

## RESEARCH REGARDING NEW RACES OF *PLASMOPARA HALSTEDII* IN FUNDULEA AREA

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### Abstract

One of the most important disease of sunflower crop is downy mildew, which is caused by *Plasmopara halstedii* fungus (Farl.) Berl. et de Toni (syn. *Plasmopara helianthi* Novit). Downy mildew is a common disease in many areas of the world where sunflower is grown and infects young plants in spring when soil moisture is high and temperatures range from 15 to 18°C. Loss of young plant caused by pathogen may be up to 80%. The pathogen could be controlled growing resistant genotypes and that is why the major objective of this study was to develop sunflower hybrids genetically resistant to dominant races of downy mildew in Fundulea area. To prevent losses caused by downy mildew, we start create hybrids genetically resistant to this pathogen, parental lines were backcrossed to introduce new genes using resistant sources from maintainer line HA 335 and restorer line RHA 274. We test resistance for our genotypes artificial infested in collaboration with Institute of Field and Vegetable Crops Novi Sad. In the future we proposed to test our hybrids in Romania using the international set of differentials for *Plasmopara halstedii* pathogen races. Is absolutely necessary to create resistant varieties for the newest Romanian races, considering downy mildew is one of the most important disease for sunflower crop.

**Key words:** backcross, downy mildew, *Helianthus annuus*, pathogen, races.

### INTRODUCTION

Downy mildew caused by the fungus *Plasmopara halstedii* (Farl.) Berl. et de Toni is one of the most harmful sunflower diseases. Downy mildew is widespread in all sunflower-growing countries with the exception of Australia. It had been first discovered on sunflowers in the United States in 1883, and in 1892 it was found on *Helianthus tuberosus* in Russia. In Romania, first described in 1964 by Radulescu in Cluj area, next year had been mentioned in South part of Romania (Baneasa-Ilfov), north of Moldova and Banat. In Yugoslavia, had been identified in sunflower crops in 1949 by Piersic and described by Nikolic (1952). Inoculum nature, plant stage, temperature and humidity, interaction between host plant-parasite and plants nutrition are the most favorable factors for downy mildew development. Considering the way fungus mycelium is developing and extended through cells tissues of plants, it exists two infections: primary (systemic) and secondary. While the primary infection causes significant yield losses, secondary infection has no importance for the production of sunflower. Primary

infection is realized during seed germination in the soil and the emergence of sunflower seedlings. It may be caused by fungus mycelium or oospores present on infected seeds, or by oospores present in infected soil into which healthy seeds are sown. The fungus penetrates the root, stem, cotyledons and reaches the meristematic tissue at the top of young plants. Classic symptoms of systemic infections are leaf chlorosis and sporulation, stunted growth, short internodes and with a platform head. On the infected plant parts, the root, cotyledons, the stem and especially the leaves, there occurs abundant white mycelium, which is typical for this disease. The mycelium occurs also on the reverse side of the leaves and it contains the vegetative organs of the fungus conidiophores and conidia (zoosporangia). Secondary infection has no important impact on sunflower yield, it is originate from summer conidia, it is transported by wind and only affects certain organs or portions as angular spots, chlorotics, which appear on leaves. To prevent downy mildew losses it is necessary to include cultivation practices, like healthy seeds for planting, seed treatment with fungicides against downy mildew, crop rotation, intervals

of 4-5 years between two sunflower crop in the same field, distance of 500 m away from a field planted with sunflower the previous year, sowing at optimum time and deep plowing of the field after sunflower harvest. Seeds treatment with metalaxyl represent one of the most important measures for downy mildew control. The chemical treatment protects sunflower crop of the primary infection, at early stages of development of sunflower. It could be used different post-emergences chemicals treatments also for downy mildew control. However, the most effective control of this fungus is genetically resistance, which represent our objective to develop sunflower genotypes resistant to dominant races of downy mildew in Romania.

## MATERIALS AND METHODS

For this experiment we used following inbred lines developed in North Central Regional Plant Introduction Station USDA-ARS-PIRU, Iowa State University, were used as donors of downy mildew resistance genes:

- B – lines: HA 335 (PI 6), HA-R4 (PI 16)
- Rf – lines: RHA 340 (PI 8), RHA 348 (PI 8)

The experiment start in 2009, we crossed each source of resistance with three of ours B – lines: 9149B, 9041B and 5017B and each resistant restorer line with three of ours Rf – lines: 5037C, 01007C and 01009C. We used back-cross method. The plants that served as female component in the crosses were manually emasculated early in the morning. The experience was located in Fundulea area, were it was sown on rows with 4.8 m length. Downy mildew resistance was tested in

laboratory conditions in collaboration with Institute of Field and Vegetables Crops of Novi Sad using M. Rahim, C. C. Jan and T. J. Gulya method. Seed were surface-sterilized in 1% sodium hypochlorite solution for 10 minutes, soaked in 0.8 ml/l 'ethrel' (2-chloroethyl phosphonic acid) for 18-24 h, washed with tap water, placed between moist blotting papers and put in a high-humidity germinator at 25°C. Two to three-day-old seedlings with a radicle length of 10-20 mm and visible root hairs were inoculated by immersion for 3 h at 18°C in a suspension containing 3-4 x 10<sup>4</sup> freshly harvested zoospores/ml. Care was taken to choose seedlings whose radicle length were similar to prevent the possibility of disease escapes. Inoculated seedlings were grown in a mixture of sand Perlite (3/2 v/v) in a greenhouse (24±3°C, 16 h photoperiod) for 10-14 days. Under these inoculation and seedling growth conditions, 100% infection was achievable in susceptible race/sunflower line combinations. The seedlings were then placed overnight in a dark chamber, maintained at 100 % relative humidity and 18°C, to initiate sporulation on the cotyledons and the first true leaves of susceptible seedlings. Seedlings classified as resistant displayed no sporulation on either cotyledons or leaves, nor did they show stunting typical of systemic infection.

## RESULTS AND DISCUSSIONS

In table 1, we can see results from laboratory infestation, tested in collaboration with Institute of Field and Vegetable Crops of Novi Sad Serbia:

Table 1. Results from laboratory infestation

No.	Genotype	Total number of plants	Healthy plants	Plants with downy mildew symptoms	Resistance (%)
1	9149B x HA 335	26	26	0	100
2	9041B x HA 335	30	24	6	79
3	5017B x HA 335	25	25	0	100
4	9149B x HA R-4	29	25	4	85
5	9041B x HA R-4	30	26	4	88
6	5017B x HA R-4	30	23	7	75
7	01007C x RHA 348	30	26	4	88
8	01009C x RHA 348	30	23	7	75
9	5037C x RHA 348	29	29	0	100
10	01007C x RHA 340	27	24	3	90
11	01009C x RHA 340	29	25	4	85
12	5037C x RHA 340	29	22	7	75

We can see in the table that all lines had been resistant during laboratory infection. Results from Fundulea are related in table 2. The experience was sown on plots of two rows, 4.8m length, in three replications. For field

experience in Fundulea area we used internationally set of sunflower differential lines to different physiological races of *Plasmopara halstedii*.

Table 2. Results from Fundulea

No.	Cross	Generation	Total number of plants	Resistant plants	Susceptible plants	Resistance %
1	9149B x HA 335	BC <sub>3</sub>	21	21	0	100
2	9041B x HA 335	BC <sub>3</sub>	22	17	5	75
3	5017B x HA 335	BC <sub>3</sub>	19	19	0	100
4	9149B x HA R-4	BC <sub>3</sub>	20	17	3	85
5	9041B x HA R-4	BC <sub>3</sub>	18	16	2	88
6	5017B x HA R-4	BC <sub>3</sub>	21	16	5	75
7	01007C x RHA 348	BC <sub>3</sub>	21	18	3	87.5
8	01009C x RHA 348	BC <sub>3</sub>	22	17	5	75
9	5037C x RHA 348	BC <sub>3</sub>	20	20	0	100
10	01007C x RHA 340	BC <sub>3</sub>	19	17	2	90
11	01009C x RHA 340	BC <sub>3</sub>	19	16	3	85
12	5037C x RHA 340	BC <sub>3</sub>	22	17	5	75

Genes of resistance to new races of downy mildew have been determined in wild sunflowers and they have been transferred into cultivate sunflower genotypes. Resistance to downy mildew is controlled by several single dominant genes named *Pl- genes*, which are racially specific and which provide vertical resistance.

## CONCLUSIONS

Downy mildew disease could produce losses of 80% in sunflower crops. In Romania, from approximately 35 years, there existed only two races, but in the last decade appeared five new races of the pathogen. Chemical treatment with metalaxyl represent one of the most important measures for downy mildew control, but it is absolutely necessary to create genetically resistance. These might be a good reason to create new material with genetically resistance for new races of *Plasmopara halstedii*. To control new races of the pathogen we created new resistant lines which are used to create resistant hybrids.

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