Combining Chemistry & Genetics for *Plasmopara* Resistance Management

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ABSTRACT

- Sunflower downy mildew, caused by the pathogenic fungus *Plasmopara halstedii* (Farlow, Berlese et de Tony) has become a major disease for sunflower crops worldwide. The goal of this research is to discuss options (genetic and chemical) for effective control of this pathogen
- Literature and the latest field monitoring data were used to review new virulent *P. halstedii* races in diverse parts of the world. Greenhouse and field trials were conducted to assess the performance of various seed treatment offers with different hybrids under a range of field conditions
- The results show that in recent years, an increasing number of new *P. halstedii* races have appeared worldwide. This paper presents the benefits of new offers, such as combining new *Plasmopara*-resistant seed varieties with novel seed treatment offers, to eliminate or reduce the chance of new *P. halstedii* pathotypes developing in the future
- As a discussion point, this paper have summarizes various situations for *P. halstedii* resistance risk and corresponding *P. halstedii* control solutions. The presence of increasingly virulent races in diverse countries clearly calls for the combination of new genetics with enhanced downy mildew resistance, new seed treatment, and new mode of actions
- This paper raises industry awareness of potential *P. halstedii* resistance risk and proposes sustainable solutions that address one of the major concerns of sunflower growers.

Key words: Downy mildew; Plasmopara halstedii; resistance management; seed treatment; Mefenoxam; mode of action

INTRODUCTION

Sunflower is grown on every continent, with Russia, Ukraine and the European Union (EU 27) being the largest producers. The combined sunflower seed production worldwide is estimated at 38.1 million tons (OilWorld Statistics, 2011).

Significant growth, driven by area planting and yield/ha increase, is expected on all continents in the next 15 years. This underscores the importance of (i) continuously improving hybrid genetics, and (ii) providing novel seed treatment offers to protect genetic yield potentials. Sunflower diseases are of major concern worldwide, as they result in considerable yield losses or harvest quality decreases. There are a number of pathogens known to attack sunflower; mainly *Plasmopara*, *Sclerotinia*, *Diaporthe*, *Alternaria;* as well as the parasitic plant *Orobanche*. Downy mildew is considered the most significant disease from an economic standpoint as it is the most disclosed in the literature (Viranyi, 2003).

Because *Plasmopara halstedii* (*P. halstedii)* shows high phenotypic diversity, effective disease control depends on profound knowledge of the biology of the pathogen, its physiological capacities and requirements, and the molecular mechanisms involved in the interaction between the host and the environment. A review of the latest advances in *P. halstedii* research is given by (Viranyi, Spring, 2010).

Worldwide, 36 different *P. halstedii* races have been identified, four of which are dominant (700, 710, 730, 770) (Guyla et al. 2011). Presently, *P. halstedii* infections in sunflower are controlled either by seed treatment fungicides or by one or several dominant resistance genes. The races are defined by their ability to attack a plant harboring different resistant genes. The goal of this presentation is to:

1. Highlight the worldwide trends on the appearance of new *P. halstedii* races
2. Review the different solutions for controlling downy mildew
3. Propose methods for sustainable resistance management and long-term control of this disease

MATERIALS AND METHODS

<u>EAME and US *Plasmopara* races monitoring</u>

A field survey was carried out to determine the race constitution of naturally occurring field infection in Southern Spain. Race identification was conducted at the Koipesol Semillas S.A. facility in Seville. Similarly, in two surveys conducted in 2009 and 2010, *P. halstedii* samples were collected randomly (49 and 160, respectively) in various US states (ND, SD, MN, NE. CO) to determine the distribution of new *P. halstedii* races. The samples were sent by NSA surveyors and seed company personnel to the USDA sunflower unit (Gulya et al., 2011).

<u>Argentina seed treatment trials</u>

Lab trials were carried out by the Professional Services for Seed Industry (Eng. E. Teyssandier, Pergamino, Argentina). Trials were conducted under artificial inoculation with *P. haltsedii* race 770 as randomized complete block design with four replicates; assessment was done 15 days after inoculation.

<u>US and Europe seed treatment trials</u>

In 2010 and 2011 field trials (broad acre conditions) were implemented in the US, France, Hungary and Spain with susceptible varieties. Plot size varied between 15 and 30 m2 and trials were carried out as a randomized complete block with four to six replicates. Some of the field sites had Mefenoxam-sensitive *Plasmopara* populations, some others had predominantly resistant ones. Field trials were evaluated at several intervals after planting, as % incidence of *P. halstedii* and % severity of infection.

RESULTS

<u>Appearance of new virulent *Plasmopara halstedii* races worldwide</u>

US:

In the US the situation could be classified as a ‘low resistance risk’ until 2008 according to the results from the USDA monitoring unit (~350 samples processed). In 2009 however the first *P. halstedii* race to overcome PI_6 was identified (*Plasmopara* race 724); and the situation has been evolving rapidly, with new *P. halstedii* races appearing in the last two years. Between 2009 and 2010, more than 200 samples were taken in a vast downy mildew survey that was carried out by the USDA, seed companies and additional industry researchers from ND, SD, MN, NE and CO (Gulya et al., 2011). The following five new virulent races were identified in that survey.

Table 1. New virulent pathotypes of *Plasmopara halstedii* identified in US survey 2009-2010

		DM 314	DM 704	DM 714	DM 734	DM 774	Total
2009	#isolates	-	-	8	4	-	12 out of 49
2010	#isolates	3	1	6	3	3	17 out of 160

Table 2. Reaction of new races to standard hybrids (RHA 265), PI_6-hybrid (HA 335), PI_7 hybrid (HA 337) and new released USDA lines PI_8 hybrid (RHA 340)

	DM 314	DM 704	DM 714	DM 734	DM 774
RHA 265	S	S	S	S	S
HA 335	S	S	S	S	S
HA 337	S	S	S	S	S
RHA 340	R	R	R	R	R

The new virulent races were able to overcome the PI_6 (HA 335) and PI_7 (HA 337) genes.

Europe:

The situation in Europe is also changing rapidly with new races appearing every year in key countries. For instance, in France new *P. halstedii* races have appeared in the last 10 years (Delmotte et al., 2008). The first race to overcome Pl_6 (304) was found in 2000. Between 2001 and 2008 six more new races were identified (307, 314, 334, 704, 707, 714). In Russia, growers are facing a similar situation (Antonova et al., 2008); and in Bulgaria, the appearance of three new virulent pathotypes (330, 712 and 731) has been reported in 2007-2008 (Shindrova, 2010). In Spain, we conducted a *P. halstedii* survey in 2010 and 2011 with the following results.

Table 3. Possible new *P.* races in Spain and their potency to hybrid genetics (106 samples for each race)

		100	300	700	730	770	310	330	710	703	304	307	314	334	704	714	707
		Situation in 2010									Possible new races in 2011 that have been found in Europe						
RHA 273	PI_2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
RHA 274	PI_2+PI_9+PI_{10}	R	R	S	S	S	S	S	S	S	?	?	?	?	S	S	S
DM2	PI_{12}+PI_5	R	R	S	S	S	R	R	S	S	R	R	R	R	S	S	S
HA 335	PI_6	R	R	R	R	R	R	R	R	R	?	S	S	S	?	S	S
HA 340	PI_8	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

In summary, an increasing number of new virulent *P halstedii* races are appearing every year worldwide, particularly in USA and in Europe. There is an increased resistance risk situation in several sunflower producing countries that needs to be urgently addressed.

<u>Current and new seed treatment solutions to control downy mildew challenge</u>

Seed treatment products help to ensure good crop establishment and reduce primary systemic infections from soil-borne zoospores of *P. halstedii* which result in plant loss or stunted plants. As *P. halstedii* is considered a high risk pathogen special attention must be paid to minimize risks by combining resistant sunflower varieties with seed treatment offers.

Currently, molecules from the chemical classes of phenylamides and strobilurins are used as seed treatment in sunflowers against *P. halstedii*. Some resistance to Mefenoxam has been reported (Garcia-Lopez et al., 2009), (Molinero, 2008). As the pathogen is known to be at high risk to develop resistance toward fungicides, there is a need to find new chemicals with different mode of actions to maintain a high level of downy mildew control in sunflower.

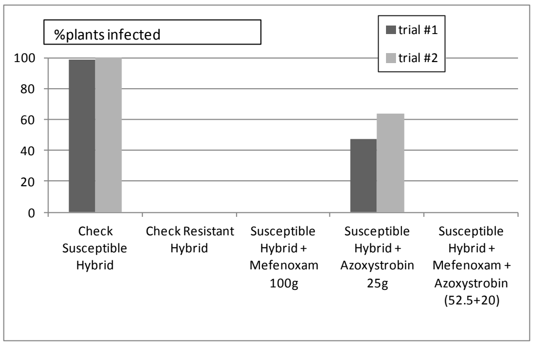
Argentina

Lab trials in Argentina (Graph 1) using a Mefenoxam sensitive *P. halstedii* isolate of pathotype 770 showed dual protection from the resistant hybrid cultivar as well as from the seed treatment Mefenoxam or a Mefenoxam + Azoxystrobin mixture.

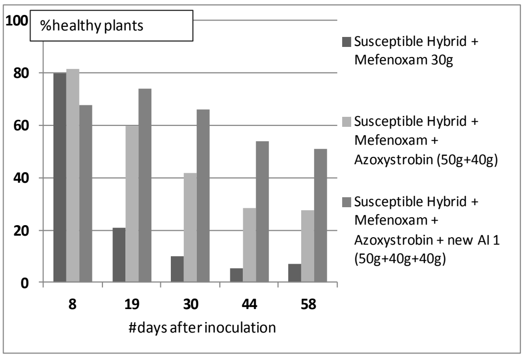
US

In the US new virulent *P. halstedii* races appear every year as described above. With Mefenoxam-resistant *Plasmopara* isolates (Graph 2) control with Mefenoxam alone was poor. Adding Azoxystrobin improved performance significantly, but the level of performance dropped after 30 days. Adding an additional active ingredient with a new mode of action (Mefenoxam + Azoxystrobin + new AI 1) not only resulted in higher control levels overall, but also enhanced the lasting activity of the seed treatment remarkably.

Graph 1 et 2. Efficacy trials of various seed treatment offers in Argentina (Graph 1) and USA (Graph 2).



Graph 1

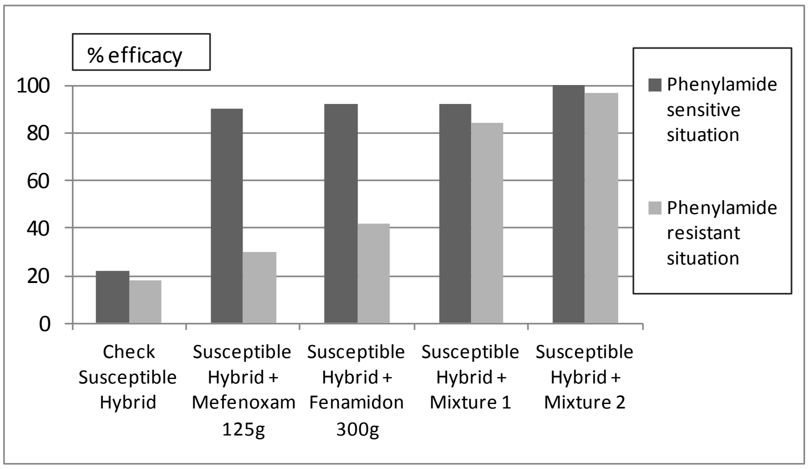


Graph 2

Europe

In 2009 and 2010 a number of field trials were set up in France, Hungary, Bulgaria and Spain to evaluate the activity of current market standards and new product combinations under either phenylamide-sensitive or phenylamide-resistant Plasmopara populations field conditions. In conditions with resistance to phenylamides Mefenoxam and Fenamidon did not provide a satisfactory level of control. However, Mixture 1 and Mixture 2 (combining active ingredients with different mode of actions) showed a significant improvement over Mefenoxam or Fenamidon alone under the same conditions.

Graph 3. Efficacy of various seed treatment offers in (i) Phenylamide sensitive situation; (ii) Phenylamide resistant situation (Mixture 1: Mefenoxam + new AI 1; Mixture 2: Mefenoxam + new AI 1 + new AI 2)



DISCUSSION AND CONCLUSIONS

New, virulent *Plasmopara halstedii* races

Recent surveys and monitoring in the US and Europe, as well as recent reports confirm that new, virulent *Plasmopara halstedii* races are appearing every year across the world. Combining new hybrids with chemical seed treatment provides the best control against the various *P. halstedii* races.

Hybrid development to counter new races of *Plasmopara halstedii*

The development and release of new sunflower cultivars resistant to the various *P. halstedii* races are of extreme importance to farmers. Although genetic resistance is very frequent in sunflower hybrids, new races of the pathogen that overcome the resistant genes are appearing in most sunflower-growing countries (Garcia-Lopez et al., 2008). It is therefore critical to bring new combinations of genes to the market as "pro-active resistance management"'. New genetics are often combined with seed treatment to prevent possible pathogen races mutations and development of resistance. In Spain, downy mildew historical data show the importance of combining breeding efforts with seed treatment.

Table 4. Evolution of hybrid and seed treatment in Andalucía with resulting *Plasmopara* infection level. Races identified from survey. (*) Certain *Plasmopara* races showed tolerance to Mefenoxam

Year	Hybrid	Seed Treatment	Infection level	*Plasmopara* races
70's	PI_0	-	Some primary infection	100
80's-90's	PI_2	-	No infection	100,300
2000-2003	PI_2	Mefenoxam	No infection	310, 330,710,730
2004-2007	PI_2	Mefenoxam	Some level of infection*	310, 330,710,730
2008-2009	PI_6	Mefenoxam	No infection	310,330,710,730
2010-2011	PI_6	Mefenoxam	Some level of infection*	310,330,710,730, new races

This stresses the importance of innovation and the combination of genetics with seed treatment to overcome new virulent *P. halstedii* races rather than allowing the pathogen to mutate and develop tolerance to Mefenoxam, or overcome the resistant genes in an alternate sequence.

Table 5. Mutation of *Plasmopara halstedii* may be prevented by Mefenoxam

	Plasmopara races	Possible mutation
Seed variety	710	New
Level of control	R	S
Mefenoxam	C	C

Current and new seed treatment offers

As *P. halstedii* races are known to have a high risk tof developing resistance to chemical seed treatment, innovation will be essential in order to maintain a sustainable level of control against this major disease. New mixtures combining active ingredients with different mode of actions will provide a very effective way of controlling the new virulent *P. halstedii* races in every part of the world.

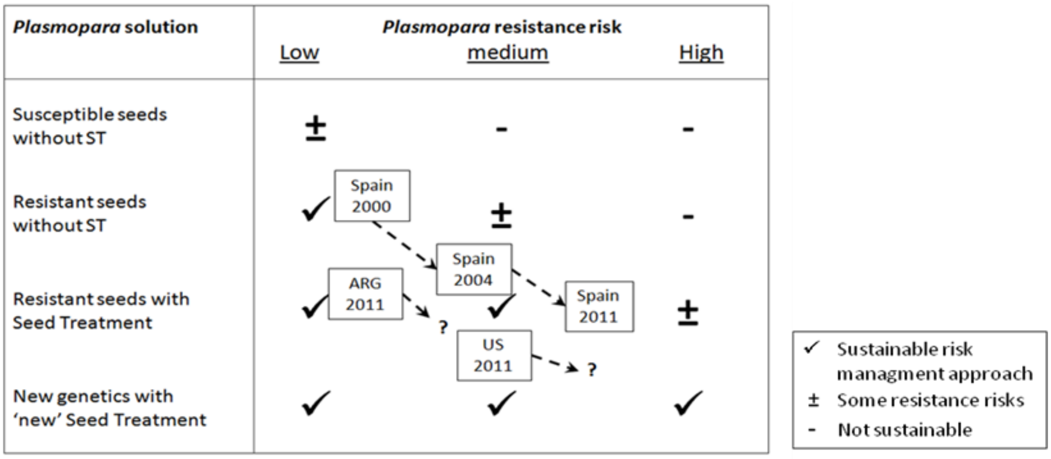
Resistance management

With the appearance of an increasing number of new virulent *P. halstedii* races it is crucial to have a sustainable risk management strategy in place in all parts of the world. It is critical to select the right combination of hybrid seeds and seed treatment offer. This clearly calls for:

1. developing new genetics with enhanced downy mildew resistance
2. developing new seed treatment offers with new mode of actions
3. combining new genetics with new seed treatment offers

As a matter for discussion, we have summarized the various situations and solutions in Graph 4.

Graph 4. Effectiveness of *Plasmopara* resistance strategy under various resistance risk situations (resistance against fungicides and genetics). Our understanding of the situation in some countries is also illustrated. The evolution of the situation in Spain is highlighted.



In conclusion, the combination of new *Plasmopara*-resistant genes with different seed treatment mode of actions provide the best downy mildew control strategy in every parts of the world. This sustainable resistance management strategy will help address one of the major concerns of sunflower growers and also ensure sustainable sunflower production over the coming decade – an objective shared by all sunflower industry partners.

Certain agricultural measures, such as crop rotation are also important to long-term sunflower disease control. The recent development of new pathotypes of *P. halstedii* have been found across the world with the exception of Argentina, where the situation does not seem to have changed much during the last decade. One important difference found there, in comparison with other countries, is that more than 80% of the sunflower is planted under "no tillage". Oospores, which are resistant spores that keep the soil infected for years, are the result of sexual fecundation that could lead to recombination in the next generation and are therefore an important step in the *P. halstedii* race development. We question whether under non-tillage conditions, the non-buried parts of the infected sunflower plants could represent a good method to prevent the appearance of new races through the limitation of oospores buried in the soil.

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