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BIOLOGICAL CONTROL OF THE WEED SPECIES *CIRSIUM ARVENSE* (L.) SCOP. USING FUNGAL AGENTS – AN OVERVIEW

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Abstract

In Romania, the field weed species Cirsium arvense (L.) Scop. is native. In the last decade, the creeping thistle has become increasingly difficult to control due to agricultural systems based on monoculture and minimal or no tillage. The invasive nature of the species and its resistance to current herbicides make it increasingly difficult to control, especially in organic farms. Therefore, there is currently great interest in controlling the species with the help of biological agents. This review is based on extensive documentation on the use of fungal biological agents in the control of the creeping thistle. The studies reviewed show that there is interest in biological control, particularly in organic farming. Ten fungal biological agents with the potential to initiate infections in the creeping thistle are currently being investigated. The fungi tested are Puccinia punctiformis, Phomopsis cirsii, Sclerotinia sclerotiorum, Alternaria cirsinoxia, Phoma destructiva, Phoma exiqua, Stagonospora cirsii, Septoria cirsii, Phyllosticta cirsii and Fusarium sp. Of these, only Puccinia punctiformis, Phomopsis cirsii and Septoria cirsii have high and very high specificity for creeping thistle. The fungus Puccinia punctiformis stands out by the highest specificity and can be successfully used in the biological control of Cirsium arvense. However, the mass production of spores is tricky because there are no methods to cultivate this biotrophic fungus. Studies published between 2013 and 2024 show the high potential of Puccinia punctiformis to initiate infections that destroy or reduce the density of the creeping thistle in agricultural crops.

***Key words**: biological agents, Puccinia punctiformis, Cirsium arvense, fungi, biological control*

INTRODUCTION

Cirsium arvense is a weed widespread on all continents. New updates show the presence of this invasive weed in countries on several continents: Africa, Antarctica, Asia, Europe, the Americas, Oceania (CABI, 2021) - Figure 1.

In Romania, the segetal species *Cirsium arvense* (L.) Scop. is native. In the last decade, it has become increasingly difficult to control due to agricultural systems based on monoculture and minimal or zero tillage, to which strip-till is now being added. The invasive feature of the species and its resistance to current herbicides make it increasingly difficult to control, especially on organic farms. The invasive feature of *Cirsium arvense* is also highlighted in other published studies, together with *Sorghum halepense* or *Asclepias syriaca* (Chifan *et al*., 2019; Sărățeanu *et al*., 2020; Ștef *et al*., 2022).

Moore (1975) pointed out that the creeping thistle is a problem weed in its area of origin (presumed to be south-eastern Europe). It subsequently spread throughout Europe and became a common weed.

Its presence is reported all over Romania, both in agricultural crops, where it causes great damage and in fallow land (Berca, 2004; Manea *et al*., 2015). Given its rapid spread and resistance to herbicides, the creeping thistle is increasingly difficult to control. In addition to the above-mentioned aspects, the difficult management of this species is related to its high reproductive capacity, both sexual and vegetative, but also to its deep root system that allows rapid regeneration even after the application of different control methods (Tyler, 2010).
It is recommended that agro-technical, mechanical, chemical and biological measures for weed control be used in integrated management in a balanced way (Morishita, 1999; Pișcoran *et al*., 2024).

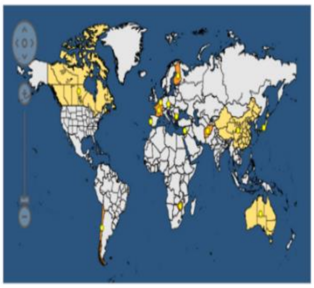

Figure 1. Distribution of the segetal species *Cirsium arvense* (yellow - areas where present; blue - transitional areas)
(Source: EPPO, 2024, available on *https://gd.eppo.int/taxon/CIRAR/distribution*)

The increase in areas under organic farming and the decreasing efficacy of herbicides has led to an increased interest in the use of biological agents, especially fungi. Studies on the biological control of *Cirsium arvense* using fungal biological agents in the last decades have identified 10 fungi with low or high potential for control, depending on their specificity (Müller & Nentwig, 2011).
The 10 fungi with the potential to initiate infections in *Cirsium arvense* are *Puccinia punctiformis* (Kluth *et al*., 2003), *Phomopsis cirsii* (Leth *et al*. (2008), *Sclerotinia sclerotiorum* (Brosten and Sands, 1986), *Alternaria cirsinoxia* (Berestetskii *et al*., 2010), *Phoma destructiva* (Kruess, 2002), *Phoma exiqua* (Scott *et al*., 1975; Bilder and Berestetsky, 2006), *Stagonospora cirsii* (Yuzikhin et al., 2007), *Septoria cirsii* (Leth, 1985; 1990), *Phyllosticta cirsii* (Berestetskiy *et al*., 2005) and *Fusarium sp.* (Gronwald *et al*., 2004) - as cited by Müller & Nentwig, 2011.
This paper is an overview of the research carried out to date on the higher or lower potential of some fungi to control the weed *Cirsium arvense*. The main aim was to compare the studies published in recent years worldwide and to highlight fungi with a real potential to control this problematic weed in agricultural crops and beyond.

MATERIALS AND METHODS

This paper was based on several scientific papers published between 1894 and 2024. They have been analysed and compared to see which biological agents can be successfully used in the biological control of the segetal species *Cirsium arvense*. Among the recently published articles, the most relevant to the topic of this paper were identified. The information was summarized and then integrated into this review.
The methods used were the classical, familiar ones. Semi-systematic, integrative, systematic, bibliometric as well as text mining techniques were addressed. In general, these methods are used in research to highlight the best studies in a field as well as the progress made (Wong *et al*., 2013; Snyder, 2019; Pan *et al*., 2020).
Very importantly, the mentioned methods can highlight possible differences between studies and even some gaps.

RESULTS AND DISCUSSIONS

Reports of biological control of the weed *Cirsium arvense* have been around for a very long time (over 100 years). The first data date back to 1893 and refer to the potential of the fungus *Puccinia punctiformis* to initiate infections in *Cirsium arvense* (Halsted, 1894). This was followed by studies which emphasized the difficulties in collecting spores to achieve infections (Cockayne, 1915) but also on the efficacy of this rust (Ferdinandsen, 1923). Ferdinandsen (1923) analysed the development of rust on creeping thistle in a pasture for three years and found a high percentage of plant disease (70%). Since then and up to the present, several fungal biological agents have been tested.

Cirsium arvense is currently difficult to control by mechanical and chemical methods alone, due to its aggressive invasiveness, and there is a desire to integrate biological methods into integrated pest management systems. On the other hand, the prohibition of herbicides in organic crops makes biological measures, together with other non-polluting methods, mandatory.

Analysing the published scientific works, it can be observed that, in the last two decades, tests with fungal and non-fungal biological agents have been on the increase, with the aim of identifying those with the highest potential to control this weed.

The fungi currently being tested for use in biological control are *Puccinia punctiformis*, *Phomopsis cirsii*, *Sclerotinia sclerotiorum*, *Alternaria cirsinoxia*, *Phoma destructiva*, *Phoma exiqua*, *Stagonospora cirsii*, *Septoria cirsii*, *Phyllosticta cirsii*, and *Fusarium* sp. (Müller & Nentwig, 2011). The ten fungal biological agents have different efficacy and specificity, as assessed by Müller & Nentwig (2011). In the opinion of these authors, the poor efficacy of these fungi in controlling the weed *Cirsium arvense* is largely due to their lack of specificity. On the basis of tests carried out over time by researchers, the specificity of the ten fungi is as follows: very high for *Puccinia punctiformis* (Kluth *et al*., 2003) and *Septoria cirsii* (Leth, 1985; 1990); high for *Phomopsis cirsii* (Leth *et al*. (2008); low for *Alternaria cirsinoxia* (Berestetskii *et al*., 2010), *Stagonospora cirsii* (Yuzikhin *et al*., 2007) and *Fusarium* sp, 2004); very low for *Sclerotinia sclerotiorum* (Brosten and Sands, 1986) and *Phoma exigua* (Scott et al., 1975; Bilder and Berestetsky, 2006); unknown for *Phyllosticta cirsii* (Berestetskiy *et al*., 2005); unclear for *Phoma destructiva* (Kruess, 2002). Of course, in addition to the authors cited, there are other authors who have reported the host specificity of these fungi.

Although Müller & Nentwig (2011) conclude that the effectiveness of pathogens in biological control is overestimated, in contrast, Cripps *et al*. (2012) argue that this is not the case. They show that specificity is not a real problem in biological control, as very good bioherbicides have been on the market for 30 years that are not host-specific and can infect a wide range of plants. The authors provide well-documented arguments for all fungal pathogens with the potential to infect *Cirsium arvense*.

Figure 2. Plant of *Cirsium arvense* with *Puccinia punctiformis* pustules (Cotuna & Sărățeanu, June 2013, Sacoșul Turcesc, Timiș)

Among the pathogens tested, the fungus *Puccinia punctiformis* stands out with a very high specificity, capable of producing systemic infections that ultimately totally or partially destroy the host (Chichinsky *et al*., 2023). This fungus has been reported to occur in all temperate zones of the world (Cripps *et al*., 2014; Kentjans *et al*., 2023) - Figure 2, 3, 4.

Figure 3. *Cirsium arvense* plant drying out due to rust (Cotuna & Sărățeanu, June 2013, Sacoșul Turcesc, Timiș)

Figure 4. Plant of *Cirsium arvense* attacked by *Puccinia punctiformis* (Cotuna & Sărățeanu, June 2013, Sacoșul Turcesc, Timiș)

In Romania, *Puccinia punctiformis* has been identified since 1924. Samples were collected by Gürtler C. from Cluj on May 26, 1924 and subsequently sent to the University of Gothenburg, where the fungus was identified by Tiesenhausen M. (*Mycology Collection Portal*).

In 1945 samples were sent from Timișoara, collected by Todor I. (on May 5, 1945) to the same university where the identification of the fungus was made by Bechet M. (Mycology Collection Portal).

More recent reports have existed since 2000, when the fungus was identified in the Vlădeni locality in Iași County (Tănase & Asoltanei, 2000). In 2013, Cotuna & Sărățeanu identified the fungus in Sacoșul Turcesc locality in Timiș county, where they observed that the creeping thistle plants were diseased (unpublished data).

Systemic infection produced by *Puccinia punctiformis* leads to leaf drying, reduced flowering and reduced root shoot development. Repeated infections can lead to reduced plant density of *Cirsium arvense*. Therefore, the use of this fungus in biological control seems to be challenging as it is difficult to propagate being an obligate biotroph (Cripps *et al*., 2012; Kentjens *et al*., 2023).

Other authors argue that this fungal biological agent may be a viable candidate in the biological control of creeping thistle due to its host specificity and ability to achieve systemic natural infections that affect growth and reproduction (Thomas *et al*., 1994).

A study conducted in Colorado (USA) and published in 2024 shows that in about 77% of the areas where treatments were carried out, the density of the creeping thistle decreased, and in 17%, no aboveground infections were observed. Control success was correlated with the amount of inoculum used (large amounts of infected *Cirsium arvense* leaves), the timing of inoculation, and the method used. The experiment lasted 8 years and the results show that the density decreased from about 88% to 45% (Bean *et al*., 2024).

The importance of application timing is also brought to the fore in other studies. Cripps *et al*. (2014) show that the application of leaves with teliospores in the fall has been very effective in initiating systemic infections. Thus, the management of *Cirsium arvense* weeds in pastures could be successful. It is recommended that biological control be used in conjunction with other methods, such as mowing (Bourdot *et al*., 2011; Cripps *et al*., 2014).

Could *Puccinia punctiformis* be a success in the fight against creeping thistle? It's a question that has yet to be answered. So far, results in protected areas are better than in the field, where they depend on several factors (climatic, pedological, technological *etc*.). The conclusion of several studies is that this fungus could be an effective biological agent for biological control, especially since it does not require high costs (Chichinsky *et al*., 2023). However, the fact that the fungus can only be grown on living plants is a serious drawback in its integration into biological control (Müller & Nentwig, 2011). On the other hand, Cripps *et al*. (2012) assess that the potential of bioherbicides in control is high, but the interest in their development is low. Concerning *Puccinia punctiformis* they state that the main obstacles in biological control are the high specificity for the creeping thistle and the obligate biotrophic nature. Considering these aspects, the fungus can be successfully utilized through natural spread that can be increased by mowing, especially during rainy periods (Demers *et al*., 2006; Bourdot *et al*., 2011; Cripps *et al*., 2012).

Another fungus with high control potential is *Phomopsis cirsii*. Its high specificity and the fact that it can be grown on artificial substrates have aroused the interest of researchers (Leth *et al*., 2008). This fungus produces symptoms on leaves (blackening of veins), stems (necrotic lesions), and shoots (drying, wilting). The preparations obtained are based on conidia and mycelia. Infections are conditioned by the presence of water for about 18 hours. By mixing with alginate the time can be shortened (Leth & Andreasen, 1999; Leth *et al*., 2008). The results obtained by the above-mentioned researchers are different. In one experiment, it was shown that the weight of green shoots decreased by 50%, and in another, the control efficacy was 100% dead *Cirsium arvense* plants. These results led him to conclude that the differences may be due to the different virulence of *Phomopsis cirsii* isolates. Realizing the high potential of this fungus for biological control, Leth (1985, 1990) patented its application as a mycoherbicide. Unfortunately, due to a lack of interest in the development of this bioherbicide, the patent would have expired by 2004 - 2005, as Bo Hammer Jensen states in a personal communication (cited by Müller & Nentwig, 2011).

The third fungus with good potential in the biological control of creeping thistle is *Septoria cirsii*. Due to its high specificity and the phytotoxin it produces (β-nitropropionic acid), it inhibits seed germination and root growth. At the leaf level, it produces chlorosis and necrosis (Hershenhorn *et al*., 1993). The good results obtained during experiments led Leth (1985) to consider this pathogen as a biological agent and to obtain a patent for it. The application as a biological agent was patented at the same time as *Phomopsis cirsii* (Leth, 1985; 1990). The two patents were not considered and the products never appeared on the market (Müller & Nentwig, 2011).

In Romania, *Septoria cirsii* is present (Andrianova & Minter, 2004).

We have highlighted these three fungi because they have been widely studied in recent years, as can be seen from the articles analysed (over 40). This is not to say that there are no other pathogens of interest to researchers. Depending on their efficacy they have been categorized as follows: *Puccinia punctiformis* - limited efficacy (Kluth et al., 2003); *Phomopsis cirsii* - high efficacy (Leth *et al*. (2008); *Sclerotinia sclerotiorum* - limited efficacy (Brosten and Sands, 1986); *Alternaria cirsinoxia* - limited efficacy (Berestetskii *et al*., 2010); *Phoma destructiva* - high efficacy (Kruess, 2002); *Stagonospora cirsii* - high but restricted efficacy (Yuzikhin *et al*., 2007); *Phoma exigua* - unstable efficacy (Scott *et al*., 1975; Bilder and Berestetsky, 2006); *Septoria cirsii* - high efficacy (Leth, 1985; 1990); *Phyllosticta cirsii* - unknown efficacy (Berestetskiy *et al*., 2005); *Fusarium* sp. - unstable efficacy (Gronwald *et al*., 2004) - cited by Müller & Nentwig, 2011.

High efficacy means that the pathogen is able to kill *Cirsium arvense* plants and limited efficacy means that it cannot kill them.

Other fungal pathogens that have been studied are *Pustula andropogonis*, and *Ramularia cirsii* (Leth *et al*., 2008). For the biological control of creeping thistle in meadows, the fungi *Sclerotinia sclerotiorum*, *Verticillium dahliae*, *Plectosphaerella cucumerina* as well as species of *Rhizoctonia*, *Cylindrocarpon* and *Phoma* are tested (Skipp *et al*., 2012). Results obtained by Skipp *et al*. (2012) show unexpectedly good efficacy of the fungus *Verticillium dahliae* against creeping thistle. They inoculated cut shoots with conidia using wet blades. Following infection, the leaves turned yellow and the shoots withered. The authors argue that the spread of verticillium wilt from one plant to another is favoured by mowing during wet weather, thus explaining the phenomenon of the disappearance of the creeping thistle during rainy periods.

The use of fungal biological agents in the biological control of creeping thistle has attracted the interest of researchers over the years. In recent decades, more and more fungi have been tested for use in biological control, and some have even been patented.

It is known in biotherapy that the results depend on several factors. Hence the oscillating effectiveness. This does not mean that bioherbicides cannot be developed. A shortcoming of bioherbicides is that they are not sufficiently tested and compared with chemical herbicides, Crispp *et al*. (2012) point out. This is also our opinion.

In Romania, we found no studies on testing fungi or other biological agents for the biological control of creeping thistle, although this weed is problematic in agricultural crops and beyond. They may exist and may not be public in accessible databases or libraries. The first tests with biological agents on *Cirsium arvense* will be done at the University of Life Sciences ”King Mihai I” from Timișoara, starting in 2025.

CONCLUSIONS

This review is an overview of studies published to date to identify fungi with high potential for the biological control of creeping thistle. The analysis of the articles (more than 40) shows the high or limited efficacy of the tested fungi. Of the tested fungi of higher interest: *Phomopsis cirsii, Septoria cirsii, Puccinia punctiformis,* and *Verticillium dahliae*.

Also interesting are the conflicting opinions of some researchers. Some argue that the efficacy of biological agents (fungal and bacterial) is greatly exacerbated, while others show that all can be effective if the correct application methods are approached and treatments are timed optimally.

Mycoherbicides could be successfully introduced in integrated pest management systems for creeping thistle, and even low-dose combinations of herbicides are recommended. In our view, biological control should not be abandoned and is necessary on organic, ecological as well as conventional farms. They could replace chemical herbicides that are overused. Overdosage is a problem in modern agriculture and farmers are responsible for it. As a result, soils are increasingly polluted, and the microbiome is deeply affected. Added to this is the contamination of plant products with pesticide residues above the allowed limits. Pesticide contamination is a threat to human and animal life and food safety. Together with other contaminants (mycotoxins, heavy metals *etc*.), they are triggers of both degenerative (Parkinson's, Alzheimer's) and proliferative (cancers) diseases. This is why we believe that bioherbicides should be given an important place in the integrated management of creeping thistle and other weeds.

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