

Control of Broomrape *Orobanche cumana* Wallr.

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We set the urgency of controlling *Orobanche cumana* Wallr. – a parasitic weed. The weed distribution is described, the regions of Ukraine with great number of *Orobanche cumana* are indicated. We also described the harmfulness of *Orobanche cumana*, loss of parts or all of the yield of sunflower, as well as the possibility of complete death of plants. We specified the morphological structure, biological characteristics, methods of distribution of seeds of *Orobanche cumana*, and germination and maturation. We underlined that parasite weeds penetrate into the root of the host plant and absorb water and nutrients from it with the help of gaustories. We suggested that these factors contribute to the infection of the sunflower by *Orobanche cumana*, the spread of the parasite and its racial composition. We presented the survey results regarding the most productive measures of *Orobanche cumana* control. We described the classical methods of struggle against *Orobanche cumana*, which include: crop rotation, soil cultivation, and biological methods. We suggested that the modern, effective measures of struggle are the use of hybrids that are genetically resistant to *Orobanche cumana*, the use of the production system "CLEARFIELD", and cultivation of sunflower using the technology "SUNEO".

Key words: *Orobanche cumana* Wallr.; plants; sunflower; yield; roots; gaustories; methods of extermination; hybrids; genetic resistance; herbicides; industrial technology

Introduction

Sunflower is one of the major oilseeds in the world. The big problem of its cultivation is the control of *Orobanche cumana* Wallr. – parasitic weed. The extermination of this weed is complicated by its biological and morphological features. Broomrape is not demanding to the soil conditions, as well as to the provision of solar radiation because it sprouts under the influence of sunflower roots, attaches to them and absorbs nutrients from the sunflower plant. Due to such features it is impossible to clearly identify the main phenological phases, as the sunflower root dipped into the ground, new germinating weeds are attached to it, so Broomrape may be on the root of a single cultivated plant during all phases of its development. Because of this, germination and maturation of Broomrape seeds can be observed throughout the period of vegetation of sunflower. The size of Broomrape seed is small and easily transported by water and wind, which promotes its rapid spread (Labrousse et al., 2001; Plakhine, Joel, 2010; Joel et al., 2011; Louarn et al., 2012; Pérez-Bueno et al., 2014; Krupp et al., 2015).

The problem of the Broomrape extermination is becoming increasingly widespread. In addition to the Black Sea and Mediterranean regions, Broomrape has started to be recorded in other parts of the world, particularly in African countries (Amri, 2012; Pineda-Martos et al., 2014; Guchetl et al., 2014; Martín-Sanz et al., 2016; Rîşnoveanu et al., 2016; Nabloussi et al., 2018). Agrotechnical methods of controlling weeds are not effective for sunflower Broomrape. The main method of combating is the selection of genetically stable hybrids of sunflower to this weed (Burlov, 2010; Hladni et al., 2010; Marinkovic et al., 2013; Antonova, 2014; Seiler, Jan, 2014; Markin et al., 2016). Also, companies producing sunflower seeds are developing production technologies using herbicides containing active substances of imidazolinones and hybrids of sunflower resistant to these substances (Shaner, 2003; Lagan, 2006).

Orobanchaceae family

One of the largest groups of underground parasites – *Broomrape* – in comparison with other genera of the family is distinguished by an exceptional diversity of species composition (there are about 120 species). This is explained by the wide range of distribution and a large range in the choice of host plants. Representatives of the genus Broomrape parasitize on wild, cultivated and bitter plants. One of the most affected plants is sunflower. There are more than 40 species of Broomrape, including five parasites of cultivated plants. The most harmful are the following species that infect technical, fodder, ornamental, vegetable, melon cultures: sunflower – *O. cumana*, walnut bran, or hemp, – *O. ramosa*, Egyptian or melon, – *O. aegyptiaca*, *O. mutellii* and *O. lutea*. (Terèhin, Nikiticheva, 1981; Pineda-Martos et al., 2014; Rîşnoveanu et al., 2016).

***Orobanche cumana* Wallr.**

Orobanche cumana Wallr. is a parasitic plant that infects the root system of sunflower (*Helianthus annuus* L.) and damages significantly the productivity of the plant, which can lead to a complete loss of crop with field's damage.

Morphological structure

The stem is straight, unbranched, the height is about 10-50 cm, thickened at the base, rusty, mild, rarely covered with whole-pointed or jagged scales of brown color, which are rudimentary leaflets. Inflorescences are flowers in the axils of the scales which are collected in a cylindrical inflorescence. Corolla tubular, crankbent, whitish at the base with a purple-blue, two-edged offset. The root has turned into a suction cup – the gaustation, which Broomrape uses to attach to the root of the plant-feeder and sucks nutrients from it (Fig. 1).

Seeds

Fruit is a box. The form is incorrect. Fruit size – length 8-10 mm. The size of the seed is small, the length of the seed is 0.25-0.3 mm, the width and thickness is 0.15-0.25 mm. The color of the seeds is dark brown. The mass of 1000 seeds is 0,008-0,01 g.

Biological features

The maximum fertility of one plant – 60-100 thousand of seeds, which are easily transferred to significant distances by wind. Sustainability in the soil – up to 20 years. The depth of germination is 20-25 cm and the bud is attached to their roots with planting plants (Novopokrovski, 1928; Terèhin, Nikiticheva, 1981; Plakhine, Joel, 2010).

Spreading

Broomrape seed sprouts under the influence of root extracts of corn, soybean, flax, but cannot solidify on the roots of these plants. It affects sunflower, occurs on tomatoes, potatoes, tobacco, mahnokes, and among weeds are on wormwood, chamomile and other.

This plant does not have a sheet device that prevents photosynthesis. It does not develop a normal root system, but forms an organ which is called goostoria, which penetrates the root of the host plant and absorbs water and nutrients from it.

The first report about a serious defeat by Broomrape was recorded in Russia in the city of Voronezh in 1866 (Oldamov, 1866). Later, the parasite spread throughout the Black Sea and the Mediterranean. In Ukraine, about 70 % of the territory where sunflower is grown, is infected by Broomrape (Novopokrovskij, 1928; Shindrova, Penchev, 2012; Pineda-Martos et al., 2014; Guchetl et al., 2014).



Fig. 1. The appearance and morphological structure of *Orobanche cumana*.

Several different races were discovered in Ukraine with the subsequent description of the interaction from one gene to another. Based on these criteria, five different races (A, B, C, D, E) were discovered initially and are now widely known as "old races". In the 80s, breeders were able to create hybrids resistant to these "old races", using five genes called Or1, Or2 to Or5, which were found in the old sunflower varieties from the former USSR and Romania. These genes provide complete (vertical) resistance to the "old races", usually due to the necrosis of the parasitic structures at an early stage of the development. In recent years, a significant increase in the sunflower growing areas in the combination with short crop rotations has led to the mutation of Broomrape in more virulent races (F and G) that are not controlled by the Or1-Or5 genes (Guchetl et al., 2014).

Nowadays, there are about 7 million hectares of total 25.64 million hectares of sunflower are affected in the world (according to Oil World in 2016/17 marketing year). The most dangerous races of this parasite are spread in Spain, Turkey, Bulgaria, Romania, Russia and Ukraine. In the south of Russia, a new race of Broomrape F is distributed and there is some information about the appearance of races G and H.

In our country, the most dangerous areas, according to the spread of the parasite and its racial composition, are Donetsk, Luhansk and Zaporizhzhia regions. Nearly 50 % of the areas in these areas have a high risk of spreading new races. It is about 190 thousand hectares in Donetsk, 150 thousand hectares in Lugansk and 120 thousand hectares in Zaporizhzhia region. Also, the potential distribution risk persists in Odesa, Dnipro and Kropyvnytsyi regions (Fig. 2).



Fig. 2. Map of the *Orobanche cumana* distribution in Ukraine.

■ – Areas where *Orobanche cumana* Wallr. is common.

The damage of Broomrape is very high. Plants affected by it, lag behind growth and development, as a result the yield can decrease by 30-70 % or more. In case of severe damage, plants do not form baskets at all. In the presence of 30 pedicels on one sunflower plant, the yield of seeds from it decreases sevenfold, and when there are 60 and more flowering plants there is no crop at all.

Germination, the suction of Broomrape to the plant's root nutrition and its initial development occur secretly, in the soil (Fig. 3). During the germinating a slightly wound sprout with a pin-shaped thickening at the end comes from the seed, and grows in the direction where the concentration of root extracts of the plant is higher. Touching the root, which is sensitive to Broomrape plant, the thickening begins to grow, and the other part of the sprout is atrophied, turning into a thin thread - then the connection with the seed coat is interrupted.

In a short period of time, thickening at the root of the host plant is covered with mounds, and it looks like a star. The trachea that develops within the gasturia merges with the leading elements of the host plant into a single whole so that it is difficult to find a border between them. At the opposite end of Broomrape, a kidney is formed, covered with numerous scales, later transformed into modified leaves. The kidney develops in a pedicel, carries inflorescences on the surface of the soil.

Germination of the seed of Broomrape, which is scattered in the soil, its suction and development occur gradually with the growth of the root system of the feeding plant. Therefore, it is possible to observe all phases of the formation of a parasite - from germination of seeds to maturation of boxes, at the roots of one host plant. From the moment of germination, the seeds of Broomrape to the appearance of its plants on the soil surface lasts for at least 1.5-2 months. It is possible to evaluate the hybrids of sunflower on the resistance to Broomrape without waiting for the exit of flower stalks from the soil, according to the existence of *Orobanche Cuman* formations on the host plant's roots. (Novopokrovskij, 1928; Barcinskij, 1936; Terèhin, Nikiticheva, 1981; Plakhine, Joel, 2010).

Factors which contribute to the infection of the sunflower:

- Temperature conditions. Broomrape prefer dry weather, but the heat is as damaging for it as coolness. The best temperature for the seeds' germination is from 16 to 25 degrees above zero. Humidity of the soil - within 70-80 %.
- Non-observance of the crop rotation's terms. The seeds of Broomrape are easily spread by the wind and can stay in the soil for up to 20 years, waiting for the appearance of symbionts. That is why the reduction of the period of the sowing rotation contributes to multiple infections.
- Improper soil treatment. Failure to comply with agrotechnical rules of soil treatment - favorable conditions for weed damage. Measures of the Broomrape extermination can be divided into classical and modern.



Fig. 3. Root of sunflower which are affected by *Orobanche cumana*.

Classic actions

- the sowing of hybrids and sunflower sorts that are resistant to all races;
- the deep plowing with a flow rate of 32 - 35 cm once in 10-12 years;
- the return of sunflower to the crop rotation at least in 6-7 years;
- the sowing of corn for several years in one field provokes germination of 70-80 % of the Broomrape seeds;
- the use of provocative flaxseed crops, soybeans that stimulate germination of the Broomrape seeds, but which are not affected by it;
- the use of *Phytomyza orobanchia*, which larvae are fed by the Broomrape seeds;
- the use of the infection by *Fusarium orobanche*;
- the sowing of the sunflower, which is not resistant to Broomrape, and then it's moving in 35-40 days after the ladder (silage, siderate).

Among the classic methods one can distinguish both methods of agrotechnology and biological methods of the Broomrape extermination.

Crop rotation

It is scientifically grounded that adherence to the crop rotation with sowing of varieties and hybrids of sunflower for the 8th-10th year can be considered. At the same time, the main problem is the observance of this norm by all farmers in view of the Broomrape seeds spreading from highly infected fields by means of drainage of water, blasting with wind, transportation by technique. The greatest effect of the struggle in crop rotation has been shown by the crops that provoke germination of the Broomrape seeds, but which are not its owner - "crop traps", which include corn, millet, sorghum, *Sorghum sudanense*, cotton. The root's excretion of these cultures provokes the germination of the seeds of Broomrape in different layers of the soil, but they are not affected by it, as a result, the number of the Broomrape seeds decreases. However, according to the laboratory of immunity and molecular marking of RSRIO, the varieties and hybrids of these cultures vary greatly in their ability to provide roots of substance (such as streptolactones or sesquiterpenes) which stimulate the germination of the Broomrape seeds. Therefore, for using as a "culture-trap" the varieties with the maximum stimulating ability should be selected.

Soil cultivation

The similarity of the Broomrape seeds can persist for many years - it has been scientifically proven that seeds could germinate after 20 years of storage. In conditions of the soil the Broomrape seeds are affected by moisture, disease (fusariosis). Because of this, the similarity of the seeds may decrease, but not significantly. However, Broomrape has a colossal fertility. Nowadays, one plant of Broomrape can lead to the formation of 500 thousand seeds, some of which can be stored in the soil for more than 10 years. The use of soil treatments in the economy at different depths, the turnover of the formation leads to "conservation" of the seeds. When applying treatments in a layer of 0-15 cm, the effect on reducing the similarity of the seeds is maximized.

It is believed that mechanical removal of the parasite (weeding, trim) is one of the methods of extermination, however, as a rule, the defeat of Broomrape begins deep in the ground and before the surface the parasite has time to harm the sunflower

plant up to its death. It is also impossible to remove Broomrape mechanically, as any damage of it can lead to the growth of new sprouts from the root's remainders (not one, but many, like the thistle, when cut it). As a result of the trauma of nodules during the cultivation of row spaces the multiple points of growth were laid in them, and additional stems appeared, if the main sprout was threatened with death. The same phenomenon causes the defeat of the main sprout by fusariosis. Subsequently, the sign of multifoldness of Broomrape could be genetically inherited, as a mutation which is useful for the species, and in this field there is already a certain percentage of Broomrape plants capable of passing such a trait inherited.

Biological methods

Phytomyza orobanchiae Kalt. lays eggs in the flower of Broomrape, and the larva eats most of the seeds, while the sunflower harvest is still under threat. In addition, this manual labor, the measure of collecting, storing and growing *Phytomyza orobanchiae* Kalt. for production conditions does not always justify itself in practice. As an additional measure for reducing the amount of seeds of Broomrape should be applied systematically.

Fusariosis, is caused by fungi of the genus *Fusarium*, is one of the most common diseases of *Broomrape*. From the samples of the parasite, selected on fields in Rostov region, Morozivsky district, as a result of the mycological analysis, the species *Fusarium sporotrichioides* was selected and identified. This type of fusarium, depending on the conditions can significantly damage the flower stalks of *Broomrape*. However, the use of varieties of fungus-fusarium as a measure of extermination of *Broomrape* can be dangerous for ecology of agrocenoses. It should be remembered that sunflower is also affected by fusariosis, and *Broomrape* is conditionally a source of preservation and spread of infection (Barcinskij, 1936; Bejlin, 1947; Louarn et al., 2012; Pérez-Bueno et al., 2014).

Modern measures of extermination:

- the use of hybrids that are genetically resistant to Broomrape;
- the use of the production system "CLEARFIELD";
- the sunflower growing using the "SUNEO" technology.

The use of hybrids genetically resistant to Broomrape (Fig. 4).

Two types of protective reaction of resistant forms of sunflower of different origin are described. The first type of protective reaction is characterized by damage of the cataract parenchyma cells, the gastoral organ of Broomrape was dying in cortical cells of the root, and did not reach the vessels of xylem due to the reaction of supersensitivity. The second type of protective reaction reveals elements of xylem of sunflower root. Lignin accumulates in the damaged sunflower root cells, is formed under the influence of peroxidase, which is secreted by a parasite. The varieties of peroxidase isolated from Broomrape were proposed as an explanation of the different virulence of races A and B. Different mechanisms of resistance were described for the differentiator lines, resistant to several races.

In addition to the accumulation of lignin in resistant sunflower genotypes, deficiency of germinator growth germs can be observed. Several mechanisms of resistance to *Orobanche* were also described: insufficient amount of germination stimulants allocated to the root of the host plant; mechanical or chemical barriers (lignification or hypersensitivity reaction) accumulation of toxic components that slow down the growth of tubers and their association with the host's vascular system (Antonova et al., 2013; Marinkovic et al., 2013; Guchetl et al., 2014; Markin et al., 2016).



Fig. 4. Sunflower seeds which are affected by *Orobanche cumana* in comparison with resistant sunflower

The use of the production system "CLEARFIELD".

The "CLEARFIELD" shows excellent results in the extermination of Broomrape. The main tool of the system is the use of selective action herbicides "Euro-Lightning" - special chemicals used to destroy weeds.

Herbicides of the type "Euro-Lightning" and its analogues ("Vitalight", "Impex Duo", "Euro-Lang"), those containing the active substances of the imidazolinone group, are the only drugs that can destroy most dicotyledonous and cereal weeds. Now, there are five active substances which are registered in Ukraine: imazamox, imazapyr, imazethapyr, metazachlor and less known chlorimuron-ethyl. The safety of these herbicides for beneficial insects is also important, including pollinating bees, and low-toxic for farm animals (Means of Plant Protection BASF Ukraine).

The action of imidazolinones against Broomrape.

Herbicides of the IMI group are the most effective in the Broomrape extermination, as they are capable of destroying all races of this parasite. The sunflower crops are cultivated in the phase 4 of true leaves, usually 1.2 liters/ha. It is necessary to carry out the preventive treatment of crops from a parasite in the areas of Broomrape danger, as it is possible to detect its presence only after the germination of the Broomrape sprouts, which occurs in the second half of the sunflower growing period. By this time, the culture may already be significantly harmed. Imidazolinones are also concentrated in the juice of the sunflower roots, which destroy the germs that penetrate into its roots.

The mechanism of imidazolinones action.

The general mechanism of action for the group of drugs is based on the violation of the production of alpha-amino acids (isoleucine and valine). Imidazolinones quickly penetrate into the structure of plants through vegetative mass and roots, accumulate at growth points. The first signs of weed damage are visible on young sprouts of plants. Even after a complete cessation of nutrition from the soil, weeds can remain green for a long time, as imidazolinones do not affect the process of photosynthesis.

Getting on the soil herbicides form, the so-called "Screen", preventing the sprouting of weeds. One-time application of herbicides-imidazolinones is sufficient to ensure the crop's cleanliness throughout the entire growing season of sunflower. They are not washed away by rain an hour after treatment (Shaner, 2003; Lagan, 2006).

Special hybrids of sunflower.

Not all sunflowers are resistant to imidazolinones, but only specially derived hybrids. The company "BASF" was the first to register the "Euro-Lightning" herbicide on the basis of imazapyr and imazamox, which make it easy to get rid of weeds, including those that are difficult to remove: all races of Broomrape, species of regweed, field bindweed, *Amaranthus*, burdock, *Avena fatua*, *Sonchus*, *Poligonum*, *Sinapis*, *Galeopsis*, *Galium*, *Panicum*, chamomile, etc.

Sunflower hybrids which are resistant to imidazolinones are not products of genetic engineering and are derived using traditional breeding methods. Imidazolinones can be used in the classical, "no-till", "mini-till" or "strip-till" cultivating technologies.

The level of soil acidity and water hardness do not affect the effectiveness of the drugs. On a very depleted or on sandy soils the minimum rate of expenditure of the drug should be used-1 liters/ha.

The sunflower cultivation using the "SUNEO" technology.

The SUNEO technology is a comprehensive harvest protection that combines sunflower resistant hybrids created on the basis of the best genetics of the company "Limagrain" (have the last gene of resistance to aggressive races) and the protective method "CLEARFIELD" developed by BASF. It means that new technology was born in partnership of two big companies.

The main advantages of the technology "SUNEO" developers calle:

- Double protection for Broomrape control. Thanks to two levers of influence – genetic stability and chemical control – full protection from Broomrape during the growing season from early to late attacks is achieved.
- Improved control of weeds. Since the hybrid of sunflower from Broomrape is protected genetically, the herbicide "EuroLightning" can be directed at the control of weeds in crops with the introduction of optimum time choosing the phase of the greatest vulnerability of weeds to the action of the herbicide.
- Reduction of production costs while maintaining efficiency. The control of crop shedding in optimal terms will allow the introduction of the minimum recommended doses of herbicide, and therefore, farmers have the opportunity to save on drugs, while not losing, and even increasing the yields.
- Optimal solution for arid zones. Flexible dose, aimed at protecting from weeds, that is, a smaller aftereffect and good control of Broomrape at the genetic level.

The use of "SUNEO" technology is recommended in zones where there are more aggressive *Broomrape* races than the 5th race "E" ("Innovative sunflower" arrives in Ukraine: the latest technology of SUNEO harvest from Limagrain).

Conclusion

In many sunflower growing countries, there is a problem with the Broomrape extermination. This weed-parasite infects about a quarter of world sunflower crops. Sunflower, damaged by Broomrape, is lagging behind in growth and development, the harvest is reduced by 30-70 %, and sometimes plants do not form baskets or die. The searches were made and an analysis was conducted on how to fight against Broomrape.

The classic methods for controlling Broomrape Wallr. are in crop rotation, soil cultivation and biological methods. They have a large amount of work, designed for a long time to execute, and for their implementation, and a lot of money is required. In addition, these methods are not always effective and can have a minor effect on the Broomrape plant.

Among the methods which have been studied, three modern measures to combat Broomrape should be distinguished. The first method is based on the selection of sunflower hybrids that are genetically resistant to the defeat of all races of Broomrape. The second is the use of the production system "CLEARFIELD", based on the use of herbicides with active substances of the imidazolinone group, which are able to disrupt the production of alpha-amino acids and lead to the death of Broomrape and weeds. With such technology it is necessary to use hybrids resistant to these herbicides. The third method involves the use of the production system "SUNEO", which combines previous methods of genetic resistance of sunflower and the use of herbicides with the active substances of the imidazolinones group. Such a system offers the possibility of double protection of sunflower seeds from the damage of Broomrape, and improved control of other types of weeds.

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