RESEARCH ON THE PATHOGEN COMPLEX CAUSING SUGAR BEET ROOT ROT IN THE NORT-EAST OF MOLDOVA

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Abstract

The root rot diseases of sugar beet (*Beta vulgaris* L.) caused by a complex of pathogens includes *Rhizoctonia solani, Macrophomina phaseolina, Phoma betae, Aphanomyces cochlioides, Pythium aphanidermatium, Phytophthora drechsleri, Rhizopus stolonifer, Sclerotium rolfsii, Fusarium oxysporum, F. solani, F. avenaceum*, etc. The disease leads to significant economic losses associated with a decrease in root yield (up to 50-60%), a drop in the percentage of sugar and a deterioration of sugar juice purity (low white sugar recovery). Many of these pathogens also cause post-harvest losses in storage piles (up to 100%). Until now, there is no disease resistant cultivars and the pathogens causes beet root rot in hybrids of all breeding companies on the market. Heat and drought contribute to the spread of the disease. The fight against the disease is very complicated by the fact that the fungus, under certain conditions, switches to saprotrophic nutrition, thereby maintaining its population.

Key words: root rot, sugar beet, *Macrophomina phaseolina*

Sugar beet (*Beta vulgaris* L.) represents the primary raw material for white sugar production in the temperate zone. Belonging to the family *Chenopodiaceae,* sugar beet is a biennial root crop renowned for sugar production, second only to sugarcane (Mall A.K. *et al*, 2021).

In 2023, the cultivated area for sugar beet in Romania reached 15,664 hectares, with the majority (68.3%) located in the north-eastern region (10,697 hectares). Achieving large and high-quality productions is a mandatory requirement for the economic efficiency of the crop, given the significantly higher expenses per unit of surface area compared to other agricultural crops in the region, such as wheat, corn, sunflower, rapeseed, and barley. Consequently, safeguarding the sugar beet crop against weeds, pathogens, and pests is crucial for the success of the cultivation.

The new sugar beet hybrids from the European Union exhibit a high production potential (70-90 t/ha), along with elevated sugar content in the roots (17-22%). These hybrids also demonstrate a high tolerance to herbicides, particularly through the Conviso Smart technology, and show good resistance to diseases such as rhizomania, cercosporiosis, and rhizoctoniosis (Puiu I. *et al*, 2022).

In the 1960s, beet root rot was observed in several European countries, including Romania, Bulgaria, Hungary, and Serbia. Initially, it was attributed to abiotic factors such as high temperatures and drought. However, subsequent research, particularly in Serbia, revealed that beet root rot is caused by a complex of pathogens, including *Candidatus Phytoplasma solani* and *Macrophomina phaseolina* (Tassi) Goid. These pathogens can lead to the complete destruction of beet roots (Ćurčić Z. *et al*, 2021a, 2021b; Duduk N. *et al*, 2023).

Among other reported pathogens (*Fusarium* spp., *Rhizoctonia solani*), *Macrophomina phaseolina* is currently considered the most dangerous root rot fungal pathogen of sugar beet (Kosovac A. *et al*, 2023), causing charcoal root rot (Budakov S. *et al*, 2015; Karadimos D.A. *et al*, 2002; Kaur M. *et al*, 2012). This disease is complex, as numerous disease complexes have been described in other hosts (Aryan A. *et al*, 2014; Lamichhane and Venturi, 2015) and occurs as a consequence of rubbery taproot disease (RTD), caused by the plant pathogenic microorganism *Candidatus Phytoplasma solani* (*Mollicutes, Acholeplasmataceae*) (Quaglino F. *et al*, 2013).

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Macrophomina phaseolina is a soil-borne, necrotrophic pathogen present worldwide, affecting more than 500 plant species across over 100 families (Abass A.N. *et al*, 2021; Marquez N. *et al*, 2021). It survives for at least 2 years as sclerotia, formed in host plants, soil, or leftover host tissue (Collins D.J., 1988).

Reports of *Macrophomina phaseolina* in sugar beet have been documented in the United States, Russia, India, Iran, Egypt, Greece, and Hungary. It is considered a minor root rot pathogen affecting weakened, injured, or stressed plants (Cooke D.A., Scott R.K., 1993; Jacobsen B.J., 2006; Karadimos *et al*., 2002).

MATERIAL AND METHOD

The research was conducted across 28 commercial farms situated in the northeast of Romania, specifically in the counties of Botosani, Iasi, and Vaslui, where cultivated beets exhibited symptoms of the disease. The soil type for beet cultivation was chernozem, and the cultivation process followed conventional practices: after harvesting the preceding crop, the soil was deforested and then plowed. Sowing took place in April, at the optimal time, with a row distance of 45 cm and a seed density of 110,000 seeds/ha.

Upon emergence, the plant density ranged from 87,000 to 98,000 plants. The seeds were treated with fungicides (potassium phosphite) and insecticides (thiamethoxam, tefluthrin). Throughout the vegetation period, beets were treated with herbicides, fungicides, and insecticides in accordance with the recommended agricultural practices, ensuring the crop remained free from weeds, diseases, and pests.

Climatic data, including temperature and rainfall, were obtained from the weather station at the experimental farm Ezareni of Iasi University of Life Sciences, located in the center of the research area. Two evaluations of the beet areas affected by drought were conducted on August 10, 2023, and August 25, 2023.

The research aimed to:

- Establishing the size of the surfaces with rotten beet;
- Establishing the degree of attack of the disease (frequency and intensity of the attack);
- Identification of phytopathogenic agents causing rotting of beet roots.

The incidence of root rot disease was estimated for the agricultural season 2022/2023 for all beet fields, where the plants presented symptoms. The incidence of the disease was evaluated by collecting the beets on 5 square meters (about 450 plants) that were randomly captured from each sugar beet field. Representative sample was performed according to the collection method based on observing symptoms of diseased plants which are randomly taken from each field.

The assessment of disease incidence was determined by formula: F%= n x100/N in wich, F% is disease incidence (frequency) percentage, n is total number of symptomatic plants and N is total number of inspected plants. The method of determining disease severity (intensity) was visual assessment, expressed as a root rot percentage (Nutter J. *et al* 1991). The degree of attack of the disease was calculated by: DA % = (F x I)/100 in which, DA (%) is degree of attack percentage (%), F% is incidence (%) and I is severity (%). During the research, the degree of attack of the disease (DA, %) was determined for 3 times, respectively: 04.09.2023, 03.10.2023 and12.10.2023.

Sugar beet roots displaying symptoms were assessed to determine the presence of fungi. Fungi isolation was conducted from both the edges of healthy and rotten tissue in roots exhibiting signs of rot. Following disinfection with 70% ethanol, root fragments were washed with running water for 30 minutes and then left to dry on filter papers. Subsequently, the tissues were placed on Potato Dextrose Agar (PDA) Petri dishes (90 mm).

After an incubation period of 4–5 days at 25±2°C in a 12/12h light/dark regime, the developed fungal colonies were transferred to a pure culture for morphological assessment. The description of fungal morphology was carried out using a compound microscope (Leica DM 500, Germany). This study was conducted at the laboratory of the Plant Pathology Department at Iasi University of Life Sciences.

RESULTS AND DISCUSSIONS

Symptoms of sugar beet rotten root first appear in the second half of August: yellowing and necrosis of the leaves and loss of turgor in leaves. The drought assessment revealed that on August 10th, 6,540 ha with beets were affected by drought and high temperatures (*figure 1*):

- 5,310 ha had 10-20% of dry leaves;
- 920 ha had 20 - 50% of dry leaves;
- 310 ha had 50-70% of dry leaves.

Figure 1 **Sugar beet fields affected by drought and high temperatures, 10.08.2023**

Sugar beet leaves undergo necrosis, resulting in the complete decline of the plant, and the taproots begin to rot partly or fully after the aboveground parts of the plant have deteriorated.

Beet infections were isolated in the fields in the middle of August, but the drought persisted, affecting 8,140 hectares (76.1% of cultivated sugar beet in the northeast of Romania) until the end of August. Here are the results for affected beets as of August 25, 2023:

- 7,110 ha had 10-50% of dry leaves;
- 420 ha had 50 - 70% of dry leaves;
- 610 ha had 70-85% of dry leaves (*figure 2; 3*).

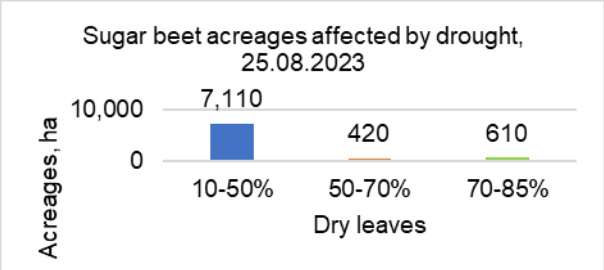

Figure 2 **Sugar beet fields affected by drought, 25.08.2023**

Figure 3 **Sugar beet fields affected by drought and high temperatures, 25.08.2023**

The beet fields that showed clear symptoms of root rot were measured, then the degree of attack based on disease incidence and severity was determined ((*figure 4; 5; 6*).

Figure 4 **Isolated sugar beet plants with symptoms of rotten roots**

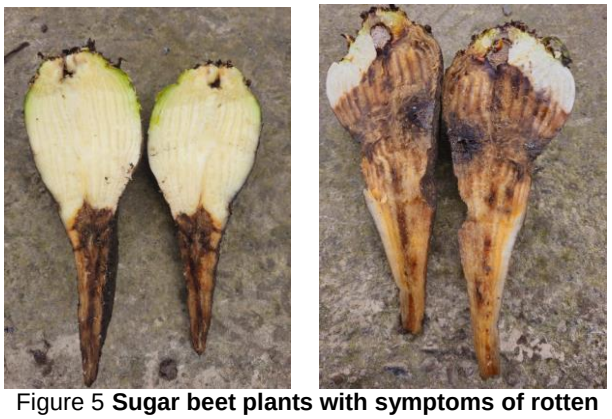

Figure 5 **Sugar beet plants with symptoms of rotten roots**

Figure 6 **Sugar beet plants with symptoms of rotten roots**

The results of the first evaluation from 04.09.2023 show that the degree of attack on the surface of 572 ha was 10-50%, on 1,043 ha it was 5-10%, and on an area of 1,725 ha the beet was affected on a small scale: 1-5% (*figure 7*).

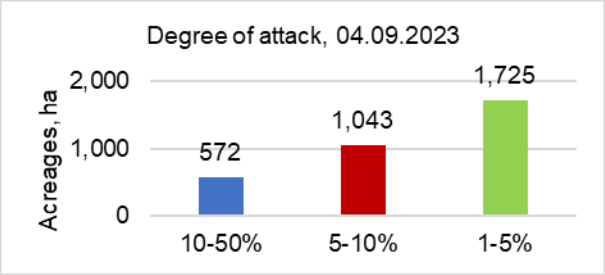

Figure 7 **The first evaluation of the degree of attack, 04.09.2023**

On 03.10.2023, the second assessment of the degree of attack was carried out and it highlights the significant increase in the beet surfaces heavily affected by root rot (*figure 8*).

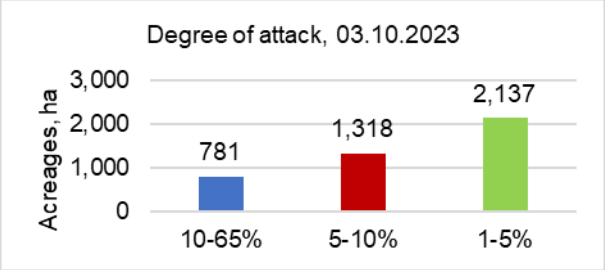

Figure 8 **The second evaluation of the degree of attack, 03.10.2023**

The degree of attack on the surface of 781 ha was 10-65%, on 1,318 ha it was 5-10%, and on a surface of 2,137 ha the beet was 1-5% (*figure 9;10*).

Figure 9 **Sugar beet surfaces heavily affected by root rot, 03.10.2023**

Figure 10 **Sugar beet surfaces heavily affected by root rot, 03.10.2023**

The final assessment of the degree of attack was conducted on October 12, 2023, and indicates the following values: 10-75% on the surface of 962 hectares, 5-10% on 1,551 hectares, and 1-5% on the surface of 3,537 hectares (*Figure 11*).

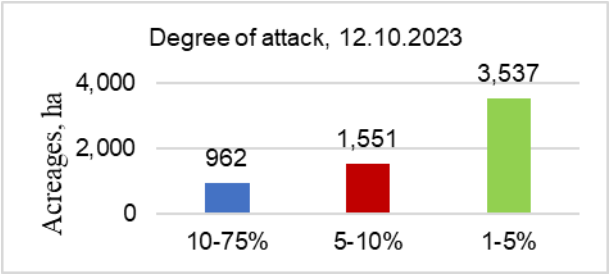

Figure 11 **The final evaluation of the degree of attack, 12.10.2023**

On many plots in the studied area, the beet was completely rotted, black-colored, and the frequency of the disease was around 60-70%, demonstrating the devastating effect of the disease under conditions of drought and high temperatures (*Figure 12; 13*).

Figure 12 **Completely rotted roots of sugar beet**

Figure 13 **Sugar beet fields with a high degree of attack (60-70%)**

The sugar content in the partly rotten roots was between 5,3-12,2% (average for healthy beet was 17,4% sugar) and the beet has to be processed very soon. In any case, the beets cannot be stored more than one day. After that, the roots of beet fermented and the sugar juice drains (*figure 14*). The loses in this fall are 100%.

Figure 14 **Sugar beet roots partly rotted, after 2 days of storage in the factory**

Most of the affected fields are heavy soils with a pH value between 6.3 and 6.8. The previous crops before sugar beet were soybean or corn, and weed control was carried out using herbicides (no hoeing). We observed no effect of fungicides used for *Cercospora beticola* control against *Macrophomina phaseolina*.

The year 2023 is considered very dry, as a precipitation volume of 336.4 mm was recorded from January 1 to September 30, 2023. This is 117.9 mm lower than the multi-year average at the Ezareni farm (454.3 mm). In May, only one significant rain event was reported on May 8 (11.2 mm), and another on June 16 (16.8 mm), making the spring very dry. Starting from August 1, 2023, a pronounced drought began, lasting over two months and negatively influencing sugar beet production (a decrease of 35-40%). The temperatures during the summer of 2023 reached very high values: 34.3°C in June, 36.9°C in July, 38.8°C in August, and 32.6°C in September.

The results of isolation and identification revealed various species of fungi isolated from sugar beet roots, including *Macrophomina phaseolina*, *Fusarium* spp., *Verticillium dahliae*, *Rhizoctonia solani*, *Penicillium* spp., *Aspergillus fumigatus*, *Rhizopus stolonifera*, etc.

Microscopic examination showed that the mycelium of *Macrophomina phaseolina* is initially branched, multi-septate, and hyaline. As it matures, it turns light green, then brown, finally forming dark grey to black colonies with numerous black sclerotia, ranging in size from 100µm to 1 mm. The conidia are hyaline, unicellular, and ellipsoidal, with sizes ranging from 16 to 23 x 6-9 µm. Pycnidia are spherical and black, with dimensions ranging from 150 to 200 µm.

CONCLUSIONS

The fungus *Macrophomina phaseolina* was identified in all beet fields with rotten roots in the commercial farms located in the northeast of Romania, specifically in the counties of Botosani, Iasi, and Vaslui.

The beets were weakened and stressed due to the very high temperatures and severe drought. The latest assessment of the degree of fungus attack, conducted on October 12, 2023, indicates that 962 hectares were affected, ranging from 10% to 75%.

The charcoal rotten rot disease can be devastating, as on many plots in the studied area, the beets were completely rotted, and the frequency of the disease was around 60-70%. The fields with a high degree of attack have heavy soils with a pH value between 6.3 and 6.8, and the

previous crop before sugar beet was soybean or corn.

Fungicides used for the control of *Cercospora beticola* have shown no significant effect on *Macrophomina phaseolina*. Currently, *Macrophomina phaseolina* is the only pathogen that can completely compromise the sugar beet crop in Romania, reducing the sugar content to a level lower than 10%, making it impossible to store and efficiently process in sugar factories.

Intensive research is needed to establish an effective plan for the prevention and control of the disease.

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