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Sunflower white blister rust – Host specificity and fungicide effects on infectivity and early infection stages

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ABSTRACT

White blister rust is an economically important emerging disease of sunflower caused by *Pustula helianthicola*. Knowledge on the host range of the pathogen particularly on different *Helianthus* species and its relative cultivars is of high relevance for disease management. Natural infection in the field on 13 sunflower genotypes with differentiating resistance against sunflower downy mildew (*Plasmopara halstedii*) showed high susceptibility to *P. helianthicola* which was independent from the resistance against downy mildew. Similarly, numerous commercial sunflower lines used in Europe showed high susceptibility to white blister rust in the field. Leaf disk inoculation with sporangia of a single spore strain of *P. helianthicola* confirmed full susceptibility of these genotypes, whereas wild species of *Helianthus* could not be infected under the controlled conditions except for a single incident observed with *Helianthus praecox*. Investigation of pre and post penetration events on the leaf surface of selected *Helianthus annuus* cultivars and wild species revealed that zoospore discharge, encystment and germ tube development occurred similarly on cultivars and wild species. But in the latter no germ tube penetration of the stomata was found and no hyphal or haustoria development was observed after 72–96 hours post inoculation (hpi) in wild species. Studies on the effect of different fungicides showed that the phenylamide metalaxyl-M and the strobilurin azoxystrobin inhibited the infection of seedlings at 1 and 2 μg/mL concentration, respectively, whereas the carboxylic acid amide dimethomorph required ca. a 10 fold higher concentration. With the plant strengthener BABA more than 40% of the plants were infected even at 200 μg/mL treatments. These results corroborated with the effects on early developmental stages of the pathogen (zoospore discharge, encystment, and germ tube development) in host independent experiments. Our results suggest that fungicides provide an effective measure for controlling sunflower white blister rust. However, considering the residual effect of chemicals and the possibility of developing fungicide resistance, breeding for resistant genotypes appears to be a more promising way for long lasting and sustainable disease management.

1. Introduction

Pustula helianthicola Rost and Thines (syn. *Albugo tragopogonis* (Pres.) Gray) is an obligate biotrophic oomycete causing white blister rust (WBR) in sunflower (*Helianthus annuus* L.). In recent years, the disease is emerging as a serious threat to commercial production of sunflower as it became evident from sudden outbreaks in several countries such as Australia, Argentina, South Africa, Kansas, Germany across different continents (Allen and Brown, 1980; Delhey and Kiehr-Delhey, 1985; van Wyk et al., 1995; Gulya et al., 2002; Thines et al., 2006). Several chemical control methods are recommended (Schwinn and Staub, 1987; Majoros et al., 1993; Garbelotto et al., 2009) for disease management of oomycetes. However, excessive use of chemicals can have negative side effects such as residual contamination in the crop and soil, and fast occurrence of resistance in the pathogen population (Cohen and Coffey, 1986; Gisi and Cohen, 1996). Alternatively, a systematic study of the pathogen and its host preferences could help to find a long term sustainable solution without hazardous effects on the environment. Hence our previous studies had focused on central aspects in the life cycle of *P. helianthicola* such as the formation and infectivity of the oospores (Lava and Spring, 2012), or the role of seed contamination for overwintering and long distance propagation (Lava et al., 2013). However, the pathogen's host range and the susceptibility of sunflower crop lines are still understudied. A previous field trial with 30 North American sunflower accessions

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revealed that none of them were resistant (Castano et al., 2005). No such experiments have yet been reported for European accessions.

Moreover, in contrast to many studies on economically important downy mildew pathogens, only few reports have been published which tested the effectiveness of fungicides on white blister rusts. Most of the fungicide work has been done on the local infection of oomycetes pathogens on host plants (Crute et al., 1977; Cohen and Coffey, 1986; Sudisha et al., 2010) and in some studies specific effects of fungicides in particular stages of development were performed for downy mildew pathogens (Cohen and Perl, 1973; Rooke and Shattock, 1983; Viranyi and Oros, 1991; Deepak et al., 2006). For some white blister rusts, the effectiveness of metalaxyl-M and azoxystrobin has been shown (Ypema and Gold, 1999; Damicone and Hammer, 2000; Damicone, 2003), but no such reports exist for *P. helianthicola*.

In the current study, we assessed the infectivity of *P. helianthicola* on different *Helianthus* wild species and *H. annuus* cultivar lines. In addition, we investigated early infection stages of *P. helianthicola* on leaves by comparing zoospore discharge, encystment and germ tube development on the epidermis of wild species and *H. annuus* cultivars. The effect of different fungicides was recorded in host independent early infection stages of the pathogen as well as in experiments with application on whole seedlings.

2. Materials and methods

2.1. Natural infection of sunflower genotypes with P. helianthicola in the field

To assess natural infection with *P. helianthicola*, a field plot was chosen near Plieningen Stuttgart, Germany, where commercial cutting sunflowers had been cultivated for three consecutive years prior to our field experiment. Natural infection of sunflower with *P. helianthicola* was observed to be common in the previous year. Thirty seeds each of the sunflower downy mildew differential lines (see Tourvieille de Labrouhe et al., 2000) HA 304, HA 821, RHA 265, RHA 274, PM 13, DM 2, HA 799-2, PM 17, HA 803-1, HAR 4, HAR 5, QHP 1, HAR 335 and of 17 different commercial breeding lines # 1–17 (kindly provided by Dr. V. Hahn, Landessaatzuchtanstalt Eckartsweier, Germany) were planted in the plot in July. Ten to 30 plants per genotype were germinated and raised to record the number of naturally infected plants. Plants showing the typical white blister symptoms on leaves were observed about three weeks after sowing. In the same period, spontaneous systemic infection with the downy mildew pathogen *Plasmopara halstedii* killed part of the plants. The monitoring was finished after four weeks past sowing.

2.2. Cultivation of H. annuus genotypes and Helianthus wild species

Fourteen differential lines of *H. annuus* (see listed above, plus HA 300), the cultivar Giganteus, and ten different *Helianthus* wild species from the Botanical Garden of Hohenheim University (*H. annuus*, *Helianthus praecox*, *Helianthus debilis*, *Helianthus bolanderi*, *Helianthus* x *laetiflorus*, *Helianthus* x *multiflorus*, *Helianthus decapetalus (2n)*, *H. decapetalus (4n)*, *Helianthus salicifolius*, *Helianthus tuberoses*) were used in this experiment. The cultivar Giganteus served as a susceptible control in all infection experiments. To obtain leaves for the infection studies, four to six seeds of each differential line and of the four annual wild species *H. annuus*, *H. praecox*, *H. debilis*, *H. bolanderi* were sown in sterile soil under controlled conditions in a growth chamber at 20 (±2) °C temperature, 80% humidity with a 14 h photoperiod. After three weeks, the plants were transferred to an experimental field plot where no infected sunflower had been observed before and were subsequently cultivated under natural conditions for further use. The remaining six perennial wild species were cultivated in the Botanical Garden of Hohenheim University.

2.3. Pathogen and plant material used and maintenance of the pathogen

The genetically homogenous single zoospore strain SLS 1000-SS-2-2010 of *P. helianthicola* (Lava and Spring, 2012) was used for infection studies on detached leaves and fungicides bioassay on seedlings. In addition, this strain was also used to assess the developmental stage response of the pathogen (host independent) to various fungicides. Furthermore, we used the field isolate OS-1236 (voucher deposited at the herbarium HOH) from Lauffen, Heilbronn, Germany for testing the sensitivity to the fungicides metalaxyl-M, dimethomorph, azoxystrobin and BABA in a bioassay on seedlings of *H. annuus* cv. Giganteus. The maintenance of the pathogen and propagation of sporangia was carried out as described by Lava and Spring (2012).

2.4. Leaf disk inoculation on different genotypes and wild species

Fresh leaves of different *Helianthus* wild species, differential lines and the cv. Giganteus (control) were washed in tap water to avoid microbial contamination. Leaf disks of approximately 1.0 cm^2 in size were cut from the lamina, washed in deionized water, and placed upside down on a wet filter paper (90 mm in size, VWR International buba Haasrode Research Park, Geldenaasksebaan, Leuven, France) in 9 cm Petri dishes. Fresh sporangia of the strain SLS 1000-SS-2-2010 were suspended in deionized water and the density was determined with a Fuchs Rosenthal hemocytometer. About 1×10^4 sporangia per leaf disk were applied on each of 25 leaf disks used per sample. The Petri dishes were sealed with parafilm and incubated in darkness for 24 h at 16 °C in a climate chamber, followed by further incubation with a 14 h photoperiod. The course of infection was checked daily until the typical blisters with sporangia were observed. The experiment was conducted three times with a total of $n = 50$ leaf disks.

2.5. Infection structures of P. helianthicola on sunflower leaves

In a similar way as described above (2.4), leaf disks of *H. annuus* cv. Giganteus, the differential lines HA 300, HA 304, PM13 and HA 335, and the wild species *H. annuus* (wild type), *H. decapetalus* (2n), *H. salicifolius*, *H. tuberoses* and *H.* x *laetiflorus* were inoculated with 10,000 sporangia per disk and incubated for the first 24 h in darkness at 16 °C, followed by incubation for 14 h light per day. After different time intervals (6, 24, 48, 72, 96 h) of incubation, leaf disk samples ($n = 5$) were transferred into a Rolland glass containing Carnoy solution (96% ethanol: acetic acid: chloroform i.e. 3: 1: 2) for fixation of infection structures and depigmentation of the leaf. After 24 h at 4 °C, the Carnoy solution was removed and leaf disks were washed with distilled water at least three times. The disks were placed on a microscopic slide and samples were stained with 1% (v/v) Blankophor in 15% KOH (Diez-Navajas et al., 2007). Short treatment with vacuum enhanced the penetration of staining solution into the intercellular space. The developmental stages of the pathogen were investigated on a Zeiss Axioplan (Zeiss, Oberkochen, Germany) microscope. The experiment was conducted four times with a total of $n = 15$ leaf disk per plant sample.

The different developmental stages of the pathogen in each cultivar and wild species were recorded by defining an area at the point of inoculation in which a total of 50 sporangia were present. In this area (approximately 0.3 cm^2) the number of discharged