced by a broomrape plant. For modeling purposed we have considered 1 plant to produce 30.000 seeds. Hence
Am= 30.000 x **Nm**

Y= Survival seed rate/year = This variable is considered 90% of previous year soil seed bank for modeling purposes i.e.,
Survival seed bank in year **n** = **Am** x $\mathbf{Y}^{n}$
Example: if only N = 1, the number of broomrape seeds in the field would decline until the next sunflower crop is planted, being **n** the number of years after the sunflower crops

year 1 = **A x Y** = 30.000 x 90%;
year 2 = **A** x $\mathbf{Y}^{2}$ = 30.000 x 81%
year 3 = **A** x $\mathbf{Y}^{3}$ = 30.000 x 73%

Z = Successful infection rate variable. For modeling purposes 2% has been used. i.e., out of the total broomrape soil seed bank only 2% infect the crop.

In **table 3,** the results of applying these formulas in 2 and 9 year rotation periods, In both cases, there is a sudden growth of the broomrape seed bank in the soil in the 4^{th} year to numbers well above the potential capacity of the sunflower crop to tolerate so many infections (see **figure 3**). We can conclude that

<u>**Period to reach Level 4 = (3 or 4) x Nº of years between sunflower crops**</u>

These results are very coincident with our observation in actual fields, and are similar to the results obtained by other mathematical models showing a fivefold increase of the seed bank every four seasons (Goldwasser Y. and J. Rodenburg 2013)

5. SOLGUARD™: The Syngenta´s sustainable broomrape management program

The SOLGUARD™ program has been launched by Syngenta in several CIS and Eastern Europe countries, with three main objectives. Long term reduction of damage on infected farms; prevent the spread of broomrape seeds and a gradual de-infestation of fields. Based on three pillars, Genetic resistance, chemical control and good agronomic practices, the benefits of this integrated approach include: Prevent broomrape introduction into non infected fields, avoid dispersion and race evolution, reducing the seed stock in the soil of infected fields, contributing to limiting the new virulent races of broomrape and integrate weed management and broomrape control. The main tool for the implementation of SOLGUARD™ program is based on Syngenta's exclusive App that includes tailored recommendations to farmers including personalized advice based on field by field diagnosis. It also allows data collection on farmers' broomrape infestation levels and race, creating a country map on broomrape and developing our knowledge of broomrape by area to deliver better recommendations year on year.

SOLGUARD™ broomrape race diagnosis per farm plot includes: Before planting diagnosis; field survey diagnosis during the crop growth and laboratory test after the crop is harvested.

The before planting diagnosis is based on a heuristic procedure through the use of a questionnaire, Syngenta's broomrape country/province risk map and other elements and allows to get the most probable race present in a given field. The App also produces a personalized recommendation plot by plot that is sent to the farmer. This heuristic diagnosis, has been proven to be correct so far in the great majority (>95%) of the fields we have tested.

The Apps also has the possibility to make a field diagnosis during or after flowering of the sunflower crop with the participation of Syngenta's technical experts. This can be done either to confirm or correct the results of the heuristic diagnosis or to evaluate a field that has never been diagnosed. The App utilizes both the field expert observations through a questionnaire plus the heuristic procedure used in the heuristic diagnosis. The level of accuracy of this field diagnosis is near to 100%. The App is also designed to help the field experts to assess the nature of infection in those cases where some infection is found due too many causes other than the buildup of new broomrape races. i.e., off types, escapees from herbicide treatment, etc.

Whenever required, Syngenta's field experts can collect broomrape seeds in any farm plot and send it to our laboratories for precise race diagnosis through the broomrape test using differential sunflower lines.

The use of the three pillars, genetic resistance, chemical control and good practices, differentiates Syngenta's SOLGUARD® program.

5.1 Genetic resistance.

Many growers, under the advice of seed companies, often decide to plant the variety or hybrid with resistance to the largest number of races. Due to the lack of accepted set of differentials, this has created an inflation of new letters, driven by marketing of seed companies, to define the latest races and the resistance to them. The results we can observe is a growing number of broomrape races or letters plus a large confusion on how to control them. Using the latest resistance gene may not be required in many cases, and using them alone in fields very heavily infected (level 4) only facilitates the appearance of new races. Furthermore, hybrids with the highest broomrape resistance may not be the best one in terms of adaptation and yield for a given region.

SOLGUARS™ recommends using the best adapted sunflower hybrid with the highest yield potential in the region with the capability to control the suspected dominant race in the farmer's field. Using the right resistance along with other control methods, may be enough to reduce the broomrape seed bank in the soil and keep the latest resistance to be used when really needed.

5.2 Chemical control

Clearfield® herbicides have been proven to control broomrape, while also providing good weed control. In many cases, when Clearfield® herbicide is applied it is difficult to determine the broomrape race present in the field. Also, there are examples where broomrape control is only done with Clearfield® herbicides. SOLGUARD™ app can provide heuristic broomrape race diagnosis, even when the herbicide has been applied. The service also allows monitoring of the potential buildup of weeds and broomrape resistance to Clearfield® herbicides.

5.3 Good practices

Often these are mentioned as educational tools but fail to be incorporated into the integrated control. Syngenta's SOLGUARD™ program incorporates some of these good practices in the recommendations sent to famers, along with the above mentioned genetic resistance and chemical control. There are two main situations where these good practices are incorporated in the recommendations. (Figure 4)

1. Preventing primary infections either in non-infected regions of the country or in certain plots within large farms.
2. Keep the primary infection for as long as possible at Level 1 or even move it to level 0

6. Is it possible to eradicate broomrape once a field is infected?

The United States Department of Agriculture (USDA) has been successful in the eradication of a parasitic weed similar to broomrape, *Striga* in corn. The first corn field infected by this parasitic weed was observed in US in 1956. The eradication program spent 250 mio US $ during 45 years (1956-2001). By 1999, the US corn area affected by *Striga* was 2800 Ha and after 2012 the parasitic plant is considered eradicated. (Eplee R.E. 1981; Parket C, 2012) This example demonstrates, eradicating parasitic weeds is possible.

But so far the attempts to eradicate broomrape have had limited success. There have been programs for the eradication or control broomrape such as the "Emergency plan for broomrape control in Israel" and "The branched broomrape eradication program in Australia" (Joel M., and Manor H. 2005; Warren Philip, 2005). The national branched broomrape program in Australia started soon after a small area was discovered in 1992 infected by branched broomrape in South Australia. The eradication program was State funded using methyl bromide, as soil fumigant, to destroy broomrape soil seed bank in the infected area. When it was found that the area affected was more widely distributed than first thought the National program was established in 1999 and the eradication program was adopted in 2001/02. Eradication remained the optimum strategy objective for years. But in 2011 the Australian Branched Broomrape National Management Group agreed that it was no longer technically feasible to eradicate the parasitic weed and the eradication program was wound up by end 2011.

I can bring an example of broomrape eradication in a farm in Spain. In 2002, one of the sunflower hybrids seed producing farms observed a plot with level 4 infection that was probably first time contaminated in 1995 during a drought as the farm is normally under irrigation but had limited water resources in that year. Under our advice, the farm adopted a very rigorous integrated approach. Sunflower seed production crops were only planted every five years and always using Clearfield® hybrids where application of the Clearfield® herbicide was mandatory. Furthermore, in the 5 year rotation at least 2 trap crops were included. i.e., Corn and cotton. In 2016, after 3 cycles using the above mentioned approach, we planted a hybrid seed production of a conventional hybrid. The plot was inspected several times without observing a single broomrape shoot. The farm may be re-infected in the future, as broomrape is present in the area, but keeping the two trap crops in the rotation and using Clearfield® resistant hybrids, at least once every two sunflower crops, should be enough to reduce the broomrape soil seed bank or keep the plots free of broomrape in non-infected areas.

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Figure 1. Preexisting broomrape mutations may be screened and selectively multiplied to become "new races" by the introduction of resistanthybrids

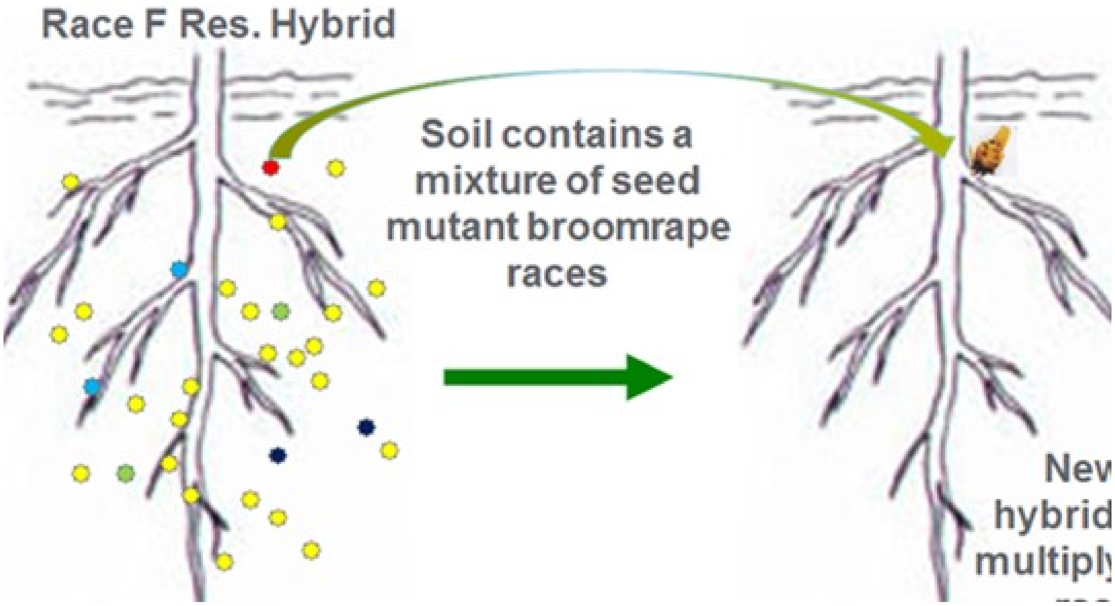
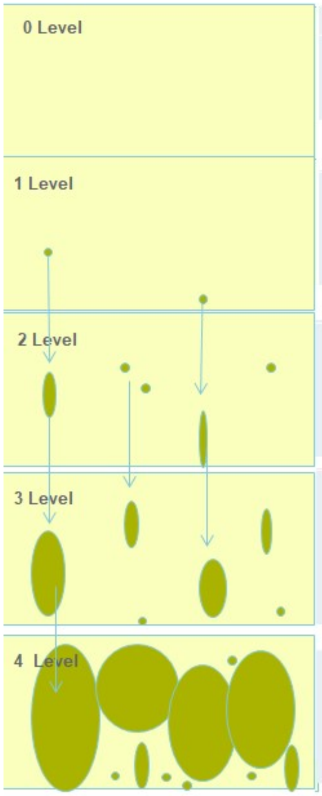


Table 1. Recent *O. Cumana r*ace G from Spain can be differentiated from race G of several East Europe countries (adapted from Martin-Sanz *et al.,* 2016*)*

	Broomrape race	
Sunflower differential	G Spain	*G East Europe*
Resistant to F	Susceptible	*Susceptible*
P-96	Resistant	*Susceptible*

Table 2 & Figure 2. Sunflower's broomrape epidemiology and field infection levels in subsequent sunflower crops



Infection level	Description	Crop Damage
0	There is no broomrape in the field	No crop damage
1	Infection only in few individual plants per Ha, not in groups, randomly distributed.	No crop damage
2	Small groups of plants infected in the same row. Isolated infected individual plants also present	Insignificant crop damage
3	Infections in small patches. More broomrape shoots/plant in the center of patch. Field damage may depend on the number and size of patches	Some crop damage
4	Infection becomes generalized with large patches with heavy infection overlapping. Plants in the center of patches have short height and delayed flowering	Important crop damage

Table 3. Sunflowers broomrape seed bank increase in the soil in two and nine year rotation schemas considering, 30.000 seed/shoot, a survival of 90% per year of previous broomrape seed soil stock and 2% of this seed bank infecting sunflowers the next time it is planted.

		Potential		
Rotation cycle	Sunflower crop	Broomrape Shoots/Ha	Broomrape Shoot/plant	Seed produced
2 year rotation	1rst	1	0	30.000
	2nd	486	0,010	14.580.000
	3rd	236.196	5	7.085.880.000
	4th	114.791.256	2.296	3.443.737.680.000
9 year rotation	1rst	1	0	30.000
	2nd	232	0,005	6.973.569
	3rd	54.034	1,1	1.621.022.061
	4th	12.560.343	251	376.810.295.780

Figure 3. Graphic representation of broomrape seed bank increase in 4 consecutive sunflower crops under a 9 year rotation schema considering 30.000 seed/shoot, a survival of 90% per year of previous broomrape seed soil stock and 2% of this seed bank infecting sunflowers the next time it is planted.

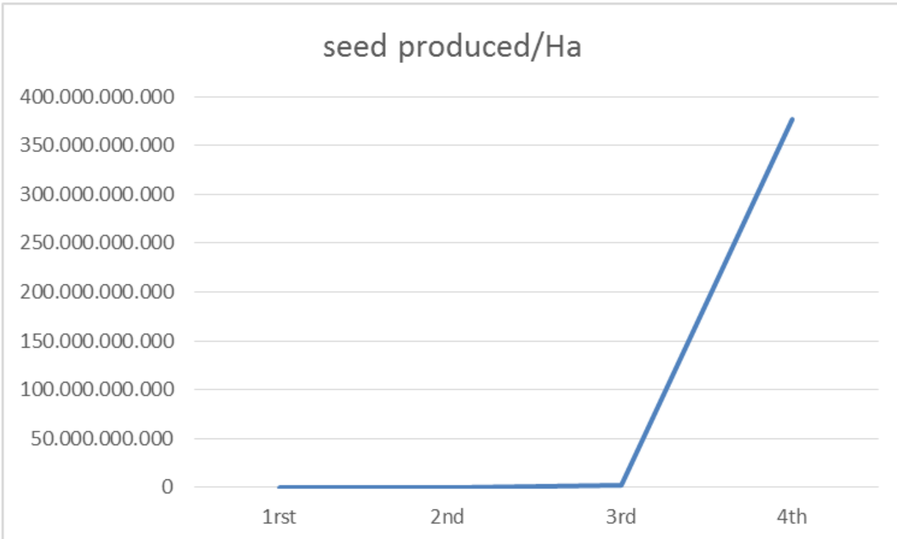
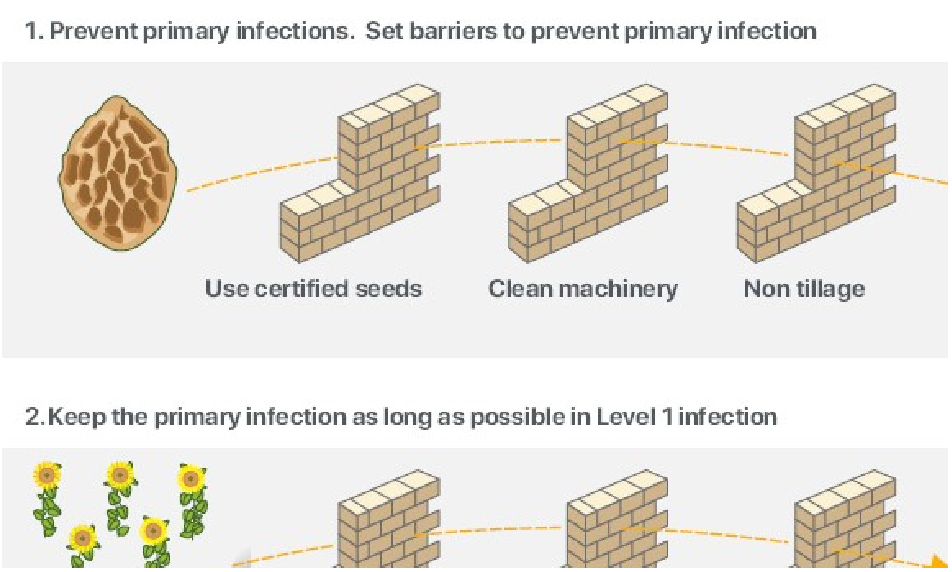


Figure 4. SOLGUARD™® incorporates the good agronomic practices in the integrated broomrape management



IMI HERBICIDE RESISTANCE STUDIES IN SUNFLOWER IN TURKEY

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Abstract

Sunflower grows many parts of world because of higher adaptation capability in different climates, less labor use and most preferable vegetable oil especially Eastern Europe, Asia and other parts of the world. However, sunflower grows in summer then mostly influenced more from environmental conditions especially from severe drought in hot summer season. Weeds and broomrape (*Orobanche cumana* Wallr.) parasite are the most limiting factors in sunflower growing areas in especially in Eastern Europe and Black Sea Region which have more than 60% of world sunflower planted areas. Clearfield technology with Imidazolinone (IMI) resistant hybrids and IMI herbicide (Imazomox) as post emergence application has been used commonly to control broomrape and common weeds. Both classical IMI originated by USDA and also other IMI tolerance source known as CL Plus and developed by seed mutagenesis and selection types are commonly in the sunflower seed market commonly both in Turkey and also in other sunflower growing countries. Furthermore, recent trends are combined both two types IMI genes with broomrape resistance to new races and also new races of downy mildew which are the most devastating disease with broomrape in sunflower. Now, these types hybrids increase selling and other than sunflower hybrids are not covering these three combined genes will not have market share in near future. Our study is conducted in Edirne, Turkey to develop these types of sunflower hybrids plus higher seed yield and oil content with larger adaptation capability. On the other hand, higher oleic acid types will be also valuable so combining this gene to new hybrids will be promising ones in the next years too. Furthermore, MAS selection and other molecular studies are using commonly in the breeding program to accelerate and apply precise selection breeding program in our study. Similarly, other tolerant genes to such as drought, some diseases and stay green trait etc. will be combined to this breeding lines utilizing molecular tools such as QTL etc.

Keywords: sunflower, Imidazolinone, herbicide resistant, hybrid breeding

IMPROVED *OROBANCHE CUMANA* CONTROL IN CLEARFIELD® PLUS SUNFLOWERS

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Abstract

Broomrape (*Orobanche cumana* Wallr.) in sunflower is one of the most important constraints in sunflower production in Europe. In the last decade, the herbicidal control option within the Clearfield® Production System in sunflower and the relevant Clearfield® herbicides has been an important control strategy and complemented the genetic resistance against broomrape. The commercial formulation Pulsar® (Imazamox 40 g/l) exhibits, besides broad-spectrum weed and grass control, a unique efficacy on broomrape in sunflower. Season long broomrape control is depending on a lethal concentration of Imazamox in the host plant over the time broomrape is attacking. The uptake of Imazamox and therewith the herbicidal concentration in sunflower is strongly influenced by the herbicide formulation. Field trials were conducted at different locations in Bulgaria, Hungary, Romania and Spain to evaluate the broomrape efficacy of Pulsar® Plus (Imazamox 25 g/l), containing a new and improved adjuvant complex, for the recently introduced Clearfield Plus system. The Pulsar Plus herbicide formulation was evaluated at full and reduced dose rates and compared to Pulsar, at the same active ingredient rates. Pulsar Plus outperformed Pulsar, both in *O. cumana* control and sunflower yield.

Keywords: Clearfield, Clearfield Plus, dose-response, *Helianthus annuus*, Imazamox

EFFICACY AGAINST BROOMRAPE AND SELECTIVITY OF IMAZAMOX-CONTAINING HERBICIDES AT SUNFLOWER

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Abstract

During the period of 2016-2017 a field trial for evaluating the efficacy of imazamox-containing herbicide products for control of sunflower broomrape (*Orobanche cumana* Wallr.) was conducted. The experiment was carried out in the experimental base of the department of ”Agriculture and Herbology” of the Agricultural University of Plovdiv, Bulgaria. The selectivity of the herbicides to the sunflower plants was also evaluated. The grown sunflower hybrid was „Lucia CLP”. Variants of the trial were: 1. Untreated control; 2. Pulsar® 40 - 1200 ml ha^{-1}; 3. Pulsar® 40 - 2400 ml ha^{-1}; 4. Pulsar® 40 - 1200 ml ha^{-1}; 5. Pulsar® 40 - 2400 ml ha^{-1}; 6. Pulsar® Plus - 2000 ml ha^{-1}; 7. Pulsar® Plus - 4000 ml ha^{-1}; 8. Pulsar® Plus - 2000 ml ha^{-1}; 9 Pulsar® Plus - 4000 ml ha^{-1}. At variants 2, 3, 6 and 7 the herbicides were applied in phenophase 4^{th} – 6^{th} true leaf of the sunflower (BBCH 14-16), and at variants 4, 5, 8 and 9 – in phenophase 8^{th} – 10^{th} true leaf (BBCH 18-19). The highest efficacy against the broomrape was reported for the treatments of variants 4 and 9. For the control the highest broomrape density was reported – 13.65 specimens per 1 sunflower plant average for the period. It was observed that Pulsar® Plus is more selective to the sunflower hybrid in the study in comparison to Pulsar® 40. After the treatment with Pulsar® Plus at rate of 2000 ml ha^{-1} in phenophase 8^{th} – 10^{th} true leaf the highest sunflower seed yield was recorded. It was proven that the seed yield from the plants treated with doubled herbicide rates was lower from the plants treated with the registered rates. The untreated control had the lowest yield. A similar tendency for the indicators 1000 seeds weight and hectoliter seed mass was observed.

Keywords: *broomrape, sunflower, imazamox, efficacy, selectivity*

INTRODUCTION

The most distributed weeds in the Bulgarian sunflower fields are the weed species *Amaranthus retroflehus* L., *Sinapis arvensis* L., *Chenopodium album* L., *Xanthium strumarium* L., *Setaria* spp., *Echinochloa crus-galli* L., *Sorghum halepense* L., *Cirsium arvense* L., *Convolvulus arvensis* L. and the root holoparasite *Orobanche cumana* Wallr. (Neshev at al., 2017). *O. cumana* L. is distributed all over Bulgaria (Stoyanov et al, 1967). The broomrape is one of the main factors limiting the sunflower production (Tsvetkova et al., 1998). This parasitic plant is naturally distributed from central Asia to south-eastern Europe, where it parasitizes wild Asteraceae species. It is also an important parasitic weed of sunflower crops (Stoyanov et al, 1967; Pineda-Martos et al., 2014). The control of the sunflower broomrape is mainly driven by the creation of sustainable and tolerant varieties, as well as the cultivation of resistant to the herbicide imazamox varieties. It is considered that the integration of different methods and tools implies best parasitic control options (Tonev et al., 2018). Shindrova (2006) reported that the highest sunflower broomrape infestation has in south-eastern Bulgaria. In the different areas there is up to 80% infestation and a parasite density of 50-60 specimens per sunflower plant. In the south-eastern and north-eastern regions of the country, the degree of infestation is up to 50% and the parasite density from 15 to 20 specimens per plant. The measurements for decreasing the degree of broomrape infestation include preventive measures, improvement of the phytosanitary conditions of the crops and control (Kleifeld, 1998). As a result of growing broomrape resistant-sunflower varieties, "the old" parasite races disappear and in their place ”new” more virulent ones appear (Alonso, 1996; Pacureanu-Joita et al., 1998). In a study conducted by Domínguez et al. (2014) an excellent broomrape control after the treatment of the plants imazamox is recorded. Making hybrids resistant to imazamox and tribenuron-methyl enabled an efficient control of the main broadleaf and grassy weeds, including parasitic species from genus Orobanche (Malidza et al., 2003; Fernandez-Martinez et al., 2009).

The aim of the study is to establish the efficacy against the broomrape and the selectivity of imazamox-containing herbicides in Clearfield® Plus sunflower hybrid.

MATERIAL AND METHODS

The experiment was situated in the experimental field of the base for training and implementation of the Agricultural University of Plovdiv, Bulgaria. The trial was conducted by the randomized block design in 4 replications. The size of the experimental plot was 28 m². The grown sunflower hybrid was „Lucia CLP" which is susceptible to parasitation of *O. cumana*. Variants of the trial were: 1. Untreated control; 2. Pulsar® 40 - 1200 ml ha^{-1}; 3. Pulsar® 40 - 2400 ml ha^{-1}; 4. Pulsar® 40 - 1200 ml ha^{-1}; 5. Pulsar® 40 - 2400 ml ha^{-1}; 6. Pulsar® Plus - 2000 ml ha^{-1}; 7. Pulsar® Plus - 4000 ml ha^{-1}; 8. Pulsar® Plus - 2000 ml ha^{-1}; 9 Pulsar® Plus - 4000 ml ha^{-1}. Pulsar® 40 is containing 40.0 g/l *Imazamox* and Pulsar® Plus is containing 25 g/l *Imazamox*. At variants 2, 3, 6 and 7 the herbicides were applied in phenophase 4^{th} – 6^{th} true leaf of the sunflower (BBCH 14-16), and at variants 4, 5, 8 and 9 – in phenophase 8^{th} – 10^{th} true leaf (BBCH 18-19). The sowing is performed in the optimal time for the region. A predecessor of the sunflower was winter wheat – for both experimental years. On the trial field deep ploughing, two times disc harrowing and two times cultivation before sowing were done. Basic combine fertilization with 250 kg ha^{-1} NPK 15:15:15 and spring dressing with 200 kg ha^{-1} NH_4NO_3 was performed. The number of the parasites per one sunflower plant was counted at the end of the vegetation. Per every repetition of each variant 2 sunflower rows were taken out from each plot and the number of parasites was recorded. The selectivity by the 9 score scale of EWRS was evaluated on the 7^{th} and the 14^{th} day after the herbicide application (at score 0 there are not damages on the crop, and at score 9 the crop is completely destroyed). The hectoliter seed mass was measured by weighing two parallel samples of 100 dm^3 air dry seeds. The hectoliter mass is calculated, as the arithmetic means of the established mass of the two samples (in grams) multiply by 100 and the resulting is divided into 1000 to obtain the mass in kilograms (Tonev et al., 2018). The absolute seed mass of 1000 clean, air-dry seeds, expressed in grams was also measured (Tonev et al., 2018). Statistical analysis of collected data was performed by using Duncan's multiple range test by the software SPSS 19. Statistical differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

The highest number of the parasite per one sunflower plant was recorded for the control (Table 1). In the first year of the experiment, the number of *O. cumana* was 9.29 specimens/sunflower. In the second experimental year, the parasite's number increased and reached 18.00 specimens per sunflower plant from the control. This increase is possibly due to the growing of the sunflower on the same field for a second year. Our data corresponded with results obtained by Tsyliuryk et al. (2018) who found that the parasitism naturally increased with the reduction of the time interval of sunflower return in crop rotation. Average for the period, the number of the parasites was 13,64 specimens per sunflower. The difference of the obtained results for the control was considered significant according to Duncan's multiple range test ($p<0.05$) in comparison to the variants with applied herbicides (Table 1).

Table 1. Number of *O. cumana* per sunflower plant

Treatments	2016	2017	Average
1	9,29 a	18,00 a	13,64 a
2	2,94 b	5,68 b	4,31 b
3	1,69 c	3,13 c	2,41 c
4	0,05 f	0,08 f	0,07 f
5	0,21 e	0,60 e	0,41 e
6	0,80 d	1,14 d	0,97 d
7	0,66 d	0,83 d	0,75 d
8	0,26 e	0,48 e	0,37 e
9	0,04 f	0,07 f	0,06 f

Figures with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$)

After the treatment with Pulsar® 40 at the rate of 1200 ml ha^{-1} (variant 2) in phenophase 4^{th} – 6^{th} leaf of sunflower (BBCH 14-16) the lowest efficacy was reported - 4.31 parasites/sunflower average for the period. The efficacy of the doubled rate of 2400 ml ha^{-1} was higher - 2.41 specimens average for the period. For treatments 4 and 9 the application of Pulsar® 40 (at rate of 1200 ml ha^{-1}) and Pulsar® Plus (at rate of 4000 ml ha^{-1}) the number of the parasites at the end of the sunflower vegetation was 0,07 and 0,06 specimens respectively average for the period of the study (Table 1). The efficacy of the treatments at the other variants (5, 6, 7 and 8) was satisfactory.

The visual evaluation of the herbicide phytotoxicity is presented in table 2. The highest herbicide toxicity was observed for variants 3 and 5 (score 3). It was determined as moderate phytotoxicity. This was due to the treatment of the doubled rate of Pulsar® 40. The content of the active substance in the herbicide product is higher and it was more aggressive to the sunflower hybrid that is bred to be grown by the Clearfield Plus technology. Sulfonylureas (tribenuron-methyl) and imidazolinones (imazamox) are herbicides inhibiting the ALS enzyme (Acetolactate synthase), which is involved in the biosynthesis of the vital amino acids valine, leucine and isoleucine. The visual phytotoxic symptoms at the sensitive plants or non-target crops are growth retardation, the leaves lost its turgor, the plants lose their vitality, etc. (Fedke and Duke, 2005).

Table 2. Visual phytotoxicity at the sunflower (by the visual scale of EWRS)

Variants	2016	2017	Average
1	-	-	-
2	2	2	2
3	3	3	3
4	2	2	2
5	3	3	3
6	1	1	1
7	2	2	2
8	1	1	1
9	2	2	2

Balabanova and Vassilev (2015) concluded that the treatment with the herbicide imazamox causes an inhibition of growth and photosynthetic performance in IMI-R Clearfield sunflower hybrids. The inhibition is less pronounced in the plants treated with the recommendable dose (120 ml/da Pulsar 40) and significantly higher in the plants treated with the exceeded imazamox dose.
The phytotoxicity was lower for treatments 2 and 4 (score 2) and it was determined as weak. After the treatments for variants 6 and 8 the phytotoxic symptoms were classified as very weak (score 1). This was probably due to treatment with Pulsar® Plus in the registered rates. At variants 7 and 9 the phytotoxicity was score 2 (weak).

The absolute seed mass of 1000 seeds is a very important indicator. The results are presented in table 3. The seeds with bigger values of the indicator have a higher price. According to a lot of authors this indicator has crucial for the formation of the yields (Georgiev et al., 2014). Hladni et al. (2008) found that seed yield was most positively correlated with thousand seed weight. At high levels of broomrape infestation, the mass of 1000 seeds was the lowest (Gisca et al., 2017). That statement corresponds with the results from our experiment.

The lowest absolute seed mass of 1000 clean, air-dry seeds was recorded for the control (61,23 g) where the infestation was the highest.

Table 3. Absolute seed mass of 1000 seeds, g.

Variants	2016	2017	Average
1	62,09 e	60,38 d	61,23 d
2	64,19 c	63,48 b	63,84 b
3	62,65 de	61,26 cd	61,96 d
4	67,35 a	64,98 a	66,17 a
5	62,46 de	61,33 cd	61,89 d
6	67,43 a	64,98 a	66,21 a
7	62,79 d	60,69 cd	61,74 d
8	66,46 b	65,14 a	65,80 a
9	63,83 c	61,80 c	62,82 c

Figures with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$)

It should be noted that the values for this indicator are lower in the second year of the experiment in all variants. The highest 1000 seeds mass was reported for treatment 6 (Pulsar® Plus - 2000 ml ha^{-1} in BBCH 18-19) - 66,21 g average for the period. The difference of the obtained results for treatment 6 was with proved differences according to Duncan's multiple range test ($p<0.05$) average for the period in comparison to the rest of the variants. Only the results of treatment 4 where with insignificant differences compared to treatments 6. At the variants with doubled rates of the evaluated herbicides, a decrease of the values of the indicator was observed. This could be a result of the herbicide stress caused by the higher imazamox rate.

High hectoliter mass is thus preferred by the industry (Abraham Nel, 2001). The lowest hectoliter mass of the sunflower seeds was the lowest for the control - 26,19 kg, (Table 4) average for the period. Gisca et al. (2017) reported a decrease of the values of this indicator at the variants with a higher level of infestation at the variants with higher *O. cumana* infestation. The magnitude of the hectoliter mass is determined by the grain size, the presence of impurities, etc. (Tonev et al., 2018).

Table 4. Hectoliter seed mass, kg.

Variants	2016	2017	Average
1	26,33 d	26,04 e	26,19 e

2	32,63 b	32,13 b	32,38 b
3	30,45 c	29,72 cd	30,08 c
4	33,15 ab	31,95 bc	32,55 b
5	29,38 cd	27,08 de	28,23 d
6	32,24 b	31,76 bc	32,00 b
7	31,22 bc	30,06 c	30,64 c
8	34,24 a	33,20 a	33,72 a
9	30,41 c	29,43 d	29,92 cd

Figures with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$)

The highest hectoliter mass of the sunflower seeds was recorded for variant 8 - 33,72 kg. The difference of the obtained results for treatment 8 was with proved differences according to Duncan's multiple range test ($p<0.05$) average for the period in comparison to the other treatments. As well as for the 1000 seeds mass the values of this indicator were lower at all variants for the second year of the study.

Also for this indicator a tendency of decrease in the values after the application of double herbicide rates independently the phenophase of the sunflower was recorded. This could also be a result of the herbicide stress.

In infested fields, *Orobanche* causes severe yield and quality losses (Duca and Glijin, 2013). Depending on the aggressiveness of the broomrape and the climatic conditions, the yield losses vary within 5-90 % (Gisca et al., 2017). The lowest yields were recorded for the control variant - 208,35 kg da^{-1} average for the period. Pulsar Plus is superior Pulsar in regard to the control of *O. cumana* control, as well as in regard to the yield (Pfenning et al., 2016). In our study the same tendency was found.

Table 5. Sunflower seed yield, kg ha^{-1}

Variants	2016	2017	Average
1	223,33 i	193,37 i	208,35 i
2	367,73 e	345,07 e	356,40 e
3	245,03 h	225,83 h	235,43 h
4	372,60 d	353,23 d	362,92 d
5	297,61 g	269,28 g	283,44 g
6	435,18 a	404,17 a	419,68 a
7	385,20 c	368,44 c	376,82 c
8	425,44 b	431,49 b	428,46 b
9	325,19 f	315,76 f	320,48 f

Figures with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$)

The highest sunflower seed yield was recorded after the treatment with Pulsar® Plus - 2000 ml ha^{-1} in BBCH 18-19 (variant 8) - 428,46 kg da^{-1} average for the period. The difference of the obtained results for the sunflower seed yield at treatment 8 were with proved differences according to Duncan's multiple range test ($p<0.05$) average for the period in comparison to the other treatments. The variants treated with doubled imazamox rates had lower yields in comparison with those treated with registered doses. This was probably due to the obtained herbicide stress from the high rates of the herbicides. Despite this fact the yield from the stressed sunflower plants was higher than those of the control highly infested with broomrape. This in turn shows how harmful it can the sunflower broomrape can be.

CONCLUSIONS

The highest number of the parasite per one sunflower plant was recorded for the control. In the second experimental year the parasite's number increased and reached 18.00 specimens per sunflower plant.

The highest herbicide toxicity was observed for treatments 3 (Pulsar® 40 - 2400 ml ha^{-1} in BBCH 14-16) and 5 (Pulsar® 40 - 2400 ml ha^{-1} in BBCH 18-19).

The lowest 1000 seed mass, as well as hectoliter mass, was recorded for the control where the infestation was the highest.

The lowest sunflower seed yield was recorded for the control variant - 208,35 kg da^{-1} average for the period. It was found that the variants treated with doubled imazamox rates had lower yields in comparison with those treated with registered doses, but the yield from the stressed sunflower plants was higher than those of the control.

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THE EFFECT OF PROHEXADIONE CALCIUM AGAINST THE SUNFLOWER ROOT PARASITE *OROBANCHE CUMANA*

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Abstract

Orobanche cumana is a root-parasitic weed, a so called obligate holoparasite. This class of parasites doesn´t carry out own photosynthesis. It obtains nutrients and water from sunflower as host plant and thus complements its life cycle. Broomrape is a highly variable parasite. It can adapt very fast to the sunflower genotype. Therefore, the control of broomrape is very problematic. One of the few solutions to this problem is the chemical control via the Clearfield® system. It contains an imidazolinone-tolerant sunflower with a genetic modification (non-GMO) of the acetohydroxy acid synthase (AHAS). The Clearfield® sunflower can still produce leucin, isoleucine and valine despite a treatment with e.g. imazamox. The herbicide is systemically distributed to roots of the host, reaches the parasite via a physical connection (haustorium) and inhibits the AHAS of *O. cumana*. Field studies have shown that Prohexadione calcium (PHDC), a plant growth regulator, can improve the efficacy of the Clearfield® herbicide. Low rates of Imazamox did not provide season long control of broomrape. However, the number of emerged *O. cumana* plants was significant lower than in the untreated control. The combined application of Imazamox and PHDC did result in a field plot without emerged *O. cumana*. The current work is aimed to show, that a similar effect could be reproduced in pot trials.

To determine the race independent synergistic effect of the herbicide with the plant growth regulator a test system was established. In this system different sunflowers with known genotype were used and tested with a collected batch of *O. cumana* seeds from Spain.

For understanding the effect of PHDC, a distribution study of the plant growth regulator in sunflower has been conducted. Interestingly, the growth regulator showed a fast-systemic distribution to the roots.

Keywords: *O. cumana*, prohexadione calcium, Clearfield® system, *Helianthus annuus*

EMERGING PARASITIC WEED BROOMRAPE ISSUES IN CHINA AND ITS POSSIBLE MANAGEMENT SOLUTIONS

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Abstract

Sunflower is one of the most popular crops cultivated from northeast to northwest of China, due to its diverse adaptability to a wide range of soil types and climates. The sunflower cultivation area in China was about 9.23×10^5 ha, with a total yield of approximately 2.42×10^6 t in 2013. From last few years, sunflower broomrape (*Orobanche cumana* Wallr.) infestation has rapidly expanded in China and has become a major threat to sunflower production in Xinjiang, Inner Mongolia, Shanxi, Hebei, Heilongjiang, Liaoning, and Jilin provinces (regions). A large number of reserves of *O. cumana* seeds exist in infected soils, especially in Inner Mongolia. For example, only in Bayan Nur City (Inner Mongolia), 40% of sunflower fields were infested by broomrape, resulting in 25–40% sunflower yield reductions. Similarly, in Dingbian County (Shaanxi province) sunflower plantation covers around 10.000 ha, and 64% of the fields were infested by *O. cumana*, resulting in 40–50% yield losses. In China, confectionary sunflower genotypes/cultivars are considered as a cash crop because of its higher demand and economic value, but its cultivation is severely hampered by *O. cumana,* especially in the Inner Mongolian region. The decrease yield of confectionary types severely affected the outcome of both large and small farmers. Till date, five races (A, D, E, F and G) of *O. cumana* exist in different regions of China, but no consistent comparative studies have been conducted on the race determination and virulence of broomrape populations from different areas in China to clarify and unify the uncertainty. Therefore, the current racial situation of broomrape in the main infested areas remains unclear. To establish strategies to control *O. cumana* growth and restrict its spread in crop fields, it is important to learn more about this pest, studying its life cycle, its development, and its molecular mechanisms of infection. It might be helpful to develop molecular markers associated with geographical origin of *O. cumana*, which could be used as a diagnostic tool for parasite introductions into growing areas where they might represent a threat to sunflower production. Controlling *O. cumana* with trap crops may be a biologically promising and practical method in farming. It may be possible that some plants have potential to be used as a trap crop or can be processed into green herbicide formulations that can be applied in agriculture production to rapidly deplete the seed bank of *O. cumana* in soil. Composition of strigolactone both in quality and quantity is a critical aspect of the sunflower to trigger interactions with *O. cumana,* because differences in the strigolactone composition among Chinese sunflower genotypes/cultivars could also govern compatibility and host preference for *O. cumana* races. Similarly the identification of a set of genes or proteins with key functions in mediating susceptibility or resistance against *O. cumana* infestation via next-generation sequencing platforms will be essential to understand the basis of the *O. cumana*-host interaction and may advance knowledge about plant-weed dialogues.

Keywords: sunflower, broomrape, strigolactone, trap crop, next-generation sequencing

CONTROL OF SUNFLOWER BROOMRAPE WITH BIOCONTROL AGENTS AND TRAPPING METHOD IN INNER MONGOLIA, CHINA

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Abstract

Sunflower broomrape (*Orobanche cumana*) is a severe disease limiting the sunflower production worldwidely. Recently, Sunflower broomrape has become a severe problem in Inner Mongolia region of China. In 2015-2017, biocontrol field experiments were conducted in Xixiaozhao, Bayannaoer city, Inner Mongolia. After sunflower seeds were treated with biocontrol agent F1 (*Fusarium* sp.), compared with control (91.3%, 69.1) and glyphosate treatment (90.2%, 65.8), both parasitic rate (51.7%) and disease index (47.7) of *O. cumana* were decreased dramatically. SFJJ-1, another biocontrol agent, was used with a dosage of either 10 kg or 20 kg per 667m^2, and the disease index was decreased significantly in both treatments. To perform trapping method in sunflower field, a large number of sunflower seeds were planted in the field at least 20 days before the planting date. After sunflower seedling emergence, the soil was turned over and sunflower seedlings were ploughed into soil, then, sunflower seeds plant again. This method could reduce the parasitic rate (81.3%) and disease index (38.8) of *O. cumana*, and the control efficiency was 58.3%. Overall, our study provides several potential measures to control sunflower broomrape in the future.

Keywords: *Orobanche cumana*, sunflower, biocontrol, trapping method

DEVELOPMENT OF SUNFLOWER HYBRIDS RESISTANT TO HERBICIDES

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ABSTRACT

The distinctions between 18 IMI-resistant experimental sunflower hybrids were studied on the characters seed yield, seed oil content, seed oil yield, plant height, head diameter and climate conditions influence applying two-factor analysis of variance. Hybrids plasticity was established by studying their reaction to the variable climatic conditions. It was determined that hybrids 1111A x 14R; 864A x 18R; 802A x 43R; 864 x 43R exceeded the mean standard on the characters seed yield and seed oil content with the highest statistical authenticity P=0,001. The studied hybrid combinations, treated with herbicides Pulsar 40, were affected equally by the climatic conditions in the respect of the character seed oil content. The strongest was the influence of environmental conditions related to the characters seed yield and plant height. The reaction of experimental hybrids to the pathogens *Plasmopara helianthi, Phomopsis helianthi, Phoma macdonaldii* and the parasite broomrape (*Orobanche cumana*) was studied with aim to establish the resistant ones.

Key words: sunflower hybrids, imidazolinone resistance, CLHA-PLUS, pathogenes resistance

INTRODUCTION

Over the last several years, the Bulgarian oilseeds industry has invested in expanding capacities, diversifying and adding value to some of the major field crops. This trend has been most pronounced with the sunflower crop. While until recently Bulgaria was a net exporter of sunflower seeds, lately the country has increased its crushing capacity. As a result of these developments, Bulgaria is on a trend to consume most of its sunflower crop and become a net exporter of processed products. The planting areas have increased in recent years because of higher profitability, low input requirements and better exporting possibilities, but higher rates of disease and pests have severely limited the sunflower production in some years. Significant results have been recently achieved in sunflower breeding for tolerance to imidazolinone (IMI) herbicides. Using IMI herbicide resistant hybrids gave farmers, the opportunity to control broadleaf weeds such wide spread *Xanthium*, *Cirsium* sp. Imidazolinone herbicides control a broad spectrum of grass and broadleaf weeds in imidazolinone-tolerant sunflower, including weeds that are closely related to the crop itself and the key parasitic weed broomrape (Tan *et al*., 2005).

An imidazolinone-tolerant wild sunflower population that was discovered in soybean field in Kansas, USA in 1996 (Al-Khatib et al. 1998) was used as a source for insertion of imidazolinone-tolerance gene into the first imidazolinone-tolerant lines (Al-Khatib and Miller 2000).Two lines (IMISUN-1 and IMISUN-2) were developed with resistance to imidazolinone herbicides (Al-Khatib and Miller 2000). This was followed by the release of public IMISUN lines in 1998. The development of resistant IMISUN sunflower hybrids depends on both inbred lines, having IMI-resistant genes, because this resistance was controlled by two genes: a mojor gene with semi dominant type of gene action (*Imr1*) and second gene (*Imr2*) with a modifier effect (Bruniard and Miller, 2001; Sala *et al*., 2012).

Sala *et al*. (2008a, b) reported for obtaining a new source of IMI resistance, CLHA-PLUS, developed by induced mutations (ethyl-methanesulfonate mutagenesis) and selection. *CLHA-PLUS* was different from *Imr1* at the molecular level and was controlled by expression of the partially dominant nuclear allele *AHAS1-3*. This *CLHA-PLUS* gene has higher IMI resistance than the *Imr1* and *Imr2* genes and higher oil content in sunflower hybrids. This was widely known as the Clearfield System, a trademark of BASF *Co* (Evci *et al.*, 2012; Skoric, 2012; Kaya *et al*., 2013; Kaya, 2014). In recent years, IMI-Plus sunflower hybrids have been developed in many countries.

The aim of this investigation was to test experimental IMI-Plus resistant sunflower hybrids, compare their seed yield, oil content with standards and choose those with highest exceeding.

MATERIAL AND METHODS

The investigation was carried out in 2016 and 2017. Four sterile mother lines 1111A, 864 A, 802 A, 564 A and nine restorers 14 R, 18 R, 43 R, 48 R, 50 R, 51 R, 53 R, 55 R, 56 R with *CLHA-PLUS* gene were used. They were the result of implementation of long-term research program for developing herbicides resistant CMS lines, restorers and hybrids at DAI applying different breeding methods. The parental lines were morphologically uniformed. They were characterized with very good combining ability and resistance to *Plasmopara helianthi*. The standards, included in this testing were Adagio CX, LG5661 CL. Hybrid plants were treated at phase 3-5 pair of true leaves with the herbicides - Pulsar 40 (120 ml/da). Hybrids were tested at the experimental breeding fields of DZI-General Toshevo in a randomized block method in two repetitions, as the area of each repetition was 20 m^2 (Barov and Shanin, 1965). Phenological characters, conformed to UPOV characteristics, were determined. The seed oil content was determined on the method of Rushkovskii (1957). Phytopathological evaluations of F1 hybrids were carried out in laboratory conditions and in artificial infection plot. Evaluation for resistance to downy mildew (*Plasmopara halstedii* Farl. Berleseet de Toni) was carried out on the method of Vear and Tourvieille (1987). Evaluation for resistance to grey spots on sunflower (*Phomopsis/Diaporthe helianthi* Munt.-Cvet. et al.) was carried out on the method of Encheva and Kiryakov (2002) in field conditions on artificial infection plot. Evaluation for resistance to black spots on sunflower (*Phoma macdonaldii* Boerema / *Phoma oleracea* var. *helianthi-tuberosi* Sacc) was carried out on the method of Fayralla i Maric (1981) in field conditions on artificial infection plot.

The obtained data were analyzed by ANOVA 3, a statistical tool, used to develop and confirm an explanation for the obtained experimental data**.** The ANOVA F-test is known to be nearly optimal in the sense of minimizing false negative errors for a fixed rate of false positive errors. The two-way analysis of variance was applied. It examines the influence of two different categorical independent variables on one continuous dependent variable. The two-way ANOVA not only aims at assessing the main effect of each independent variable, but also, if there is any interaction between them. These two factors are YEAR (Y) and HYBRID (H).

RESULTS AND DISCUSSION

Rising temperature and altered soil moisture due to climate change is believed to decrease the yield of food crops over next 50 years. Drought is one of the environmental factors, limiting plant growth and the productivity of many crops. Sunflower is considered as comparatively drought tolerant crop. It has the ability to extract water from deeper in the soil profile than other crops, and thus it can extract more water from each soil layer. The period of investigation 2016-2017 was covered by difference on soil moisture supply. For the aim of the study, some meteorological data were summarized and analyzed (fig.1). The meteorological conditions during the vegetation period of sunflower were characterized by quantities of rainfall (mm) for the period April-October and average diurnal temperature range for that period. The agricultural and meteorological conditions were variable and that affected the plants growth. The analysis of the obtained data showed that the experimental hybrids were tolerant to both low and high temperatures, but more tolerant to low temperatures. A critical time for water stress was the period 20 days before and 20 days after flowering.

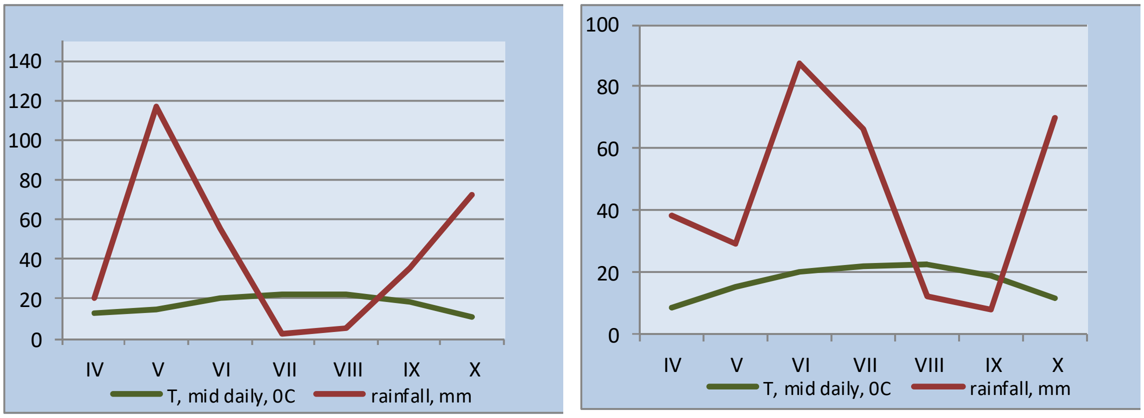

Fig.1. Temperature range (^{0}C) and rainfall (mm) during 2016-2017.

The summer weather in 2016 was unusually hot and dry for July and August (fig.1). Despite very good soil moisture reserves until middle of June, the scorching temperatures had a negative impact on sunflower yields. It also affected sunflower quality (oil content and the size of seeds). There have been above-average temperatures and scarce rainfall between mid- June and mid-August, which decreased the soil moisture content to below average levels, resulting in a 50-70 mm rainfall deficit as compared to the long-term average. Timely rains and moderate temperatures in early fall stimulated optimal planting in 2017. The summer weather remained dry and warm. Rainfall has been scarce since mid-August and September. Rains at the end of May and early July 2017 were very favorable for the crop.

Sunflower is very responsive to rainfall received during June and into September - starting just prior to flowering and continuing through seed fill. The seed yield was strongly affected when water deficiency appeared during flowering and in the following stages. A critical time for water stress was the period from beginning of button formation until the end of flowering.

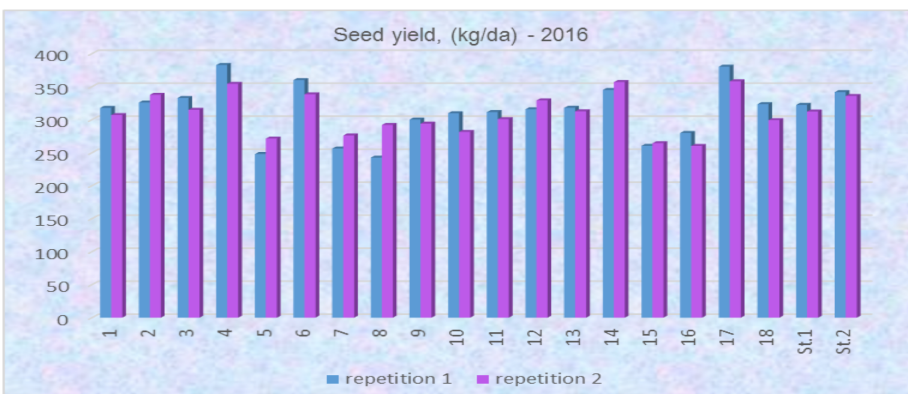

Fig. 2. The seed yield in 2016 of 18 experimental hybrids and standards

If occurring in that period, the drought causes substantial reduction of yield and yield components. That's why the seed yield of experimental hybrids and the mean standards Adagio-CX and LG 5661 CL were significantly lower in 2016 than in 2017 (fig.2, 3).

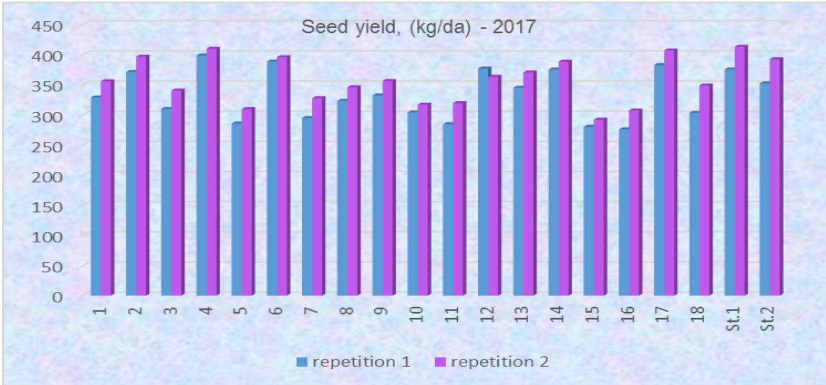

Fig. 3. The seed yield in 2017 of 18 experimental hybrids and standards

The mean standard for seed yield of replications in 2016 was 337,2 kg/da. Just three of the tested experimental hybrids exceeded it with 3,6% to 6,4%. The best results showed the hybrids 864 A x 43 R and 864 A x 55 R and 802 A x 43 R. Their seed yield was respectively 358,7 kg/da, 349,3 kg/da and 354,3 kg/ha. They also exceeded the mean standard of oil yield with 1,8% - 3,8% respectively. The mean standard for seed yield of replications in 2016 was 136,7 kg/da. The lower seed yield was due to the lack of water supply in the soil and rainfall during the vegetation period of plants. The exceeding of seed oil content was 3,6% to 12,5% for some of the studied hybrids. The exceeding regarding oil yield varied from 4,1% to 9,2%. The highest seeds oil content was determined for hybrid 1111A x 55 R – 47,5%, followed by 864 A x 43 R – 46,5%. The mean standard for seed yield of replications in 2017 was 382,9 kg/da. Four of the tested experimental hybrids exceeded it with 1,2% to 6,7%. The best results showed the hybrids 864 A x 43 R (408,7 kg/da), 864 A x 18 R (398,9 kg/da), 802 A x 43 R (391,7 kg/da) and 1111 A x 14 R (387,5 kg/da). They also exceeded the mean standard of oil yield with 2,4% - 5,8% respectively (fig.4).

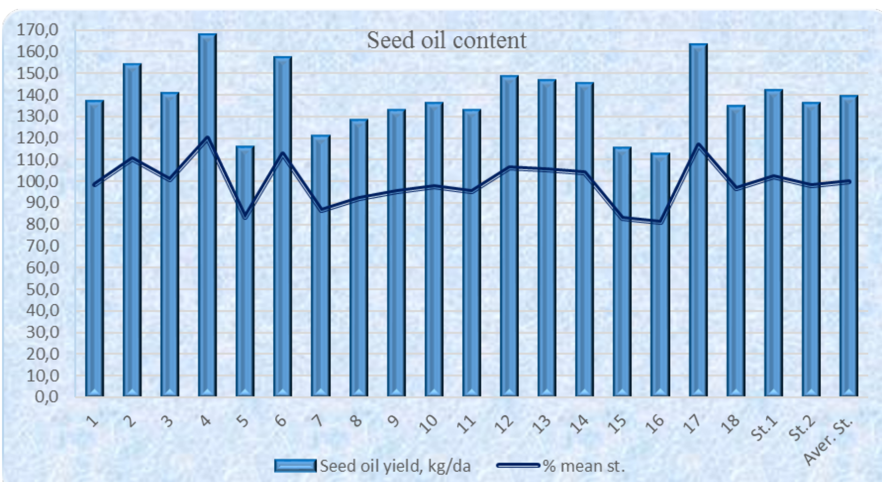

Fig. 4. Average seed oil content of 18 experimental hybrids and standards (2016-2017).

Two-way analysis of variance was applied for two independent variables with aim to determine three sets of hypothesis.

Table 1. Dispersion analysis of variance for some characters of tested hybrids, connected to seed yield.

Characters	MSh	MSy	MS yxh	MS error	LSD 5%	LSD 1%	LSD 0,1%
Seed oil content	7,15**	69,1***	1,7	0,94	0,62	0,73	1,01
Oil yield	1193,2***	4213,1***	81,4	58,4	5,16	6,71	8,17

Seed yield	3743,1**	9230,7***	328	410	12,7	15,2	21,5
Plant height	4112,3	2235***	1312,9***	33,5***	3,2	4,4	6,1
Head diameter	137,7***	31,1**	18,1*	2,1	1,1	1,4	1,4

** - statistical significant by p=0.01 , *** - statistical significant by p=0.001

The null hypotheses for each of the sets are the follow: 1.The population means of the first factor are equal. 2. The population means of the second factor are equal. 3. There is no interaction between the two factors.

The idea is that there are two variables, factors, which affect the dependent variable. Each factor will have two or more levels within it, and the degrees of freedom for each factor is one less than the number of levels. The summarized results were demonstrated on table 1. There is an F-test for each of the hypotheses, and the F-test is the mean square for each main effect and the interaction effect divided by the within variance. The numerator degrees of freedom come from each effect, and the denominator degrees of freedom is the degrees of freedom for the within variance in each case. The critical value of F is a function of the degrees of freedom of the numerator and the denominator and the significance level. If $F > F_{crit}$, the null hypothesis is rejected. An expressive interaction existed between year and hybrid in their influence on the seed yield and $F_{-criteria,}$ pointed that the hypothesis for significance of compound effects was rejected. As the compound effects of these two factors were significant, it could be accepted that the influence of factors *year* and *hybrid* on the seed yield was significant.

The reaction of experimental hybrids to the pathogens *Plasmopara helianthi, Phomopsis helianthi, Phoma macdonaldii* and the parasite broomrape (*Orobanche cumana*) was studied with aim to establish the resistant ones (Tables 2-3). The hybrid combinations 802 A x 14 R, 802 A x 43 R, 864 A x 43 R, 864 A x 51 R, 864 A x 55 R were resistant (100%) to downy mildew and the parasite broomrape. They were characterized with resistant type of reaction to the pathogens caused grey and black spots on sunflower and with up to 49% oil content in seeds. Their vegetation period was 111-118 days. Certain resistance to these two pathogens was established for the other hybrids, obtained with participation of resistant to these pathogens restorer lines. These hybrids could be successfully included in the sunflower breeding programs for developing new resistant lines.

Table 2. Phytopathological evaluation of F_1 hybrids for resistance to *Pl. helianthi* and *Orobanche cumana.*

Resistance, %	Experimental Hybrids	
Resistance 100 % to *Pl. helianthi* Novot. and 76-99% to *Orobanche cumana* Wallr.	802 A x 14 R 802 A x 43 R 864 A x 43 R	864 A x 51 R 864 A x 18 R 564A x 48R
Resistance 76-99% to *Pl. helianthi Novot*. and *Orobanche cumana* Wallr.	864 A x 18 R 864 A x 48 R 864 A x 56 R 802 A x 48 R	802 A x 14 R 802 A x 51 R 1111 A x 14 R 1111 A x 53 R

Table 3. Phytopathological evaluation of F_1 hybrids for resistance to *Phomopsis helianthi Munt.-Cvet. et all.* and *Phoma macdonaldii Boerema.*

Type of reaction	Experimental Hybrids	
Resistant to *Phomopsis helianthi* and *Phoma macdonaldii*	1111 A x 14 R 1111 A x 18 R	802 A x 43 R 1111 A x 51 R
Mid. resistant to *Phomopsis helianthi* and *Phoma macdonaldii*	864 A x 43 R 864 A x 48 R 864 A x 55 R	864 A x 50 R 802 A x 14 R

CONCLUSIONS

The results showed that the experimental hybrids were distinguished on their ecological plasticity. The lowest susceptibility to different climatic conditions were established for the hybrids 864A x 18 R, 864 A x 43 R, 1111A x 14 R and 802 A x 43 R. These hybrids were characterized with resistance to downy mildew and broomrape, highest seed oil content and oil yield respectively. They were submitted to continue the official variety testing. All other hybrids will be included in third-year testing for choosing those with high resistance to biotic and abiotic stress factors, high oil content in the seed.

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CROSS PATHOGENICITY OF *PLECTOSPHAERELLA CUCUMERINA* ISOLATED FROM SUNFLOWER BROOMRAPE AND THE OTHER HOSTS

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Abstract

Orobanche cumana Wallr. (broomrape; synonym *Orobanche cernua* Loefl.) is a parasitic plant that has become a serious threat to sunflower production in Inner Mongolia. In 2015, we observed some diseased sunflower broomrape with spindle-shaped lesions on stem in Wusutu village, Chahaeryouyi town, Wulanchabu city, Inner Mongolia. Fungal isolates were acquired for morphological and moleucular identification with ITS primer. The results showed that the pathogen was morphologically identified as *Plectosphaerella cucumerina* (Lindf.) W. Gams. Results of BLAST indicated 100% similarity with the sequence of *P. cucumerina* isolated from endive. *P. cucumerina* is well known as a soil-borne pathogen and decaying tissues of a diverse range of plants. To clarify the biological morphology and cross pathogenicity, *P. cucumerina* from sunflower broomrape, sunflower, potato and tomato were selected to study the colony and conidia morphology, growth rate, conidia production, crude toxin concentration and pathogenic specificity. The results showed that variable results on the colony and conidia morphology, growth rate, conidia production and crude toxin concentration among all tested isolates. The pathogenic specificity was studied using the stem-wound inoculation way. Fourteen days post inoculation (dpi), all isolates showed the highest virulence on sunflower, followed by sunflower broomrape, potato and tomato, for example, WST-1 which was isolated from sunflower broomrape caused the largest gray to black lesions (average length 2.40 cm) on sunflower, but the lesions on sunflower broomrape, potato and tomato were smaller, the average length were 2.28, 0.66 and 0.24 cm respectively. In conclusion, the pathogenicity of *P. cucumerina* on the different hosts is variable. All isolates showed the strongest pathogenicity on sunflower, and less pathogenicity on tomato.

Keywords: *Orobanche cumana* Wallr., *Plectosphaerella cucumerina*, cross pathogenicity

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SUNFLOWER CROP TECHNOLOGY IN SOUTH-EASTERN DOBROUDJA IN THE CONTEXT OF CURRENT CLIMATE CHANGES

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Abstract

At Sport Agra in Amzacea, in the last few years there have been experimented new sunflower crop technologies designed to face the current climate changes. This technology includes the following elements:

Application of herbicides in order to control both weeds and *Orobanche Cumana* Wallr. parasite – it was applied imazamox 40 g/l in dose of 1,2 l/ha, in fractions, in two phases (in first stage for weed control 0,7 l/ha and in second stage 0,5 l/ha, for the parasite control) in plots cultivated with various hybrids from Syngenta and Limagrain companies;

Application of last generation fungicides during the vegetation period, which will reduce the attack of the main crop pathogens. There were applied three fungicides during the two sensitive phenological phases,

Screening of hybrids with good behavior towards the main pest agent of area – *Orobanche cumana* parasite, which cause important yield losses in the south east past of Romania. There were tested hybrids from Syngenta Company and hybrids from NARDI Fundulea.

Keywords: sunflower, hybrids behavior, control