

## STUDY ON AN *Ambrosia* ISOLATE OF *Plasmopara halstedii*

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### SUMMARY

In 1998, a number of *Ambrosia artemisifolia* individuals located in the vicinity of a sunflower breeding nursery at Bicsérd, south Hungary, showed disease symptoms resembling downy mildew and/or white rust. Sporangia of both *Plasmopara* and *Albugo* have been isolated from the affected plants. Microscopical observations and subsequent inoculation experiments revealed the existence of *Plasmopara halstedii* on the affected *A. artemisifolia* plants. Following inoculations on a set of sunflower differentials, the *Ambrosia* isolates of *P. halstedii* consistently showed a virulence formula of 730 that is equal to pathotype 4.

**Key words:** *Ambrosia artemisifolia*, *Plasmopara halstedii*, south Hungary, pathotype 4

### INTRODUCTION

The downy mildew fungus, *Plasmopara halstedii*, is a highly specialized pathogen confined to the cultivated sunflower (*Helianthus annuus* L.) worldwide (Sackston, 1981). However, from its place of origin, the Americas, it has been described on a number of plant species belonging to the *Asteraceae* (Leppik, 1966). In contrast, Novotelnova (1966) in Russia presumed this fungus attacked a range of *Helianthus* species only. As a conclusion she renamed it into *Plasmopara helianthi* Novot. with three formae speciales pathogenic to different *Helianthus* species. In fact, until the 1980's in Europe, there was no indication of the natural occurrence of this fungus on plants other than the cultivated sunflower. In 1984, *P. halstedii* has been reported to attack *Xanthium strumarium* L. and the fungus isolated from that plant species was pathogenic to sunflower (Virányi, 1984) (Table 1). A similar situation occurred recently with *Ambrosia artemisifolia*, another common weed of the *Asteraceae* in Hungary (Bohár and Vajna, 1996). The latter authors identified the

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fungus on the basis of through microscopical observations but they did not make any re-inoculation experiment to prove its pathogenicity on sunflowers.

Table 1: Natural (N) vs. artificial (A) hosts of *Plasmopara halstedii* based on seedling tests (Virányi, 1984) and on field surveys

| Plant species                       | Response | Host type |
|-------------------------------------|----------|-----------|
| <b><i>Helianthus annual:</i></b>    |          |           |
| <i>H.annuus</i> (wild)              | +        | A         |
| <u><i>H.argophyllus</i></u>         | +        | A         |
| <i>H.debilis</i>                    | +        | A         |
| <i>H.petiolaris</i>                 | +        | A         |
| <b><i>Helianthus perennial:</i></b> |          |           |
| <i>H.angustifolius</i>              | -        | A         |
| <i>H.decapetalus</i>                | -        | A         |
| <i>H.divaricatus</i>                | +        | A         |
| <u><i>H.doronicoides</i></u>        | -        | A         |
| <u><i>H.grosseserratus</i></u>      | +        | A         |
| <i>H.maximiliani</i>                | -        | A         |
| <i>H.mollis</i>                     | -        | A         |
| <u><i>H.multiflorus</i></u>         | -        | A         |
| <i>H.nuttallii</i>                  | -        | A         |
| <i>H.occidentalis</i>               | -        | A         |
| <i>H.resinosus</i>                  | -        | A         |
| <i>H.rigidus</i>                    | -        | A         |
| <i>H.salicifolius</i>               | -        | A         |
| <i>H.scaberrimus</i>                | -        | A         |
| <u><i>H.strumosus</i></u>           | -        | A         |
| <i>H.tuberosus</i>                  | -        | A         |
| <b>Other Asteraceae:</b>            |          |           |
| <i>Ambrosia artemisifolia</i>       | +        | N         |
| <i>Artemisia vulgaris</i>           | +        | A         |
| <u><i>Centaurea cyanus</i></u>      | -        | A         |
| <u><i>Xanthium strumarium</i></u>   | +        | N         |

Note: Items underlined mean contradiction with literature data.

In 1998 in south Hungary, a number of *A.artemisifolia* individuals have been found to show chlorotic leaf symptoms coupled with retarded growth (Figure 1) and infected leaves supporting a white downy cover together with large superficial white pustules on the abaxial surface (Figure 2), the latter resembling the appearance of the white rust fungus, *Albugo tragopogonis*.

## MATERIALS AND METHODS

Microscopical observations of the specimens have been made by washing off sporangia from diseased plants into distilled water. Inoculation experiments have been initiated to see whether the *Plasmopara*-like fungus isolated directly from mildewed *A.artemisifolia* individuals was *P.halstedii* and whether this isolate was able to attack the cultivated sunflower. For this, the sunflower cultivar GK-70 susceptible to downy mildew was used. Three-day-old germs have been inoculated with a sporangial suspension using the WSI-method according to Cohen and Sackston (1973). About 10-12 days post-inoculation, the plants have been incubated in a humid chamber at 18°C overnight to get sporulation. The freshly developed sporangia were used for virulence tests by making inoculations on a set of sunflower differentials (Gulya *et al.*, 1998) and by using the WSI method as before. Based on reactions being either positive or negative of the differentials, a virulence formula according to Gulya (1995) has been established.

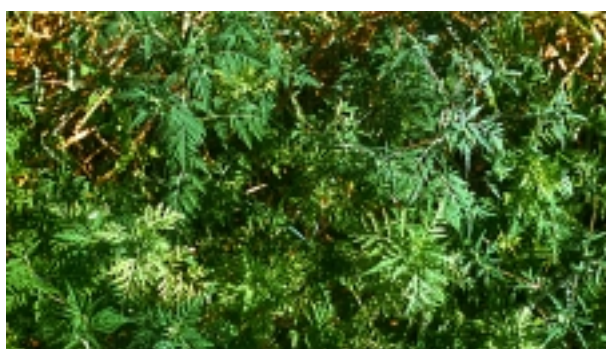


Figure 1: *Ambrosia artemisiifolia* systemically infected with both *Plasmopara halstedii* and *Albugo tragopogonis* showing leaf chlorosis and stunting



Figure 2: White pustules of *Albugo tragopogonis* on the abaxial leaf surface of *Ambrosia artemisiifolia*

## RESULTS AND DISCUSSION

Microscopical observations with freshly made sporangial suspensions from infected plants revealed the co-existence of both *P.halstedii* and *A.tragopogonis* in the same leaf tissue.

Furthermore, inoculations with these sporangial suspensions were successful, i.e., the *Ambrosia* isolates of *P.halstedii* were able to infect sunflower cv. GK-70 by inducing all the typical disease symptoms, as stunting, leaf chlorosis and sporulation on underleaf surfaces. It is interesting to note that the overall fitness and aggressiveness of those *Ambrosia* isolates were much higher than expected since the first inoculum originated from *Ambrosia* leaves was deep-frozen at  $-25^{\circ}\text{C}$  for almost a year prior to use. In comparison, *P.halstedii* sporangia routinely stored under the same conditions on detached sunflower leaves remained viable no longer than 2-3 months.

Table 2: Virulence performance of *Plasmopara halstedii* isolated from *Ambrosia artemisifolia* at Bicsérd in 1998

| Differential | No. of plants* |          | Reaction type <sup>§</sup> |
|--------------|----------------|----------|----------------------------|
|              | inoculated     | diseased |                            |
| HA-288       | 40             | 40       | +                          |
| RHA-265      | 40             | 39       | +                          |
| RHA-274      | 40             | 40       | +                          |
| DM-2         | 40             | 38       | +                          |
| PM-17        | 40             | 37       | +                          |
| 803-1        | 40             | 6        | -                          |
| RHA-4        | 40             | 0        | -                          |
| RHA-5        | 40             | 0        | -                          |
| HA-335       | 40             | 0        | -                          |

Pathotype/ virulence formula: 4/730

\*Mean of 2 experiments; <sup>§</sup> + = susceptible, - = resistant

In subsequent inoculation tests, using a set of sunflower differentials, it was found that the isolates were virulent on 5 out of 9 sunflower lines exhibiting the virulence formula of 730, in other words belonging to the pathotype 4 (Table 2). The results suggest that *A.artemisifolia* is not only a simple natural host of the sunflower downy mildew fungus but it has the ability to support the fungus being maintained in nature and keeping its virulence character over the years. In turn, the species *A.artemisifolia* appears not only to be a simple host of *P.halstedii* but it also reacts to fungal virulence factors positively or even selectively.

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### **ESTUDIO DE UN AISLADO DEL HONGO *Plasmopara halstedii* EN LA ESPECIE *Ambrosia***

#### **RESUMEN**

Durante el año 1998, en los alrededores de la capa arable para el girasol en Bicsérd, Hungría meridional, fue constatado cierto más grande número de plantas de la especie *Ambrosia artemisifolia* con los síntomas de enfermedad que recordaban al mildiu y/o a la roya blanca. De las partes de plantas infectadas, fueron aislados los esporangios de los hongos *Plasmopara* y *Albugo*. Las observaciones microscópicas y los experimentos con la infección artificial que fueron hechos después, han confirmado la peresencia del hongo *Plasmopara halstedii* en las plantas *A. artemisifolia*. La infección artificial del grupo de líneas diferenciales del girasol ha indicado que los aislados de *P. halstedii* tomados de la especie *Ambrosia* mostraron la fórmula correspondiente al patotipo 4.

### **ÉTUDE D'UN ISOLAT DU CHAMPIGNON *Plasmopara halstedii* SUR L'ESPÈCE *Ambrosia***

#### **RÉSUMÉ**

Au cours de l'année 1998 dans les environs du champ expérimental de tournesol à Bicsérd, au sud de la Hongrie, on a remarqué un grand nombre de plantes de l'espèce *Ambrosia artemisifolia*, présentant des symptômes qui rappelaient ceux de la maladie du mildiou et/ou de la rouille blanche. Les champignons sporanges *Plasmopara* et *Albugo* ont été isolés des parties atteintes des plantes. L'examen microscopique et les expériences d'infections artificielles qui ont été faites ultérieurement ont confirmé la présence du champignon *Plasmopara halstedii* sur les plantes *A. artemisifolia* atteintes. L'infection artificielle de groupes de lignes différentielles de tournesol a démontré que les isolats *P. halstedii* prélevés sur les espèces *Ambrosia* désignait sans exception la formule 730 qui correspond au pathotype 4.

