HISTORY AND PRESENT STATE OF DOWNY MILDEW IN ARGENTINA

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ABSTRACT

Until 1998, races of *Plasmopara halstedii* (Farl.) Berl. *et* de Toni present in Argentina were 300 and 330, and almost all the sunflower hybrids sown in the country were resistant by the introgression of *Pl*2 resistant gene. Since that year resistance conferred by Pl2 was broken and races 710, 730 and 770 were determined. Resistance genes to these new races were introgressed from public lines from USA and Argentinean populations (*Pl5, Pl6, Pl7, Pl8, Pl15, Pl17, Pl18* and *PlArg*). Seed treatment with metalaxyl has been also widely used in different sunflower areas, but strains tolerant to this fungicide were found in all these races. Since 2013, downy mildew has been found in hybrids containing *Pl*15 gene, indicating the presence of a new race which is not possible to be classified by the international set of differential lines. Sustainable management of this disease should be based on reducing the selection pressure over the pathogen by combining practices as the introgression of several resistance genes simultaneously, using different active ingredients for seed treatment, crop rotations and avoiding contaminated seed exchange.

Keywords: metalaxyl tolerance; *Plasmopara halstedii;* new races; differential inbred line; *Pl* 15 resistant gene.

INTRODUCTION

Sunflower (Helianthus annuus L.) downey mildew (DM) caused by the oomycete *Plasmopara halstedii* (Farl.) Berl. & de Toni is one of the major diseases of this crop worldwide. It is potentially destructive when early infections occur through the roots causing damping-off and dwarfism. Usually, *P. halstedii* can generate variants of pathogenicity (pathotypes) with high frequency. Seed exchange between countries and / or production regions may favor the spread of these new pathotypes. Currently, there are at least 36 pathotypes of *P. halstedii* worldwide, but the number of races is increasing rapidly (Virányi and Spring, 2011). Furthermore, during the 80´s and 90´s many cases of *P. halstedii* tolerant to metalaxil were report from Spain, Hungary (Oros and Virányi, 1984), Turkey (Delen *et al*., 1985), France (Lafon *et al*., 1996) and USA (Gulya *et al*., 2000) among others.

Until 1998, the races of *P. halstedii* present in Argentina were 300 and 330 (Bazzalo, *et al*., 1996), and almost all the sunflower hybrids were resistant by the introgressed *Pl*2 resistant gene. Since that year, resistance conferred by *Pl*2 was broken by new races (710, 730 and 770 races) (ASAGIR, 2003). Resistance genes to these races were introgressed from public lines from USA and Argentinian populations (*Pl*5, *Pl*6, *Pl*7, *Pl*8, *Pl*15, *Pl*17, *Pl*18 and *Pl*Arg) (Bertero de Romano, *et al*. 2010). Cultivars with genetic resistance and/or seed-coated with metalaxyl or metalaxyl–M (mefenoxam) are strategies widely used for disease control in Argentina. Crop rotation with low presence of sunflower, a delay in sowing dates in high soil density (high clay content and no tillage) can also contribute to disease control. However, between 2012 and 2015 the prevalence and intensity of DM has increased in different production regions of Argentina, indicating changes in pathogenicity of *P. halstedii*. The aim of this study was to identify new pathotypes of *P. halstedii* associated with the occurrence of epiphytotics of DM in Argentina. With this objective we have identified DM epiphytotic in different production regions of Argentina and then collected *P. halstedii* inoculum (sporangia) to determinate race and / or tolerance to metalaxyl.

MATERIALS AND METHODS

Between 2012 and 2015 seasons the presence of DM epiphytotics (> 5% incidence) were identified in different production regions of Argentina. The geographical location and the implemented strategies for DM control (genetic resistance and/or seed-coated with metalaxyl) were recorded for each case. Isolates of *P. halstedii* were collected. For inoculum multiplication, susceptible sunflower genotypes (without *Pl* genes or only *Pl*2; Paraiso 20®, Nidera Argentina; Cauquen®, El Cencerro, Argentina; HA 89 public line) were inoculated with each isolate using a protocol adapted from Viranyi and Gulya (1995). The resulting inoculum was briefly stored at -20ºC.

Twenty-eight isolates were selected and used to carry out the tests of tolerance to metalaxyl and/or race determination. Seeds of susceptible genotypes were treated with metalaxyl (46 mg per seed) to determine the tolerance of each isolate. A control without metalaxyl was included to determinate de level of tolerance. A randomized complete block design was used with two or three repetitions. Each experimental unit consisted of ten seeds planted in speedlings in a soil:perlite (1:1) substrate. The inoculation was performed according to the protocol of Viranyi and Gulya (1995). For the disease evaluation (incidence: plants with signs of DM per total plants), susceptibility was considered when sporulation on cotyledons and the first true leaves became evident to the naked eye. Occasionally, damping-off could be seen among the inoculated seedlings. Data was analyzed with ANOVA and LSD Fischer for disease incidence media comparison. For the race determination the sets of sunflower differential lines proposed by Tourvieille de Labrouhe, *et al*. (2000) were used. Seeds inoculation, seedling cultivation and disease evaluation were carried out as described above.

RESULT AND DISCUSSION

DM epiphytotics were identified in the three sunflower production regions of Argentina (Figure 1). North Santa Fé province and south Buenos Aires province were the regions with major number of cases (6 and 20 cases, respectively) between 2012 and 2015 crop seasons. In north Santa Fé, five *P. halstedii* isolates were tolerant to metalaxyl (Barro Pazos, Ceibal, Malabrigo, Reconquista and Villa Ocampo). These isolates were identified as 710, 730 and 770 races (Figure 1) (Bazallo 2014, Bazzallo and Piubello, 2015). Also, the presence of 713 race was detected near Santa Fé city (Figure 1; Table 1). It is the first registry in Argentina of *Pl*13 and *Pl*14 break (Table 1).

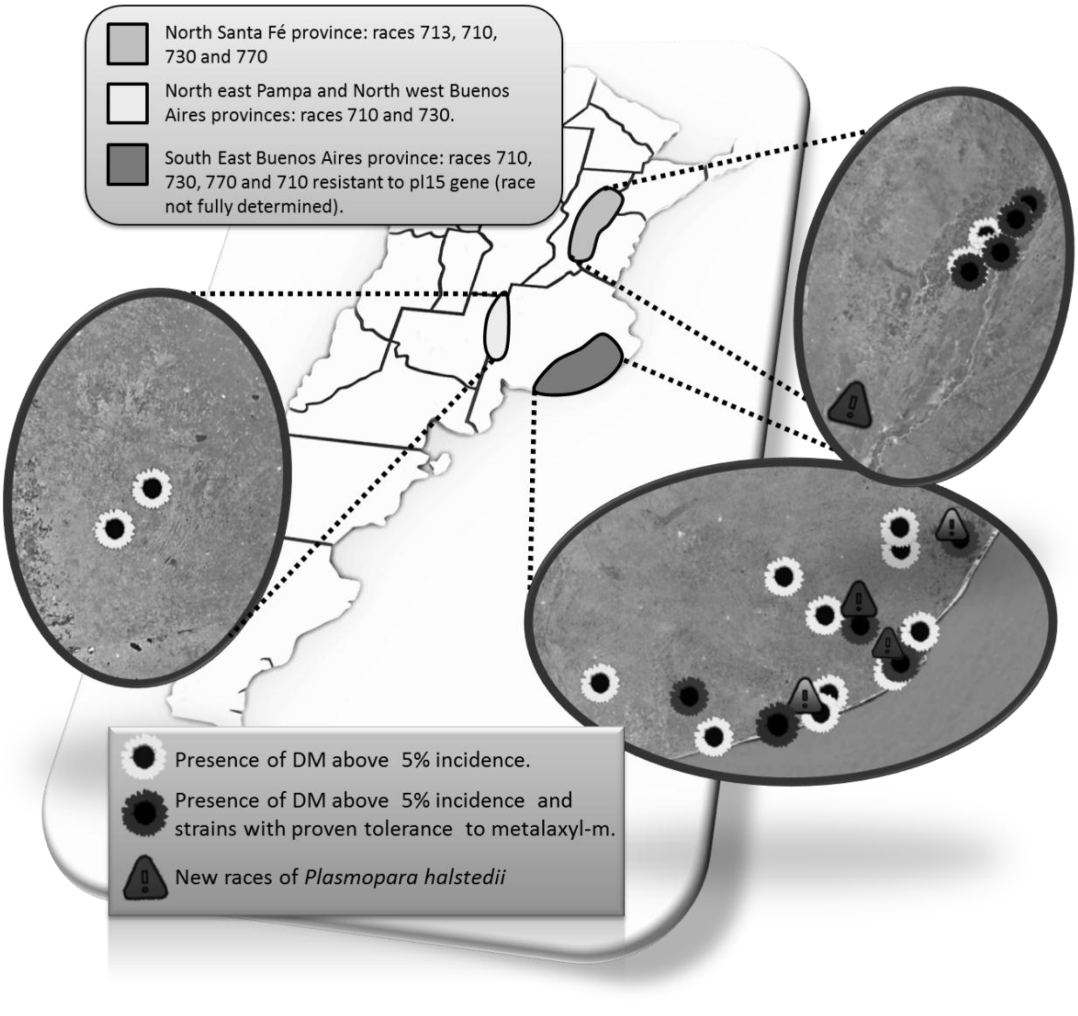

Figure 1. Location of downy mildew epiphytotics (more than 5% of incidence) caused by *P. halstedii* in Argentina between 2012 and 2015. Torelant isolates to metalaxyl and/or new races are discriminated.

In south of Buenos Aires province, twelve *P. halstedii* isolates were corroborated as tolerant to metalaxyl (Balcarce, Mar del Plata, Otamendi, Tres Arroyos among others) and were identified as 710, 730 and 770 races (Figure 1) (Erreguerena *et al*., 2013; Bazallo 2014, Bazzallo and Piubello, 2015). In this region, in 2014-2015 seasons it was found that some hybrids with resistant (*Pl*15 gene) to widespread Argentinian races (710, 730 y 770) were affected by DM. In these cases, the *P. halstedii* isolates were characterized as 710 race, although these variants compared with ordinary 710 races broke *Pl15* resistance gene (Table 1).

Table 1: New *P. halstedii* races in Argentina characterized by their reaction of sunflower differential line suggested by Tourvieille de Labrouhe, *et al.* (2000) and by an inbred line with *Pl*15 resistance gen.

					New races in Argentina	
Diferencial set		Line	*Pl* Gen	Race 710 ordinary variant (Argentina)	Race 710 variant not fully discriminated	Race 713
SET ONE	D-1	HA89		S	S	S
	D-2	RHA265	1	S	S	S
	D-3	RHA274	2	S	S	S
SET TWO	D-4	PMI-3	4	S	S	S
	D-5	PM-17	5	R	R	R
	D-6	803-1	803	R	R	R
SET THREE	D-7	HAR-4	14	R	R	**S**
	D-8	HAR-5	13	R	R	**S**
	D-9	HA-335	6	R	R	R
Inbred line with Pl 15		NI-Pl15	15	R	**S**	R

The present context of sunflower production in Argentina shows a growing dynamics towards to the generation of pathogenic variants of *P. halstedii* than the historically observed. In recent years there has been determined the tolerance to metalaxyl and the appearance of at least two new races (713 and 710 race that break the *Pl*15 resistance). *Plasmopara halstedii* pathotypes with tolerance to metalaxyl are distributed mostly in the regions of sunflower production, except for center region (east of La Pampa and west of Buenos Aires provinces). The loss of effectiveness of metalaxyl as a seed control requires its replacement with other molecules. The *Pl15* break by a new race exposes some of the Argentinian commercial hybrids to DM increasing the risk of an epyphitotic occurrence in the southern region of Buenos Aires province. For an accurate discrimination of this new race in relation to 710 ordinary race is required to expand the differential set of inbred lines suggested by Tourvieille of Labrouhe, et al. (2000). In this context, the *Pl6* and *Pl8* genes widely introgressed in Argentinian hybrids remains efficient for controlling DM. Is important to implement molecular techniques for *P. halstedii* identification in seed and avoid the incorporation of the pathogen or its pathotypes in the crop regions free of DM. Sustainable management of disease should be based on reducing the selection pressure over the pathogen by combining practices as the simultaneously introgression of several resistance genes, using alternative active ingredients for seed treatment, crop rotations and avoiding early sowing date and contaminated seed exchange.

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