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# Transition of secondary to systemic infection of sunflower with *Plasmopara halstedii* – An underestimated factor in the epidemiology of the pathogen

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ARTICLE INFO

*Article history:*
Received 18 September 2008
Revision received 3 December 2008
Accepted 17 December 2008
Published online 10 March 2009
*Corresponding editor.* Lynne Boddy

*Keywords:*
Downy mildew
Epidemiology
*Helianthus annuus*
Oomycetes

ABSTRACT

*Plasmopara halstedii*, the downy mildew pathogen of sunflower causes significant economic loss world-wide, mostly through soilborne systemic infection of seedlings. Natural infection of sunflower with *P. halstedii* was monitored in a sunflower field cultivated for ornamental purpose in soil where no sunflower had been grown before. Local and systemic infections were observed in plants of different developmental stages which were sown in five consecutive field plots between Apr. and Jul. The airborne origin of the infection by zoosporangia was concluded from field history, pathogenic symptoms, time course of infection and microscopic investigation of mycelium distribution in stems. A high potential for transition from local to systemic infection was found, at least in ornamental sunflower cultivation under the typical weather conditions in Central Europe. This questions the paradigm that economically and epidemiologically relevant sunflower downy mildew incidences are only derived from subterranean infections. Airborne secondary infections, as they may occur in all developmental stages and on all organs of the host plant, are responsible for late systemic infection and can play a key role in the production of contaminated seeds carrying the pathogen into the next season.



## Introduction

In sunflower cultivation, the biotrophic oomycete *Plasmopara halstedii* is one of the most devastating pathogens. Various modes of infection, from systemic to local and nearly symptomless latent forms, are known from this downy mildew pathogen (Cohen & Sackston 1974; Gulya *et al.* 1997; Spring 2001). The agronomically most relevant systemic infection invades all plant organs and causes the typical symptoms of dwarfing, chlorosis or early damping off. This form of infection is usually derived from oospores in soil or in achene hulls. It affects seedlings in the early stage of germination and therefore, *P. halstedii* is often characterized as a soilborne pathogen (Zimmer & Hoes 1978; Sackston 1992a; Gulya *et al.* 1997). Resistance breeding and fungicide treatment by means of seed coating are the most effective measures to protect sunflower from this type of invasion through the root system and the young hypocotyl. However, fungicide protection lasts only a few weeks and leaves of resistant sunflower genotypes may be susceptible to airborne secondary infection by mitotically derived zoosporangia, as shown by inoculation experiments with detached leaves (Spring *et al.* 1997), and observed in oilseed cultivation where late systemic infections in the flowering stage led to significant amounts of oospore contaminated seeds (Spring 2001). Recently identified resistance reactions of sunflower were found to be tissue specific in hypocotyls (Radwan *et al.* 2005), thus explaining the restriction of the pathogen to cotyledons

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1754-5048/$ – see front matter 
doi:10.1016/j.funeco.2008.12.003

and plant parts below ground (Sackston 1992b; Mouzeyar *et al.* 1993; Heller *et al.* 1997).

Once the biotrophic pathogen has established itself in the host tissue, hyphae extend through the stomata and propagation is by zoosporangia mitotically formed on sporangiophores at the plant surface. This initiates the airborne phase of the pathogen's life cycle. Zoosporangia can be dispersed over long distances (Delanoe 1972) and lead to so-called secondary infections on above-ground parts of sunflower. However, this type of infection is regarded to be of little economic impact on sunflower production, because it leads to infections which mostly remain local and transient, thus causing a few leaf lesions but not affecting plant development and crop yield significantly (Zimmer & Hoes 1978; Gulya *et al.* 1997).

Nevertheless, in periods of cool and humid weather local infections were observed which later became systemic (Viranyi 1992; Spring 2001). Moreover, zoosporangia led to almost symptomless head infections, which reduced achene weight by up to 40 %, and to the development of oospores in the infected achenes (Spring 2001). For several years we have regularly observed such airborne secondary infections affecting plants in all developmental stages, particularly in sunflower cultivated for ornamental purpose, which economically outranges sunflower oilseed production in Germany and some other European countries. Multiple sowing every two to three weeks in consecutive, adjacent field plots allowed the monitoring of downy mildew incidences on plants of consecutive development over the whole season.

Spontaneous local infection on leaves and symptoms of late systemic infection in older plants were recently observed in a field where no sunflower had been cultivated before, hence soilborne infection from oospores was not likely unless the seeds themselves carried the pathogen. The scope of this study was to trace the origin of late systemic infections and evaluate the potential of secondary infections on aerial plant parts to become systemic.

## Materials and methods

### *Field plots and cultivation*

A field of ca. 1 ha near the University of Hohenheim, used for commercial cultivation of sunflower for ornamental purpose, was selected for observation of spontaneous downy mildew incidences caused by *P. halstedii*. According to the field history and our own observation, no sunflower had been cultivated on this land before. Seeds used for cultivation were of an unknown genotype, imported from East Europe. No fungicide treatment had been applied on seeds or germinated plants. Seeds were sown densely in five parallel plots of ca. 2 × 75 m (Fig 1A). The seedlings of each plot remained covered with a horticultural fleece until the first true leaves of seedlings measured ca. 2 cm in length. Sowing dates were: Apr. 10, 21, May 2, 20 and Jun. 6 2007.

### *Infection monitoring*

In each field plot, one hundred plants randomly selected from five different sites were investigated for downy mildew symptoms when the stage of flowering had been reached. Plants with no visible signs of *P. halstedii* infection (e.g. chlorosis, sporulation, stunting, leaf lesions) were categorized as uninfected, whereas plants with chlorotic and sporulating leaf spots, but no reduced internodal growth were grouped as local secondary infections. The third category was made up by plants showing typical systemic symptoms such as sporulation and chlorosis along leaf veins and inhibited internodal growth at least in the upper part of the stem towards the capitulum.

For the investigation of putative transitions from local to systemic infection, plants of the 5th plot were monitored three times starting in the stage of two true leaf pairs (I), at an average height of 70 cm (II) and in the stage of flowering (III). To trace the soilborne or airborne origin of systemic infections, ten plants of stage II in this plot with apparent stunting symptoms in the upper stem were investigated microscopically for the occurrence of *P. halstedii* hyphae in hypocotyl, petioles and different internodes of the epicotyl. For better contrast of hyphae, cross sections were stained with resorcin which leads to blue colouring of callose around the haustoria.

Sporangia of the field isolate were collected and used for virulence studies in a set of sunflower differential lines as described elsewhere (Rozynek & Spring 2000). The isolate was classified as pathotype 710 according to the actual race nomenclature (Tourvieille *et al.* 2000).

### *Climate data*

Climate data were provided from a weather station of the University of Hohenheim, Institute of Physics, coordinates 48°43′N, 09°13′E, altitude 407 m ("©<Hohenheimer Klimadaten>"). The station was located less than 3 km from the observed sunflower field.

## Results

Spontaneous infection of sunflower with *P. halstedii* was monitored in a field where plants had been sown consecutively every 2–3 weeks from Apr. to Jul. 2007 in five parallel plots. Plants of the first and second sowing (Apr. 10 and 21) did not show symptoms of infection at their flowering stage in Jul. In contrast, local and systemic infection was observed in the third field plot and their ratio gradually increased in the fourth and fifth plots (Table 1). When sunflower of the fifth plot had reached flowering stage in Aug., no healthy plant was found and systemic infection had reached a ratio of 76 %. It was evident that the typical symptoms of systemic infection did not occur in the early stage of plant development and stunting of the stem did not affect the hypocotyl and basal internodes (Fig 1B,C). Weather data during this period (Fig 2) showed phases of precipitation lasting for 2–3 d and relatively cool temperatures (10–15 °C) within the first weeks after sowing in the third to fifth plot. However, infection correlated with a specific and unusual weather event.

The time course of infection was investigated with plants of the fifth plot (Table 2). In the stage two true leaf pairs (first leaves ca 5 cm in length), 14 % of the plants were free of symptoms and no systemic infection was detected (evaluation