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Preliminary data on *Plasmopara halstedii* pathotype distribution in central Italy in 2020–2022

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Abstract: Downy mildew of sunflower is caused by the oomycete *Plasmopara halstedii* (Farl.) Berl. et de Toni. Italy has seen an increase in mildew infections in parallel with the spread of the crop. In the present situation, there is a substantial lack of information that could help farmers and researchers control the disease. The most reasonable explanation for pathogen spread appears to be the genetic variability of *P. halstedii*. To develop an effective control strategy, we conducted a preliminary test in the central part of the peninsula (Marche Region) to gather data on this variability.

Keywords: sunflower; Downy mildew; genetic variability

Starting from year 2020, the area dedicated to the production of sunflower in Italy has always been over 110 000 ha. More than one-third of the planted area is concentrated in the central part of the peninsula, and particularly in the Marche region (Figure 1).

Plasmopara halstedii (Farl.) Berl. et de Toni, the main pathogen of sunflower downy mildew, was first found in Italy about 25 years ago. Since then, there has been an increase in mildew infections in parallel with the spread of the crop (Zazzerini & Tosi 1998; Tosi & Zazzerini 2004; Tosi & Beccari 2007; Bán et al. 2021), but over the course of the past 5–6 years, the situation has worsened significantly.

P. halstedii early primary infection occurs when sporangia enter the young root tissue, causing a systemic colonisation that usually results in severe stunting and plant death; late primary infection causes dwarfism of the plant (Gascuel et al. 2015). Secondary infections cause additional damage that is more severe the sooner they ap-

Supported by the Regione Marche (Italy), Servizio Politiche Agroalimentari, Project No. L.R. 37/99 – Bando per la selezione degli attuatori del progetto pluriennale di studio delle razze di peronospora del girasole presenti sul territorio marchigiano finalizzato al contenimento del patogeno.

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Figure 1. Map of Italy
The position of the Marche Region is indicated in red; the five districts are shown in the expanded view

pear. With conditions predisposing to the disease, the economic loss can range from 30 to 60% of the production.

Disease control by seed coating gradually decreased in efficacy with the appearance, since the 1990's, of resistance of the oomycete to the chemical Metalaxyl-M (Körösi et al. 2021). Consequently, an Integrated Pest Management (IPM) approach that relies on mechanisms of genetic resistance has been developed (Bán et al. 2023). The first attempts at breeding for resistance to downy mildew began in France in 1966. *Plasmopara* (*Pl*) resistance genes were progressively introduced into sunflower commercial hybrids in response to the pathogenic races (or "pathotypes") that gradually appeared in that country. French hybrids are currently sold throughout Europe.

In Italy, sunflower genotypes of exclusive foreign origin are grown. There is a substantial lack of information about the distribution of *P. halstedii* pathotypes across the territory. Without knowledge of which Pl resistance genes would be most valuable in blocking the spread of the disease, it is difficult to set up a targeted control program based on an IPM approach. The oomycete is therefore subjected to genetic pressure driven by the presence of commercial hybrids of foreign origin, rather than by a strategy aimed at slowing the spread of the disease.

To gather preliminary data on *P. halstedii* genetic variability, we conducted a study on the presence and evolution of pathotypes across the national territory, starting in the Marche region, the area of greatest sunflower diffusion in Italy.

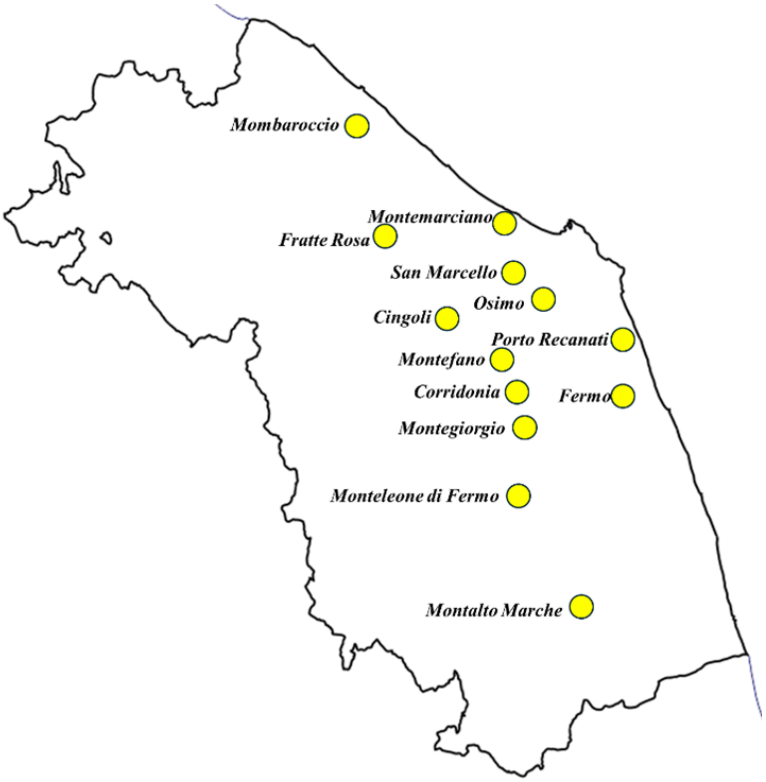

Figure 2. Map of Marche Region
The yellow dots indicate the sunflower growing locations where *Plasmopara halstedii* infected leaves have been collected

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MATERIAL AND METHODS

The territory of the Marche region is divided into five districts: Pesaro-Urbino, Ancona, Macerata, Fermo and Ascoli Piceno (Figure 1). Thirteen test areas (representative of all five districts) were chosen to evaluate the presence of *P. halstedii* pathotypes (Figure 2), and the study was carried out over three years of testing. Each test area consisted of a location where downy mildew symptoms had already been identified.

Samples consisting of infected sunflower leaves were taken to the laboratory and kept at 15 °C for about 24 h in the dark. The pathogen zoospores were collected directly from the leaves and suspended in sterile double-distilled water; the inoculum concentration was about 30 000 zoospores/mL, determined using a hemocytometer (Trojanová et al. 2017).

For the *P. halstedii* pathotype identification, 12 lines of sunflower (Table 1) maintained at the experimental farm "Settempedana" at CREA-CI of Osimo (Ancona) were used. The seeds of these lines were sterilised by immersion in a 1% hypochlorite solution for 10 min, followed by rinsing with sterile water to inhibit the growth of saprophytic fungi. The seeds were germinated on paper at 22 °C until the root reached a length of at least 2.0–2.5 cm.

Table 1. Sunflower differential lines and supplemental lines were used to determine the virulence phenotype of *Plasmopara halstedii* isolates

Triplet	Value	Lines			Resistance genes
	1	Differential	D.1	HA 304	None
1st	2		D.2	RHA 265	Pl_1
	4		D.3	RHA 274	Pl_2/Pl_{21}
	1		D.4	DM -2	Unknown
2nd	2		D.5	PM-17	Pl_{5-}
	4		D.6	803-1	Pl_{5+}
	1		D.7	HAR 4	Pl_{16}
3rd	2		D.8	HAR 5	Pl_{13}
	4		D.9	HA 335	Pl_6
	1	Supplemental		RHA 295	None
–	2			RHA 325	None
	4			RHA 340	Pl_8

For every line, the corresponding resistance gene is indicated accordingly with Gilley et al. 2020

Twenty seedlings for each differential line were inoculated by immersion for 3 h at 15 °C in the sporangial suspension obtained from the leaves.

The inoculated seedlings were placed in a growth chamber on vermiculite with a 16 h photoperiod and alternating temperatures (18–22 °C) until the development of the first true leaf (10–12 days post-inoculation). To promote sporulation of the pathogen, the plants were placed at 15–18 °C with 100% relative humidity for 24 h.

The identification of *P. halstedii* pathotypes was carried out according to the international nomenclature based on the "triplet" system (Limpert & Müller 1994; Gulya et al. 1998). For experimental purposes, a triplet of supplemental lines (RHA 295, RHA 325, RHA340) (Tourvieille de Labrouhe et al. 2012; Gilley et al. 2020; Molinero-Ruiz 2022) was added to the original triplets of differential lines (D1, D2, D3; D4, D5, D6; D7, D8, D9). The supplemental lines of seeds were kindly provided by Prof. Gulya (USDA-ARS Sunflower Research Unit Fargo, ND 58102). The differential lines were considered sensitive to the strain tested in the presence of sporulation on cotyledons and first true leaves (Molinero-Ruiz et al. 2008; Molinero-Ruiz 2022), and the test was replicated twice to avoid false-positive results.

RESULTS AND DISCUSSION

In 2020 and 2021, with both spring and summer seasons very dry, *P. halstedii* infections in the Marche region were not particularly widespread or intense; therefore, it was difficult to obtain the inoculum and to bring it to the expected concentration. Consequently, few isolates were collected. In 2022, due to favourable conditions for the pathogen's development, a greater number of isolates were collected.

Overall, 18 isolates of *P. halstedii* were recovered (Ph1–Ph18). For each isolate, the collection site and year of isolation were annotated; pathotype was determined using the triplet system. Results are summarised in Table 2.

The results of the present work were compared with findings from existing scientific literature.

Until 2021, in Italy, pathotypes 100, 300, 301, 700, 703, 704, 714, 715, and 717 have been identified (Zazzerini & Tosi 1998; Tosi & Zazzerini 2004; Tosi & Beccari 2007; Martín-Sanz et al. 2020; Bán

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Table 2. Pathotype and collection area of *Plasmopara halstedii* isolates

Isolate	Year	District	Locality	Pathotype
Ph 1	2020	Pesaro-Urbino	Fratte Rosa	730
Ph 2	2020	Ancona	Osimo	704*
Ph 3	2020	Macerata	Montefano	734
Ph 4	2020	Ascoli Piceno	Montalto Marche	730
Ph 5	2021	Ancona	Osimo	704*
Ph 6	2021	Macerata	Porto Recanati	314
Ph 7	2021	Fermo	Monteleone di Fermo	304
Ph 8	2021	Fermo	Montegiorgio	304
Ph 9	2022	Pesaro-Urbino	Mombaroccio	714
Ph 10	2022	Ancona	Montemarciano	704
Ph 11	2022	Ancona	Osimo	704*
Ph 12	2022	Ancona	San Marcello	714
Ph 13	2022	Macerata	Cingoli	700
Ph 14	2022	Macerata	Corridonia	714
Ph 15	2022	Macerata	Montefano	700
Ph 16	2022	Macerata	Porto Recanati	314
Ph 17	2022	Fermo	Fermo	714
Ph 18	2022	Fermo	Monteleone di Fermo	704

Pathotype was determined with differential lines D.1–D.9; isolates that overcome the resistance gene Pl_8 are indicated with *

et al. 2021; Miranda-Fuentes et al. 2021). Our results confirm the presence of pathotypes 700, 704, and 714, especially in 2022, when high levels of infection were recorded.

In the present work, the pathotypes 304, 314, 730, and 734 were isolated for the first time in Italy; their presence has been confirmed in most European countries (Spring 2019; Bán et al. 2021; Miranda-Fuentes et al. 2021).

In the district of Ancona, in the locality of Osimo, we identified isolates Ph 2, Ph 5 and Ph 11 (pathotype 704) that were able to induce heterogeneous sporulation on the supplemental lines RHA 295, RHA 325 and RHA 340 (Figure 3).

The high virulence of these *P. halstedii* isolates observed in the field against some hybrids is confirmed by pathotype identification on the RHA 340 line that carries the Pl_8 resistance gene. This result confirms previous findings by Martín-Sanz in 2020 in Central Italy, and accordingly, we assigned the nomenclature 704-Pl_8 to the pathotype corresponding to these isolates. The remaining 15 isolates couldn't induce sporulation on the supplemental line RHA 340.

Osimo is a site where, for many years, experimental trials have been conducted to test new sunflower hybrids. In this location, the presence of hybrids of different natures and resistance may have

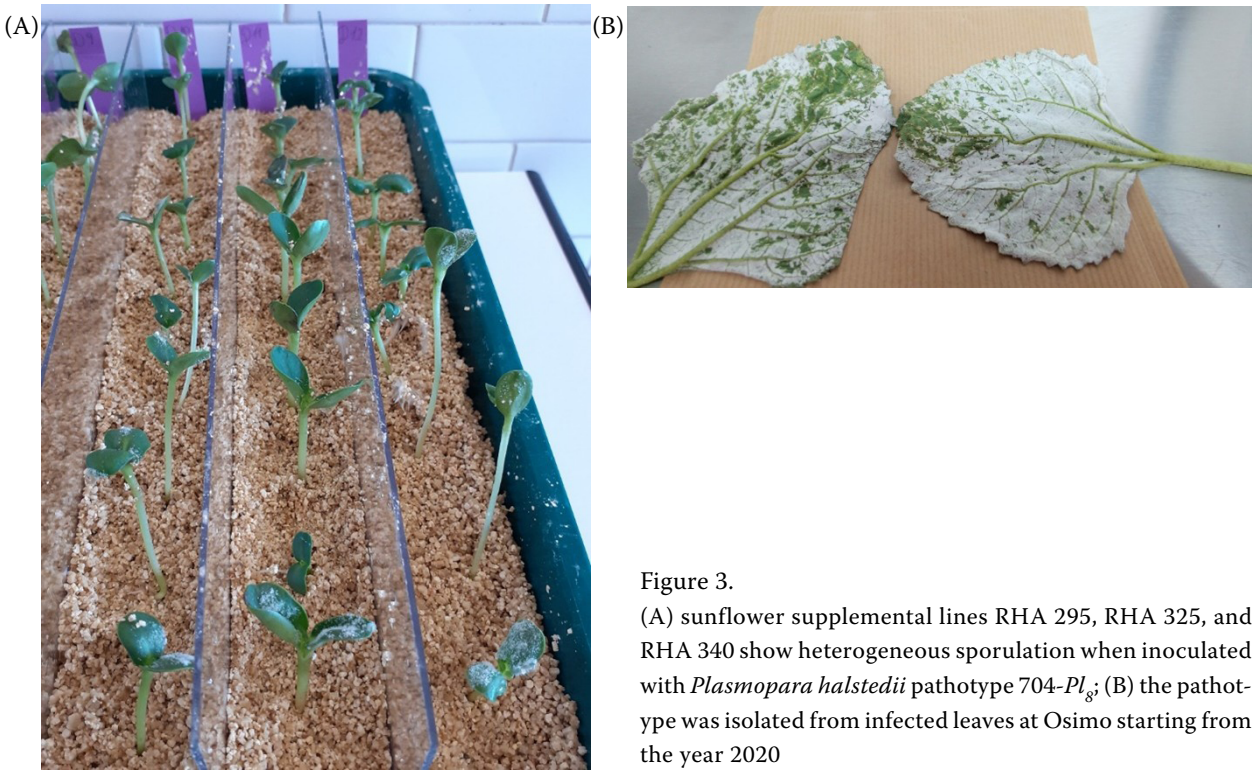

Figure 3.
(A) sunflower supplemental lines RHA 295, RHA 325, and RHA 340 show heterogeneous sporulation when inoculated with *Plasmopara halstedii* pathotype 704-Pl_8; (B) the pathotype was isolated from infected leaves at Osimo starting from the year 2020

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increased the genetic pressure on the pathogen, leading to new pathotypes. In the present work, we show that the heterogeneity of the *P. halstedii* population in the Marche region is evident, with several pathotypes detected. This result confirms the findings of numerous reports showing a similar pathogen evolution across the European territory.

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