Helia 2024, Vol. 47 (80), 47-67
https://doi.org/10.29329/helia.2024.1101.4

Original article

Update on Sunflower Diseases in Argentina

Denis Nahuel Colombo [a], Monica Irina Fass [b], Ignacio Errenguerena [c],
Carla Valeria Filippi [d], Maria Carolina Martínez [b], Matías Dominguez [e],
Ana Laura Martínez [f], Pablo Aguilera [b], Sofía Deperi [h], Nicolás Heinz [c],
Emiliano Ben Guerrero [b], Alicia Carrera [g], Sergio Alberto Gonzalez [b],
Agustín Mazzalay [c], Juan Francisco Montecchia [i], Carla Maringolo [h],
Daniel Alvarez [c], Facundo Quiroz [a], Verónica Lia [j], Carolina Troglia [h],
Norma Paniego [b] & Andres Corro Molas [k, *]

[a] Estación Experimental Agropecuaria Anguil, Instituto Nacional de Tecnología Agropecuaria (INTA), Ruta Nacional 5 Km 580, Anguil L6326, Argentina. North Dakota State University – Department of Plant Pathology, Walster Hall, Fargo, ND 58102, United States.,
[b] Instituto de Agrobiotecnología y Biología Molecular–IABiMo–INTA-CONICET, Instituto de Biotecnología, Centro de Investigaciones en Ciencias Veterinarias y Agronómicas, INTA, Hurlingham B1686, Argentina
[c] Estación Experimental Agropecuaria Manfredi, INTA, Ruta Nac. nro. 9 km 636, Manfredi X5988, Argentina
[d] Laboratorio de Bioquímica, Departamento de Biología Vegetal, Facultad de Agronomía, Universidad de la República, Avenida Garzón 780, Montevideo 12900, Uruguay
[e] Estación Experimental Agropecuaria Pergamino, INTA, Av. Frondizi Km 4.5, Pergamino B2700, Argentina
[f] North Dakota State University, USDA-ARS. Department of Plant Pathology, Fargo, ND 58102, United States.
[g] Departamento de Agronomía UNS - Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS, CONICET), Bahía Blanca (8000), Argentina
[h] Estación Experimental Agropecuaria Balcarce, INTA, Ruta 226 Km 73.5, Balcarce B7620, Argentina
[i] Advanta Semillas SACI, Estación Experimental, Venado Tuerto, Santa Fe, Argentina
[j] Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Intendente Güiraldes 2160, Ciudad Autónoma de Buenos Aires C1428, Argentina
[k] Agencia De Extensión Rural General Pico, INTA, Calle 13 N° 857, Gral. Pico L6360, Argentina

Abstract

The moderate genetic diversity of cultivated sunflower, along with a changing environment, make sunflowers subject to the attack of many pests and diseases, with great economic impact. In Argentina, fungi and oomycetes produce four of the most challenging diseases: Sunflower Verticillium Wilt, Phomopsis Stem Canker, Sclerotinia Head Rot and Sunflower Downy Mildew. Efficient crop management to reduce the impact of pathogens requires resistant hybrids, recommendations for agricultural practices, and epidemiological monitoring to aid decision-making. However, each pathosystem has its own caveats. The integration of genotypic and phenotypic knowledge of sunflower breeding resources with the characterization of pathogens is critical to effectively assisting breeders in improving plant resistance and reducing yield losses. Below, we provide an overview of the latest scientific knowledge on each pathosystem and the strategies being pursued for breeding disease-resistant hybrids.

Keywords: Molecular Breeding; Pathogen; *Diaporthe helianthi; Plasmopara halstedii; Sclerotinia sclerotiorum; Verticillium dahlia*

Received: 24 November 2024 * **Accepted:** 22 December 2024 * **DOI:** https://doi.org/10.29329/helia.2024.1101.4

*** Corresponding author:**

Verónica Lia, Prof. Dr., Facultad De Ciencias Exactas Y Naturales, Universidad De Buenos Aires, lia.veronica@inta.gob.ar
Carolina Troglia, Dr., Estación Experimental Agropecuaria Balcarce, Instituto Nacional De Tecnología Agropecuaria, paniego.norma@inta.gob.ar
Norma Paniego, Prof. Dr., Biotechnology, Instituto De Agrobiotecnología Y Biología Molecular–Iabimo–Inta-Conıcet, Instituto De Biotecnología, Centro De Investigaciones En Ciencias Veterinarias Y Agronómicas, Inta, Hurlingham, Argentina,
Email: paniego.norma@inta.gob.ar

INTRODUCTION

Fungal diseases

Sunflower Verticillium Wilt

Sunflower Verticillium wilt and leaf mottle (SVW) is a monocyclic vascular disease whose causal agent is the soil-borne fungal pathogen *V. dahliae* (Kleb.) (Vd). *Vd* is a polyphagous pathogen that infects more than 350 dicotyledonous host plants, making it difficult to control through common agricultural practices. The fungal inoculum consists of long-lived microsclerotia that remain infectious in the soil for 10 to 15 years (Pereyra and Escande ,1994). According to Creus et al., (2007) and Quiroz et al. (2012), susceptible commercial sunflower hybrids can experience yield reductions of as much as 30% due to SWD and susceptible material can experience yield losses of up to 73% (Pereyra et al. 1999). SVW occurs in most sunflower-growing regions of the world (Gulya et al. 1997). In the past, it has had a major impact in Argentina and on extensive regions in Canada, and the USA, where new pathogenic races have emerged (Harveson and Markell, 2016; Gulya, 2007; Pereyra and Escande, 1994). In addition, it has recently become a serious threat to European temperate sunflower-growing countries (Martín-Sanz et al., 2018; Mestries, 2017).

In Argentina, *Vd* is an endemic pathogen with a variety of local races (Bertero de Romano and Vázquez, 1982; Clemente et al., 2017; Galella et al., 2004). *Vd* inoculum is spread over 1.2 million hectares, covering more than 70% of the country's sunflower-growing areas (Argentinian Sunflower Association, 2002). In the south of Buenos Aires province, the main production area in Argentina, SVW prevalence has averaged 45% (± 14%) over the last few years (ReTSaVe 2020). The density of microsclerotia in the soil in this growing area is an indicator of the severity of SVW (Rojo et al. 2008). In addition, Erreguerena (2013) found that low levels of organic matter (1.5-3%) and microbiota (bacteria, fungi, and actinomycetes) were associated with higher disease incidence and that there was a tendency towards higher disease incidence in sandier soils, probably related to low resistance to root exploration. Based on these results, Erreguerena et al. (2019) proposed a model that predicts disease risk before planting in each field.

The populations of *Vd* show great variability (Berbegal et al., 2011). *Vd* has no known sexual stage and reproduces mitotically by conidia and persistent soilborne microsclerotia, having a distinctly clonal population structure (Bautista-Jalón et al., 2021; Collado-Romero et al., 2008; Dung et al., 2013; Gurung et al., 2014; Jiménez-Gasco et al., 2014; Milgroom et al., 2014). However, mating-type idiomorphs have been described in the fungus (Usami et al., 2009). It has been hypothesized that sexual reproduction was lost from *Vd* recently and that current clonal lineages arose via previous ancestral recombination events (Atallah et al., 2010; Milgroom et al., 2014).

Before the availability of molecular markers, isolates of *Vd* were classified into vegetative compatibility groups (VCGs), which comprise isolates that can form stable heterokaryons (Jimenez-Diaz et al., 2012; Jiménez-Gasco et al., 2014; Milgroom et al., 2014). Five main VCGs (VCG1 through VCG4, plus VCG6) have been distinguished. Molecular genetic markers correlate almost perfectly with VCGs, with the major exception of VCG2B, which was subdivided into genetically distinct lineages 2B334 and 2B824 (Bautista-Jalón et al., 2021; Collado-Romero et al., 2008; Dung et al. 2013; Gurung et al. 2014; Jiménez-Gasco et al. 2014; Milgroom et al. 2014), and a recently discovered recombinant lineage, 2BR1 (Milgroom et al. 2014). *Vd* strains are also classified according to their pathogenicity in race 1 or race 2. The dominant locus V1 is the main source of resistance to race 1 worldwide (Fick and Zimmer, 1974; Fradin et al., 2009; Gulya, 2007; Kawchuk et al., 2001). This monogenic resistance source is conserved among species and has been described as a Pattern Recognition Receptor (PRR) (Fradin et al. 2011). However, race 2 strains evade this resistance (Bertero de Romano and Vázquez, 1982). Therefore, new *Vd* races were identified in sunflower according to the quantitative resistance response evoked in the plant.

In Argentina, Galella et al. (2004) and Clemente et al. (2017) described the presence of four races of *Vd* infecting sunflower, designated VArg1 to VArg4. In agreement with Martín-Sanz et al. (2018), molecular PCR analyses characterized VArg1 and VArg2 as molecular race-2 due to the absence of the Ave1 effector present in the USA reference race (Short et al., 2014). This result is consistent with the different pathogenic behavior of local and foreign races observed in race-specific resistance tests in a subset of inbred lines under controlled conditions (Montecchia et al., 2017). Aguilera et al. (2023) studied the diversity and population structure of 17 isolates collected between 2001 and 2022 in the provinces of Buenos Aires and La Pampa, including races VArg1 and VArg2 and two races from France and the USA. To do this, these authors used genotyping by sequencing technique (Aguirre et al. 2019) to produce approximately 6,000 single nucleotide polymorphisms (SNP). A population structure analysis combining these results with SNP data from 34 sequenced *Vd* genomes from public sources confirmed a strongly clonal population structure. A subsequent combination of SNP from local and reference isolates with 168 isolates from different crops and geographical regions (Bautista-Jalón et al., 2021) identified the same lineages previously described by VCGs. Interestingly, isolates from Argentina were grouped together with two isolates from France in a separate putative new lineage. The results obtained strengthen the characterization of *Vd* races affecting sunflowers in the region and favor the development of molecular diagnostic methods, epidemiological and surveillance studies, as well as the development of future breeding strategies for sunflowers for disease control.

Consistent with the different races of *Vd*, the sources of genetic resistance of sunflower depend on the circulating races. In Argentina, Galella et al. (2012) reported two QTLs associated to the resistance to VArg1 and VArg2 races, but the exact location was not disclosed. On the other hand, using an RNA-

Seq approach, Guo et al. (2017) described 759 and 511 differentially expressed genes specific to resistant and susceptible inbred lines, respectively. Montecchia et al. (2021) approached the identification of resistance sources in both a bi-parental population (BMP) and a genome-wide association mapping population (AMP). The response to *Vd* in these populations was characterized across nine field trials (FTs) conducted in heavily infested fields of La Pampa and Buenos Aires Provinces (Argentina). Clustering analysis based on disease descriptors allowed the recognition of four major groups of lines, differing in their response to the pathogen, with trait heritability ranging from 35 to 45%. The association mapping (AM) study allowed the identification of 36 genomic regions related with SVW resistance distributed over 12 of the 17 chromosomes of the genome of sunflower. Validation of three of these regions was achieved by linkage mapping (LM) on the BMP (Montecchia 2019). Based on these results, Domínguez *et al.* (2022) selected parental lines for the construction of two multi-parent advanced generation intercrosses (MAGIC) populations. A subset of 400 of the F2 families of MAGIC2 was phenotyped in infested fields of INTA Balcarce during the 2020/21 season. The analysis included multispectral images for phenotyping SVW. The results obtained allowed the identification of family groups with different responses to SVW and showed the potential of this technique for high-throughput phenotyping of fungal diseases in sunflower and the implementation of both populations in future fine-mapping studies.

Phomopsis Stem Canker and Phomopsis Head Rot

Phomopsis Stem Canker (PSC) of sunflower is a disease first identified in the former Yugoslavia by Mihaljcevic et al. (1980). From there it was detected in the main sunflower production regions of the world. In some regions, there are also infections in the capitulum, known as Phomopsis Head Rot (PHR) (Colombo et al., 2024).

In endemic regions, PSC results in yield losses of 40% or more (Maširević et al. 2016). In Argentina, both healthy and diseased paired plants have shown yield losses of up to 44% in achenes and up to 22% in fat content (Ghironi et al., 2018). In confectionery sunflower, changes in seed color and size have been observed, leading to potential market rejection. However, the quantification of this damage in confectionary sunflower remains to be investigated.

PSC was first reported on sunflower in Argentina by Fálico de Alcaraz et al. (1994) and Ridao (1994). Since then, two epidemics were reported in the country in 2002 and in 2015/16 (Corró Molas et al., 2016a; Huguet, 2006). Currently, the disease is present in all sunflower production regions of Argentina.

The causal agent of the disease was initially identified as *Diaporthe helianthi* / *Phomopsis helianthi* (Fálico de Alcaraz et al., 1994; Ridao, 1994). Subsequent surveys conducted in the southeast of Buenos Aires, based on molecular studies, identified six species: *D. helianthi*, *D. gulyae*, *D. caulivora*, *D. sojae*, *D. kongii*, and *D. longicolla*. The first specie represented 94% of the isolates obtained (Zambelli et al., 2021). Colombo and Corró Molas (2022) conducted additional surveys in La Pampa and western Buenos Aires. Over 3 years, they determined a prevalence ranging from 67% to 100%. They also detected *D. ambigua* in sunflowers in Argentina for the first time, with symptoms like those produced by *D. helianthi* (Colombo et al. 2023). *D. helianthi* was also found infecting weeds of the *Xanthium sp*. genus, while *D. gulyae* was found in *Carthamus lanatus*, *Chenopodium album*, among others (Thompson et al. 2015; Vrandecic et al. 2010). To date, *D. helianthi* has also been detected in *Helianthus petiolaris* in Argentina (Cáceres et al. 2007).

The pathogen primarily overwinters on infected stem debris, producing both pycnidia (anamorph state) and perithecia (teleomorph state) on these residues. Also, infected seeds may contribute to further spread both locally and internationally (Thompson et al., 2023). Ascospores serve as the primary source of infection, enabling the disease to persist from one season to the next. While pycnidia and their conidia exist, further research is required to ascertain their biological function. In Europe, Delos et al. (2000) developed the ASPHODEL model, while Moschini et al. (2019) developed a spore release prediction model based on records from Uruguay. The use of the second model in Argentina has enabled the early detection of high-risk areas for ascospore release in regions with limited historical disease records (Corró Molas et al. 2021). In the 2022/23 season, the model has also been monitored to aid in decision-making for fungicide use by detecting locations with a high risk of ascospore release (Corró Molas et al., 2023).

Disease management is based on the use of cultivars with improved performance, crop rotation, balanced fertilization, low planting density, residue burial, changes in planting date and hybrid cycle, and the use of fungicides (Debaeke et al., 2003; Maširević et al., 2016). The latter has been recently studied by Kashyap et al. (2023) and Dangal et al. (2023) in the United States. In Argentina, ongoing studies have yielded variable results, with a greater impact of fungicides observed on symptoms than on yield (Corró Molas, personal comm.). On the other hand, the genetic background of the cultivars has the most significant impact on disease resistance. European research groups initially took the lead in searching for sources of resistance through QTL studies (Bert et al. 2002; Besnard et al. 1997). In recent years, with the re-emergence of the pathogen in the USA and Argentina, new efforts towards unraveling the genetic basis of resistance have been made, now employing whole-genome approaches. To that end, using a recombinant inbred line (RIL) population derived from a cross between HA89 (susceptible) and HA-R3 (resistant), Talukder et al. (2020) identified a total of 15 quantitative trait loci (QTL) associated with PSC resistance, most of them spanning many centimorgan (cM). Additionally, Pogoda et al. (2021) identified seven (7) PSC unique QTLs, using a Genome-Wide Association Study (GWAS) approach based on a population of 218 accessions preserved at USDA.

The complexity of the genetic resistance not only involves numerous genes with infinitesimal contributions to the resistance phenotype, but also multiple species of *Diaporthe* responsible for canker in sunflower and different entry points of the fungus (leaf, stem, head), developing a diversity of diseases. Including more variables to the pathosystem, Guidini et al. (2023) identified 28 QTLs common to two *Diaporthe* species responsible for PSC: *D. Gulyae* and *D. helianthi*. Meanwhile, in Argentina numerous studies have been dedicated to characterizing the behavior of hybrids and genetically improved lines through natural or semi-natural infections with the additional input of infected crop residues (Corró Molas et al., 2016b; Troglia et al., 2022). Using the association mapping population from INTA, Filippi et al. (2022) identified 19 unique marker-trait associations for two manifestations of *Diaporthe*/*Phomopsis* diseases: PSC and PHR. This constitutes the first report on the identification of loci associated with different *Diaporthe* diseases.

Sclerotinia Head Rot

S. sclerotiorum is the causal agent of Sclerotinia head rot (SHR), a disease that is widespread in sunflower-growing areas around the world. Outbreaks of this disease lead to 10-20% of production loss under normal climate but can lead to total production loss under favorable conditions for the fungus (Pereyra and Escande, 1994). This organism, a necrotrophic ascomycete, is a serious threat to agriculture because it can infect more than 400 plant species, including economically important crops like soybean, oilseed rape, tomato, and weeds (Agrios, 2005). Over the past three decades, there has been a greater understanding of the behavior of hybrids and growing conditions, notwithstanding this, there is no sufficiently effective method for managing the disease. In Argentina, susceptible materials represent an average of 23% of the total commercial sunflower hybrids, whereas moderately susceptible and superior materials account for 51% and 23% of the evaluated samples in Balcarce (Buenos Aires), respectively. The variability observed across different seasons can be partially attributed to the genetic advancements achieved over the years. However, the most influential factors on disease incidence are primarily linked to predisposing environmental conditions (Moschini and Pereyra, 1998). Moschini et al. (2002) investigated the environmental factors associated with *S. sclerotiorum* ascosporic infection and created prediction models to forecast the occurrence of the disease. This tool helps identify high-risk locations, which is essential for disease management. This issue is especially important considering climate change and meteorological instability.

Filippi et al. (2017) also considered environmental effects to model several variables related to Sclertonia Head Rot (SHR) response in sunflower. The analysis of 137 inbred lines showed a significant effect of the inoculation date on disease incidence (DI), disease severity (DS), incubation period (IP), and area under the disease progress curve for DI and DS (AUDPCI, AUDPCS). The inclusion of the inoculation date effect in the model improved the accuracy of disease level estimations. As expected for complex quantitative characters, moderate to low heritability was observed in all cases.

The genetic basis of SHR resistance is known to be complex, with an infinitesimal contribution from multiple loci. Biparental and association mapping studies have been conducted since 2002, leading to the identification of multiple Quantitative Trait Loci (QTL). These QTLs typically cover large genomic regions, spanning multiple megabases and containing multiple genes (Filippi et al. 2014). Using a biparental mapping population derived from a cross between RHA266 (susceptible) and PAC2 (partially resistant), Zubrzycki et al. (2017) detected 36 main effect QTLs and 13 epistatic QTLs, with a significant enrichment of QTLs in linkage groups (LG) 1, 10 and 15.

Previous research has demonstrated that sunflower has extensive linkage disequilibrium (LD) (Filippi et al., 2020a; Mandel et al., 2013). Consequently, the AM strategy has emerged as the one of the preferred approaches in this species for the purpose of identifying genes implicated in quantitative resistance mechanisms. Fusari et al. (2012) conducted the first AM study reported for sunflower. Using a candidate gene approach on a population of 95 inbred lines from the INTA breeding program, these authors identified a gene associated with reduced SHR incidence (*HaRhoBP*). Based on a population of 135 inbred lines from INTA and a more extensive genotyping strategy using SNP panels and five phenotypic descriptors (i.e., DI, DS, IP, AUDPCI, and AUDPCS), Filippi et al. (2020b) found 13 polymorphisms associated with SHR resistance.

The availability of a sunflower reference genome (Badouin et al. 2017), along with the advancement of sequencing-based genotyping strategies, such as genotyping by sequencing or whole-genome resequencing, allowed association studies on molecular markers across the sunflower genome. In the same GWAS conducted for Diaporthe/Phomopsis, Pogoda et al. (2021) found 14 QTL related with SHR. Additionally, Filippi et al. (2022) discovered 24 SHR markers based on GWAS on the INTA AM population.

In addition, while no correlation has been reported between the response to different manifestations of *Sclerotinia* in sunflower (i.e., SHR and basal stem rot, BSR), independent AM studies have identified common genes (e.g., Filippi et al. 2020b; Talukder et al. 2014), paving the way for future integrated analyses of defense patterns in relation to both diseases.

Finally, different studies have also shown a mild correlation between the response to PHC and PSC in pre-breeding materials (Bert et al. 2002; Filippi et al. 2022; Pogoda et al. 2021; Talukder et al. 2020), suggesting a generalized defense mechanism against these necrotrophic pathogens. However, the search for regions simultaneously associated with both pathogens has not been conclusive, with only one marker found in combination, explaining a low percentage of resistance (Pogoda et al., 2021), or some markers showing epistatic association (Filippi et al., 2022).

The exploration of regions physically close to the associated markers has identified R genes belonging to the group of pattern recognition receptors (PPRs). These are proteins that recognize pathogen-associated molecules (PAMPs), of the "receptor-like kinase" class. A detailed analysis of these genes may help unravel the underlying mechanism of resistance to both pathogens in sunflower (Filippi et al., 2022).

As an alternative approach to characterizing the sunflower-*S.sclerotiorum* pathosystem, Fass et al. (2020) performed transcriptome profiling in three sunflower lines with different behavior against the fungus in early infection stages. The lines showed limited overlap in differentially expressed genes (DEGs) and functionally enriched categories, indicating a genotype-specific defense structure. However, a common feature of the three lines was the overrepresentation of lncRNAs. In addition, five DEGs were colocalized with QTL regions previously associated with SHR resistance (Filippi et al., 2020; Zubrzycki et al., 2017), further supporting their contribution to defense mechanisms.

Functional validation steps are required for the integration of QTLs or genes into breeding programs. The survey of mutants using effective genotyping techniques presents novel opportunities for confirming candidate genes and unraveling the biological mechanisms associated with resistance to SHR. Following a reverse genetics strategy, Fass et al. (2018) developed mutagenized sunflower populations and searched for candidate gene mutants by using CEL1 heteroduplex analysis (Fusari et al., 2010) and microfluidics-based multiplex PCR sequencing. Two of the identified mutants were tested in the field in an artificial inoculation experiment. Increased resistance to SHR was found in plants carrying a mutant allele of the candidate gene *HA18494*, a mitochondrial calcium uptake protein that was differentially expressed in early stages of infection (Fass et al., 2018).

Oomycete diseases

Sunflower downy mildew

Downy mildew (DM), caused by the oomycete *P. halstedii* (Farl.) Berl. & de Toni (Hall 1989), is a yield limiting disease occurring in most of the sunflower growing areas in the world (Spring and Otmar, 2019). The pathogen can infect over 80 species of Asteraceae, including cultivated, ornamental, and wild sunflowers (Gascuel et al. 2015). It is an obligate biotroph that can only survive and reproduce on a living host. It is a polycyclic organism, which produces many infection cycles during a growing season, as a combination of asexual and sexual reproduction events. The primary infection (zoospores from sporangia) produces the typical symptoms of DM: dwarfism, chlorotic mosaic around the leaf veins, sporulation in the underside of the leaves and leaf curling. At the end of the growing season, horizontal sunflower receptacles with vain, absent, or small achenes are observed. As a soil-borne disease, symptoms are observed as patches in the field (Figure 1).

DM in Argentina has been increasingly reported along the sunflower growing area where the main affected regions are in the South-East of Buenos Aires province, with prevalent cool and humid weather, and in the North of Santa Fe province, where early sowing dates may cause favorable low-temperature conditions for oospore germination. Martínez et al. (2021a) studied the presence of spores in seeds from asymptomatic (healthy) sunflowers near DM patches of Buenos Aires province through a molecular diagnosis based on the amplification of effector gene sequences of *P. halstedii*. They found that normal-sized seeds can be asymptomatic carriers of the pathogen, which represents a higher risk for disease spreading when the destination of the crop is hybrid seed production or when infected seeds contaminate field machinery or transport. The detection threshold was established in 0.1 picograms of infected seed DNA per microliter. This simple PCR- based method for *P. halstedii* detection is useful for growers and nurseries to determine the presence of the pathogen in seeds when symptoms are undetectable.

Martínez et al. (2019) established a methodology for studying and monitoring DM from distant collection sites. It includes *in situ* inoculation, fast desiccation of infected plant leaves using silica gel bags and a chemical treatment to remove fungicide from sunflower seeds. This approach facilitates the transportation and preservation of plant tissues, ensuring high-quality genomic DNA for molecular analysis and enabling further fungicide experiments on DM.

Since the first report of *P. halstedii* in USA a century ago, the pathogen has evolved in several countries where sunflower is cultivated. Molecular variability can be combined with race determination to interpret the processes of introduction and evolution in each region (Bán et al. 2021). Martínez et al. (2021b) and Martínez (personal comm.) performed a population study on *P. halstedii* isolates collected from the main sunflower growing regions of Argentina between 1997 and 2018 and included genetic data of *P. halstedii* from other countries retrieved from public databases. They used EST markers (Delmotte et al., 2008; Giresse et al., 2007), SSR (Rivera et al., 2016) and effector-based markers (Gascuel et al., 2016) to obtain diversity indexes and perform clustering analysis (Kitner et al. 2023). The expected heterozygosity values corresponded with a homothallic mode of reproduction, and the Argentinian population of *P. halstedii* was genetically different from populations from other countries. Moreover, the year of collection of the isolates was the most significant factor influencing the genetic variability levels of the pathogen.

Wild sunflowers of genus *Helianthus* have been the main source of genetic resistance for sunflower breeding programs worldwide (Qi et al., 2023). In Argentina, six populations of wild *H. annuus* of Buenos Aires, La Pampa, Entre Ríos, Mendoza and Córdoba provinces were screened for DM resistance against a local strain of the pathogen with promising results (Martínez et al., 2024). The study included phenotypic and histological aspects and the development of a novel disease scale and index for wild sunflower germplasm evaluations.

The evolution of *P. halstedii* in each region is highly influenced by management practices, where sunflower genetics and applied fungicides are most important. The disease can be efficiently controlled by vertical genes named Pl, that confer resistance to a limited number of races of *P. halstedii*. More than 20 Pl genes have been described and incorporated in sunflower breeding programs worldwide (Pecrix et al., 2018; Qi et al., 2019). However, the host-pathogen interaction influenced by the continuous use of a reduced number of *Pl* genes in a territory, can lead to the overcoming of these genes with consequent disease outbreaks by the emergence of novel races. At least 45 races of the pathogen were determined worldwide (Iwebor et al., 2018). This gene-overcoming process has been documented in many countries as France (Delmotte et al. 2012), United States (Gilley et al., 2016), Czech Republic (Drábková Trojanová et al., 2018), among others. In Argentina, the use of Pl genes is limited, and several disease outbreaks attributed to the breakdown of Pl genes and the development of fungicide tolerances were documented (Bazzalo et al. 2016; Erreguerena et al., 2013; Marcellán et al., 2008; Pereyra and Escande, 2002). The race spectrum of Argentina is believed to be small and stable, being the predominant races 300, 330, 710, 730 and 770 (Harveson et al., 2016). Nevertheless, several outbreaks in sunflower fields sown with resistant hybrids occurred in different regions, indicating the overcoming of a widely used *Pl* resistance gene in the country (Huguet, personal comm.). Through a multidisciplinary effort of the INTA, universities, seed companies, the Argentinian Sunflower Association (ASAGIR), and private pathologists, the current race spectrum of *P. halstedii* in Argentina was recently characterized from 1994 to 2022 (Martínez, personal comm.). Over ten races were found (Martínez et al., 2022), demonstrating the breakdown of three *Pl* genes. In the same context, studies on *P. halstedii* isolates from Buenos Aires, Santa Fe and Chaco provinces were tested for sensitivity to the main used oomyceticides (seed-coated) for DM control, metalaxyl-m (MX) and oxathiapiprolin (OXA). Results showed a significant increase in the development of resistance/tolerance to MX and established OXA as an efficient alternative to prevent primary infections (Cohen et al., 2019; Bannister et al., 2020; Di Giano et al., 2020; Erreguerena, personal comm.). An integrated disease management program of sunflower crop in Argentina should include the use of resistant hybrids, the systematic monitoring of races and oomyceticide resistant strains, the avoidance of early sowing dates, a molecular seed testing (Martínez et al., 2021a), the use of multisite fungicides and/or bio-fungicides (Trichoderma based) (Faberi et al., 2020) and the incorporation of Pl genes in sunflower breeding according to the race spectrum in the country. The combination of these management practices/strategies is crucial to reduce DM impact on maintaining the effectiveness of resistance genes and oomiceticides/fungicides over an extended period towards crop sustainability.

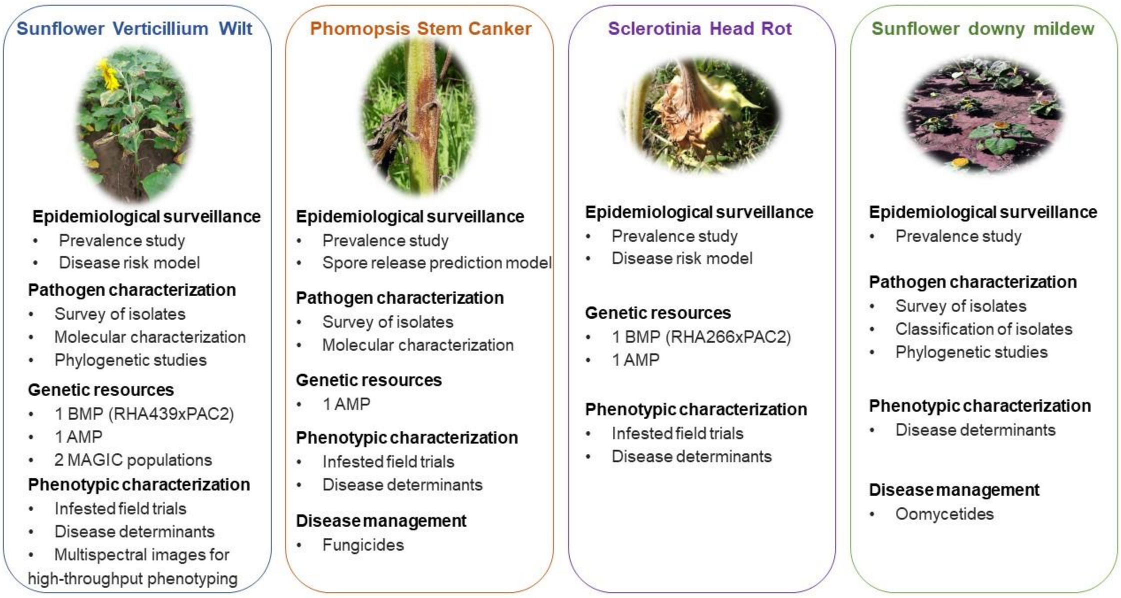

Figure 1. Strategies applied in INTA/Argentina to limit the losses caused by the four main sunflower diseases: Epidemiological surveillance, Characterization of pathogens, Genetic resources used to evaluate and find sources of resistance, Phenotypic characterization of genetic resources, Development of complementary practices to genetic resistance.

CONCLUSIONS

In recent years, crop production stability has been threatened by increasing occurrence of plant diseases. This phenomenon can be attributed to factors such as monocultures and extensive trade in plants, as well as weather instabilities that lead to the emergence of new strains and changes in the virulence of pathogens (Corredor-Moreno and Saunders, 2020; Singh et al., 2023). This fact and the demand for sustainable crop production and environmentally friendly crop protection have led to the development of various approaches to better understand plant pathosystems holistically (Demirjian et al., 2023).

In this article we present a comprehensive overview of the investigations conducted on the resistance of sunflower to diseases and the more prevalent pathogens affecting the crop (Figure 1). It is noteworthy that there is a limited overlap in the genomic regions associated with disease resistance across different research groups globally. This fact is poorly explained by the genetic differentiation among the circulating germplasm of cultivated sunflower since it was reported to be low (Filippi et al., 2020a). The diverse genotyping panels or strategies used to characterize the genome do not contribute to clarify this diversity either, considering that the genomic positions of the analyzed regions are solely based on the two reference genomes available for this specific species (Badouin et al., 2017). A significant finding from the work reviewed here is the substantial variability reported among pathogen populations in different sunflower-growing regions. Considering recent research on other pathosystems, this observation partially explains the absence of shared QTL in the studies. Briefly, a study of the *Arabidopsis thaliana-Xanthomonas arboricola* pathosystem using joint association analysis (co-GWAS) revealed specific strain resistance QTLs with strong allelic effects and generalist resistance QTLs with minor allelic effects. This study revealed that a greater proportion of the variation in quantitative disease resistance was due to genetic diversity among strains of *X. arboric*ola than to the polygenic effects of *A. thaliana* (Wang et al., 2018). Other published works on plant and animal pathosystems confirm this hypothesis (Hassan et al., 2020; Zhang et al., 2021). This emphasizes the importance of finding QTLs linked to inherent variability in pathogenicity. Simultaneously, alternative methodologies based on building and analyzing gene expression networks, as described by Ribone et al. (2023), contribute to the establishment of a comprehensive perspective on the plant's response to stressors. Finally, to obtain a comprehensive representation of the interaction between host and pathogen, it becomes crucial to study the microbiota and the pathobiota (Teixeira et al., 2019; Vannier et al., 2019), along with the interaction with environmental stressors.

ACKNOWLEDGEMENT

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

The authors state no conflict of interest.

REFERENCES

Agrios, G. (2005). Plant Pathology, 5th ed. Burlington, MA: Elsevier Academic Press.

Aguilera, P., Ben Guerrero, E., Montecchia, J.F., Troglia, C., Colombo, D., Corro Molas, A., Deperi, S., Erreguerena, I., Lia, V., Martínez, M.C. et al. (2023). Análisis de diversidad y estructura genética de aislamientos de *Verticillium dahliae* de girasol (*Helianthus annus*) de Argentina In: 2do Simposio de Mejoramiento Genético Vegetal. Córdoba, Argentina.

Aguirre, N.C., Filippi, C.V., Zaina, G., Rivas, J.G., Acuña, C.V., Villalba, P.V., García, M.N., González, S., Rivarola, M., Martínez, M.C., et al. (2019). Optimizing DDRADseq in non-model species: A Case Study in *Eucalyptus dunnii* Maiden. Agronomy 9: 484.

Atallah, Z.K., Maruthachalam, K., Toit, L. du, Koike, S.T., Michael Davis, R., Klosterman, S.J., Hayes, R.J. and Subbarao, K. V. (2010). Population analyses of the vascular plant pathogen *Verticillium dahliae* detect recombination and transcontinental gene flow. Fungal genetics and biology : FG & B 47: 416–422.

Badouin, H., Gouzy, J., Grassa, C.J., Murat, F., Staton, S.E., Cottret, L., Lelandais-brière, C., Owens, G.L., Carrère, S., Mayjonade, B., et al. (2017). The sunflower genome provides insights into oil metabolism, flowering and Asterid evolution. Nature Publishing Group 546: 148–152.

Bán, R.; Kovács, A.; Nisha, N.; Pálinkás, Z.; Zalai, M.; Yousif, A.I.A.; Körösi, K. (2021): New and High Virulent Pathotypes of Sunflower DownyMildew (*Plasmopara halstedii*) in Seven Countries in Europe. J. Fungi 2021, 7, 549.

Bannister, J., Quiroz, F., Erreguerena, I., Di Giano, F., Petruccelli, M. and Faberi, A.J. (2020). Confirmación de perdida de sensibilidad de Plasmopara halstedii a metalaxil-m en las regiones productoras de girasol de Argentina In: 1er Congreso Argentino de Semillas.

Bautista-Jalón, L.S., Frenkel, O., Tsror, L., Malcolm, G.M., Gugino, B.K., Lebiush, S., Hazanovsky, M., Milgroom, M.G. and Del Mar Jiménez-Gasco, M. (2021). Genetic differentiation of verticillium dahliae populations recovered from symptomatic and asymptomatic hosts. Phytopathology 111: 149–159.

Bazzalo, E., Huguet, N., Romano, C., Bock, F., Piubello, S., Quiroz, F. and Bertero, A. (2016). History and present state of *Downy mildew* in Argentina In: Proc. 19th Int. Sunflower Conf. Edirne, Turkey, 828–832.

Berbegal, M., Garzón, C.D., Ortega, A., Armengol, J., Jiménez-Díaz, R.M. and Jiménez-Gasco, M.M. (2011). Development and application of new molecular markers for analysis of genetic diversity in *Verticillium dahliae* populations. Plant Pathology 60: 866–877.

Bert, P.F., Jouan, I., de Labrouhe, T.D., Serre, F., Nicolas, P. and Vear, F. (2002). Comparative genetic analysis of quantitative traits in sunflower (*Helianthus annuus* L.) 1. QTL involved in resistance to *Sclerotinia sclerotiorum* and *Diaporthe helianthi*. Theoretical and Applied Genetics 105: 985–993.

Bertero de Romano, A.B. and Vázquez, A. (1982). A new race of *Verticillium dahliae* Kleb In: Proceedings of the 10th International Sunflower Conference. Toowoomba, QLD, Australia, pp.177–178.

Besnard, G., Griveau, Y., Quillet, M.C., Serieys, H., Lambert, P., Vares, D. and Bervillé, A. (1997). Specifying the introgressed regions from *H. argophyllus* in cultivated sunflower (*Helianthus annuus* L.) to mark Phomopsis resistance genes. Theoretical and Applied Genetics. 94: 131–138.

Cáceres, C., Castaño, F., Rodríguez, R., Ridao, A., Salaberry, T., Echeverría, M. and Colabelli, M. (2007). Phomopsis resistance on leaves and stems of helianthus petiolaris. Helia 30: 213–218.

Clemente, G.E., Bazzalo, M.E. and Escande, A.R. (2017). New Variants of *Verticillium Dahliae* Causing Sunflower Leaf Mottle and Wilt in Argentina. Journal of Plant Pathology 99: 445–451.

Cohen Y, Rubin AE, Galperin M (2019) Novel synergistic fungicidal mixtures of oxathiapiprolin protect sunflower seeds from downy mildew caused by Plasmopara halstedii. PLoS ONE 14(9): e0222827

Collado-Romero, M., Mercado-Blanco, J., Olivares-García, C. and Jiménez-Díaz, R.M. (2008). Phylogenetic Analysis of *Verticillium dahliae* Vegetative Compatibility Groups. Phytopathology 98: 1019–1028.

Colombo, D.N. and Corró Molas, A. (2022). Relevamiento del cancro del tallo y la pudrición seca de capítulo del girasol causadas por el complejo Diaporthe In: XVII Jornadas Fitosanitarias. Pergamino, Argentina.

Colombo, D.N., Corró Molas, A., Paniego, N.B. and Comerio, R.M. (2023). First report of *Diaporthe ambigua* causing Phomopsis stem canker on sunflower in Argentina. New Disease Reports 48: e12204.

Colombo, D.N., Figueruelo, A., Paniego, N.B., Comerio, R.M., & Corró Molas, A. (2024). Phomopsis Head Rot caused by Diaporthe helianthi: A New Disease on Sunflower in Argentina. Journal of Phytopathology, 172(5), e13416.

Corredor-Moreno, P. and Saunders, D.G.O. (2020). Expecting the unexpected: factors influencing the emergence of fungal and oomycete plant pathogens. New Phytologist 225: 118–125.

Corró Molas, A., Comerio, R., Figueruelo, A., Ghironi, E., Sanchez, E., Bertero, A. and Pérez Fernández, J. (2016a). Epiphytotic disease of sunflower stem canker in Argentina In: 19th international Sunflower Conference. Edirne, Turkey, pp.825–827.

Corró Molas, A., Ghironi, E., Sanchez, E. and Gareis, E. (2016b). Comportamiento sanitario frente al cancro del tallo del girasol. Revista del Colegio de Ingenieros Agrónomos de La Pampa: 16–20.

Corró Molas, A., Edwards Molina, J., Therisod, G., Colombo, D., Martínez, M.I., Bilbao, A., Bertero, A. and Moschini, R.C. (2021). Riesgo climático de la región pampeana respecto a la liberación de ascosporas de *Diaporthe helianthi*. Technical Report, AER General Pico, INTA.

Corró Molas, A., Colombo, D., Martínez, M.I. and Moschini, R.C. (2023). Evolución diaria de los valores predichos probabilísticos de liberaciones severas de ascosporas de Diaporthe helianthi en sitios de la Región Pampeana, en la campaña 2022/2023. Technical Report, AER General Pico, INTA.

Creus, C., Bazzalo, M.E., Grondona, M., Andrade, F. and León, A.J. (2007). Disease expression and ecophysiological yield components in sunflower isohybrids with and without *Verticillium dahliae* resistance. Crop Science 47: 703–710.

Dangal, N.K., Rekabdarkolaee, H.M., Markell, S.G., Harveson, R.M. and Mathew, F.M. (2023). Foliar Fungicides Containing FRAC 11 Mitigate Phomopsis Stem Canker in Sunflower (*Helianthus annuus*). Plant disease 107: 743–749.

Debaeke, P., Casadebaig, P. and Langlade, N.B. (2021). New challenges for sunflower ideotyping in changing environments and more ecological cropping systems. OCL. 28: 29.

Debaeke, P., Estragnat, A. and Reau, R. (2003). Influence of crop management on sunflower stem canker (*Diaporthe helianthi*). Agronomie 23: 581–592.

Delmotte, F., Giresse, X., Richard-Cervera, S., Vear, F., Tourvieille, J., Walser, P. and de Labrouhe, D.T. (2008). EST-derived markers highlight genetic relationships among *Plasmopara halstedii* French races *In*: 17th International Sunflower Conference. Córdoba, Spain.

Delmotte, F., Tourvieille de Labrouhe, D. and Ahmed, S. (2012). Emerging races arising from recombination facilitated by multiple introductions of the sunflower downy mildew *Plasmopara halstedii* in France In: 18th International Sunflower Conference., 01-VC–6.

Delos, M., Moinard, J. and Debaeke, P. (2000). ASPHODEL : modèle de simulation des épidémies de phomopsis du tournesol (*Diaporthe helianthi*) In: 15. Conférence Internationale sur le tournesol. Toulouse, France.

Demirjian, C., Vailleau, F., Berthomé, R. and Roux, F. (2023). Genome-wide association studies in plant pathosystems: success or failure? Trends in plant science 28: 471–485.

Di Giano, F., Quiroz, F., Erreguerena, I., Bannister, J., Petruccelli, M. and Faberi, A.J. (2020). Sensibilidad de base de Plasmopara halstedii a oxathiapiprolin en el cultivo de girasol In: 1er Congreso Argentino de Semillas.

Domínguez, M., Montecchia, J.F., Nicosia, S., Fernández, P., Troglia, C., González, J. and Paniego, N. (2022). Implementación de herramientas de fenotipado de alto rendimiento para evaluar el Marchitamiento por *Verticillum dahliae* en girasol. Revista de Tecnología Agropecuaria 10: 39–43.

Drábková Trojanová, Z., Sedlářová, M., Pospíchalová, R. and Lebeda, A. (2018). Pathogenic variability of *Plasmopara halstedii* infecting sunflower in the Czech Republic. Plant Pathology 67: 136–144.

Dung, J.K.S., Peever, T.L. and Johnson, D.A. (2013). Verticillium dahliae Populations from Mint and Potato Are Genetically Divergent with Predominant Haplotypes. Phytopathology 103: 445–459.

Erreguerena, I. (2013). *Relación inóculo de Verticilium dahliae con la marchitez del girasol en suelos de las provincias de Buenos aires y la Pampa (Argentina) y su asociación con características edafo-biológicas*. Master’s Thesis. Universidad Nacional de Mar del Plata.

Erreguerena, I.A., Mantecón, J. and Quiroz, F. (2013). First report of a Plasmopara halstedii isolate tolerant to metalaxyl-M in Argentina In: XXII Congreso Peruano y XVII Congreso Latinoamericano de Fitopatología [Libro de Resúmenes]. Lambayeque, Peru, 102–103.

Erreguerena, I.A., Rojo, R., Quiroz, F.J., Clemente, G.E. and Escande, A.R. (2019). Relationship between *Verticillium dahliae* inoculum and sunflower wilt in Argentina. Canadian Journal of Plant Pathology 41: 576-584

Faberi, A.J., Montoya, M.R.., Faura, A., Erreguerena, I. and Quiroz, F. (2020). *Trichoderma harzianum* como potencial agente de control de *Plasmopara halstedii* en girasol In: 1er Congreso Argentino de Semillas.

Fálico de Alcaraz, L., Visintin, G. and Alcaraz Fálico, M.E. (1994). El cancro del tallo del girasol *Diaporthe helianthi* Munt. Cvet. Ciencia, Docencia y Tecnología 8: 19–26.

Fass, M.I., Gonzalez, S., Vera, P., Puebla, A., Di Rienzo, J., Alvarez, D., Landau, A., Nicosia, S., Hopp, E., Heinz, R., et al. (2018). Development of mutagenized sunflower populations: discovery and characterization of mutants for Sclerotinia Head Rot resistance candidate genes. In: International Symposium on Sunflower and Climte Change. Toulouse, Francia.

Fass, M.I., Rivarola, M., Ehrenbolger, G.F., Maringolo, C.A., Montecchia, J.F., Quiroz, F., García-García, F., Blázquez, J.D., Hopp, H.E., Heinz, R.A., et al. (2020). Exploring sunflower responses to Sclerotinia head rot at early stages of infection using RNA-seq analysis. Scientific Reports 10: 1–14.

Fick, G.N. and Zimmer, D.E. (1974). Monogenic Resistance to Verticillium Wilt in Sunflowers 1. Crop Science 14: 895-896.

Filippi, C., Zubrzycki, J., Lía, V., Heinz, R.A., Paniego, N.B. and Hopp, H.E. (2014). Genetics and Genomics Applied to Sunflower Breeding In: Sunflowers: Growth and Development, Environmental Influences and Pests/Diseases. Nova Science Publisher, pp.61–94.

Filippi, C. V., Zubrzycki, J.E., Di Rienzo, J.A., Quiroz, F., Fusari, C.M., Alvarez, D., Maringolo, C.A., Cordes, D., Escande, A., Hopp, H.E., et al. (2017). Phenotyping Sunflower Genetic Resources for Sclerotinia Head Rot Response: Assessing Variability for Disease Resistance Breeding. Plant Disease 101: 1941–1948.

Filippi, Carla V., Merino, G.A., Montecchia, J.F., Aguirre, N.C., Rivarola, M., Naamati, G., Fass, M.I., Álvarez, D., Di Rienzo, J., Heinz, R.A., et al. (2020a). Genetic diversity, population structure and linkage disequilibrium assessment among international sunflower breeding collections. Genes 11: 283.

Filippi, C. V., Zubrzycki, J.E., Di Rienzo, J.A., Quiroz, F.J., Puebla, A.F., Alvarez, D., Maringolo, C.A., Escande, A.R., Hopp, H.E., Heinz, R.A., et al. (2020b). Unveiling the genetic basis of Sclerotinia head rot resistance in sunflower. BMC Plant Biology 20: 322.

Filippi, C.V., Corro Molas, A., Dominguez, M., Colombo, D., Heinz, N., Troglia, C., Maringolo, C., Quiroz, F., Alvarez, D., Lia, V., et al. (2022). Genome-Wide Association Studies in Sunflower: Towards *Sclerotinia sclerotiorum* and *Diaporthe*/*Phomopsis* Resistance Breeding. Genes 13: 2357.

Fradin, E.F., Zhang, Z., Juarez Ayala, J.C., Castroverde, C.D.M., Nazar, R.N., Robb, J., Liu, C.-M. and Thomma, B.P.H.J. (2009). Genetic Dissection of Verticillium Wilt Resistance Mediated by Tomato Ve1. Plant Physiology 150: 320–332.

Fradin, E.F., Abd-El-Haliem, A., Masini, L., van den Berg, G.C.M., Joosten, M.H.A.J. and Thomma, B.P.H.J. (2011). Interfamily Transfer of Tomato Ve1 Mediates Verticillium Resistance in Arabidopsis. Plant Physiology 156: 2255–2265.

Fusari, C.M., Lia, V. V., Nishinakamasu, V., Zubrzycki, J.E., Puebla, A.F., Maligne, A.E., Hopp, H.E., Heinz, R.A., Paniego, N.B. (2010). Single nucleotide polymorphism genotyping by heteroduplex analysis in sunflower (*Helianthus annuus* L.). Molecular Breeding 28: 73–89.

Fusari, C.M., Di Rienzo, J.A., Troglia, C., Nishinakamasu, V., Moreno, M., Maringolo, C., Quiroz, F., Álvarez, D., Escande, A., Hopp, E., et al. (2012). Association mapping in sunflower for sclerotinia head rot resistance. BMC Plant Biology 12: 93.

Galella, M.T., Bazzalo, M.E. and León, A. (2004). Compared pathogenicity of *Verticillium dahliae* isolates from Argentine and the USA. 16th International Sunflower Conference. (7620), pp.177-180.

Galella, M.T., Bazzalo, M.E., Morata, M., Cimmino, C., Kaspar, M., Grondona, M., Reid, R., Zambelli, A. and León, A. (2012). Pyramiding QTLs for *Verticillium dahliae* resistance In: Proceedings of the 18th International Sunflower Conference. Mar del Plata, Argentina, pp.77–82.

Gascuel, Q., Martinez, Y., Boniface, M.C., Vear, F., Pichon, M. and Godiard, L. (2015). The sunflower downy mildew pathogen *Plasmopara halstedii.* Molecular Plant Pathology 16: 109–122.

Gascuel, Q., Bordat, A., Sallet, E., Pouilly, N., Carrere, S., Roux, F., Vincourt, P. and Godiard, L. (2016). Effector Polymorphisms of the Sunflower Downy Mildew Pathogen *Plasmopara halstedii* and Their Use to Identify Pathotypes from Field Isolates. PLOS ONE 11: p.e0148513.

Ghironi, E., Corró Molas, A., Sanchez, E. and Gareis, E. (2018). Variaciones en las pérdidas de rendimiento y aceite por cancro del tallo del girasol en la región semiárida pampeana central In: Libro de resúmenes 5° Congreso Argentino de Fitopatología. Tucumán, Argentina.

Gilley, M.A., Misar, C., Gulya, T.J. and Markell, S.G. (2016). Prevalence and virulence of *Plasmopara halstedii* (downy mildew) in sunflowers *In*: Proceedings of the 38th Sunflower Research Forum. Fargo, ND, USA.

Giresse, X., Tourvieille De Labrouhe, D., Richard-Cervera, S. and Delmotte, F. (2007). Twelve polymorphic expressed sequence tags-derived markers for *Plasmopara halstedii*, the causal agent of sunflower downy mildew Molecular Ecology Notes 7: 1363–1365.

Guidini, R., Jahani, M., Huang, K., Rieseberg, L. and Mathew, F.M. (2023). Genome-Wide Association Mapping in Sunflower (*Helianthus annuus*) Reveals Common Loci and Putative Candidate Genes for Resistance to *Diaporthe gulyae* and *D. helianthi* Causing Phomopsis Stem Canker. Plant disease 107: 667–674.

Gulya, T. (2007). New strain of V*erticullium dahliae* in North America. Helia 30: 115–120.

Gulya, T., Rashid, K.Y. and Masirevic, S.M. (1997). Sunflower Diseases *In*: A. A. Schneiter, ed. *Sunflower Technology and Production*, pp.263–379.

Guo, S., Zuo, Y., Zhang, Y., Wu, C., Su, W., Jin, W., Yu, H., An, Y. and Li, Q. (2017). Large-scale transcriptome comparison of sunflower genes responsive to *Verticillium dahliae.* BMC genomics 18: 42.

Gurung, S., Short, D.P.G., Atallah, Z.K. and Subbarao, K. V. 2014. Clonal Expansion of *Verticillium dahliae* in Lettuce. Phytopathology 104: 641–649.

Hall, G. (1989). CMI Descriptions of Pathogenic Fungi and Bacteria, Set 98, Nos. 971-980. Mycopathologia 106: 183–211.

Harveson, R. and Markell, S. (2016). Verticillium wilt *In*: R. Harveson, S. Markell, C. Block and T. Gulya, eds. Compendium of sunflower diseases and pests. St Paul: The American Phytopathological Society.

Harveson, R.M., Markell, S.G., Block, C.C. and Gulya, T.J. (2016). Part I: Biotic Diseases In: Compendium of Sunflower Diseases and Pests. St. Paul, MN, USA: The American Phytopathological Society, pp.59–61.

Hassan, A.O., Case, J.B., Winkler, E.S., Thackray, L.B., Kafai, N.M., Bailey, A.L., McCune, B.T., Fox, J.M., Chen, R.E., Alsoussi, W.B., et al. (2020). A SARS-CoV-2 Infection Model in Mice Demonstrates Protection by Neutralizing Antibodies. Cell 182: 744-753.e4.

Huguet, N.I. (2006). Ocurrence of *Phomopsis helianthi* in Argentina and Uruguay. Helia 29: 121–126.

Iwebor, M., Antonova, T. and Saukova, S. (2018). Occurrence and distribution of races 713, 733 and 734 of sunflower downy mildew pathogen in the Russian federation. Helia 41: 141–151.

Jimenez-Diaz, R.M., Cirulli, M., Bubici, G., del Mar Jimenez-Gasco, M., Antoniou, P.P. and Tjamos, E.C. (2012). Verticillium Wilt, A Major Threat to Olive Production: Current Status and Future Prospects for its Management. Plant disease 96: 304–329.

Jiménez-Gasco, M.D.M., Malcolm, G.M., Berbegal, M., Armengol, J. and Jiménez-Díaz, R.M. (2014). Complex molecular relationship between vegetative compatibility groups (VCGs) in *Verticillium dahliae*: VCGs do not always align with clonal lineages. Phytopathology 104: 650–659.

Kashyap, R., Markell, S.G., Harveson, R.M., Rekabdarkolaee, H.M. and Mathew, F.M. (2023). Application of Pyraclostrobin Fungicide at Miniature Floral Head Development Growth Stage Is Efficacious Against Phomopsis Stem Canker Fungi in Sunflower (*Helianthus annuus*). Plant Health Progress 24: 24–31.

Kawchuk, L.M., Hachey, J., Lynch, D.R., Kulcsar, F., Van Rooijen, G., Waterer, D.R., Robertson, A., Kokko, E., Byers, R., Howard, R.J., et al. (2001). Tomato Ve disease resistance genes encode cell surface-like receptors. Proceedings of the National Academy of Sciences of the United States of America 98: 6511–6515.

Kitner, M., Thines, M., Sedlářová, M., Vaculná, L., Bán, R. & Körösi, K. et al. (2023) Genetic structure of *Plasmopara halstedii* populations across Europe and South Russia. Plant Pathology, 72, 361– 375.

Mandel, J.R., Nambeesan, S., Bowers, J.E., Marek, L.F., Ebert, D., Rieseberg, L.H., Knapp, S.J. and Burke, J.M. (2013). Association Mapping and the Genomic Consequences of Selection in Sunflower. PLOS Genetics 9: p.e1003378.

Marcellán, O., Escande, A., Quiroz, F., Alvarez, D. and Pereyra, V. (2008). Mildiu del girasol: análisis preliminar de la variabilidad de las fuentes de resistencia. Revista de la Facultad de Agronomía 107: 11–14.

Martín-Sanz, A., Rueda, S., García-Carneros, A.B., González-Fernández, S., Miranda-Fuentes, P., Castuera-Santacruz, S. and Molinero-Ruiz L. (2018). Genetics , Host Range , and Molecular and

Pathogenic Characterization of *Verticillium dahliae* From Sunflower Reveal Two Differentiated Groups in Europe. Front. Plant Sci. 9: 1–13.

Martínez, A.L., Anderson, F., Garayalde, A., Sabatini, P., Presotto, A., Hernandez, F., Pandolfo, C., Ureta, S. and Carrera, A. (2023). Genetic resistance of *Helianthus annuus* populations naturalized in Argentina to *Plasmopara halstedii,* the causal agent of sunflower downy mildew. In: LI Argentine Congress of Genetics. Córdoba, Argentina.

Martínez, A.L., Anderson, F., Quiroz, F., Garayalde, A., Erreguerena, I., Armando, L., Huguet, N. and Carrera, A. (2019). Methodologies for *Plasmopara halstedii* Research. Helia 42: 173–186.

Martínez, A.L., Quiroz, F.J. and Carrera, A.D. (2021a). Detection of *Plasmopara halstedii* in sunflower seeds: A case study using molecular testing. Journal of the Saudi Society of Agricultural Sciences 20: 395–400.

Martínez, A.L., Garayalde, A., Petruccelli, M., Erreguerena, I., Quiroz, F. and Carrera, A. (2021b). Genetic characterization of *Plasmopara halstedii* populations in Argentina using simple sequence repeats (SSR) and effector-based markers. *In*: *Phytopathology*. Iowa, USA: The American Phytopatological Society (APS).

Martínez, A.L., Bazzalo, M.E., Huguet, N., Bertero, A., Erreguerena, I., Faberi, A., Petrucelli, M., Bannister, J., Di Giano, F., Della Maddalena, M., et al. (2022). Races and oomyceticide tolerances of *Plasmopara halstedii* in Argentina In: 20th International Sunfower Conference. Novi Sad, Serbia.

Maširević, S., Thompson, S.M., Gulya, T.J. and Markell, S.G. (2016). Phomopsis stem canker: *Diaporthe*/*Phomopsis* spp Complex. *In*: R. M. Harveson, S. G. Markell, C. C. Block and T. J. Gulya, eds. Compendium of sunflower diseases and pests. St. Paul, MN, USA: The American Phytopathological Society, pp.38–40.

Mestries, E. (2017). Maladies du tournesol: Le Verticillium, très presént dans le soud-ouest. Perspectives Agricoles *N°444*.

Mihaljcevic, M., Petrov, M. and Cvetkovic, M.M. (1980). Phomopsis sp. novi parazit suncokretau Jugoslaviji. (Phomopsis sp., a new sunflower parasite in Yugoslavia.). Savremena Poljoprivreda 28: 531–539.

Milgroom, M.G., Jiménez-Gasco, M.D.M., Olivares-García, C., Drott, M.T. and Jiménez-Ďiaz, R.M. (2014). Recombination between clonal lineages of the asexual fungus *Verticillium dahliae* detected by genotyping by sequencing. PloS one 9.

Molinero-Ruiz, L. (2019): Recent advances on the characterization and control of sunflower soilborne pathogens under climate change conditions. OCL 26: 2.

Montecchia, J. (2019). Identificación y caracterización de fuentes de resistencia genética a la Marchitez Anticipada causada por Verticillium dahliae en Girasol, PhD Thesis. Universidad de Buenos Aires.

Montecchia, J., Clemente, G., Quiroz, F., Paniego, N. and Heinz, R. (2017). Evaluación de respuestas de líneas endocriadas de girasol a la marchitez causada por variantes locales y foráneas del hongo *Verticillium dahliae.* In: 4th Argentinean Congress of Phytopathology. Mendoza, Argentina.

Montecchia, J.F., Fass, M.I., Cerrudo, I., Quiroz, F.J., Nicosia, S., Maringolo, C.A., Di Rienzo, J., Troglia, C., Hopp, H.E., Escande, A., et al. (2021). On-field phenotypic evaluation of sunflower populations for broad-spectrum resistance to Verticillium leaf mottle and wilt. Sci. Rep. 11: 11644.

Moschini, R. and Pereyra, V. (1998). Análisis de la componente ambiental y genética para explicar la variación de la incidencia de la podredumbre blanda del capitulo de girasol In: III Reunión Nacional de Oleaginosas., pp.43–44.

Moschini, R.C., Rodríguez, M.J., Martínez, M.I. and Stewart, S. (2019). Weather-based predictive models for *Diaporthe helianthi* ascospore release in Uruguay. Australasian Plant Pathology 48: 519–527.

Moschini, R., Escande, A., Pereyra, V., Pedraza, V., Quiroz, F., Troglia, C. and Iriarte, L. (2002). Predicción de la incidencia de la podredumbre blanda del capítulo de girasol basada en factores ambientales y genéticos. Rev. Arg. De Agrometeorología 2: 51–57.

Pecrix, Y., Penouilh-Suzette, C., Muños, S., Vear, F. and Godiard, L. (2018). Ten broad spectrum resistances to downy mildew physically mapped on the sunflower genome. Frontiers in Plant Science 871: 414483.

Pereyra, V., Quiroz, F., Agüero, M. and Escande, A. (1999). Relación del rendimiento de girasol con la intensidad de síntomas provocados por *V. dahliae In*: *Proceedings of the X Jornadas Fitosanitarias Argentinas*. Jujuy, Argentina, p.35.

Pereyra, V.R. and Escande, A. (1994). Enfermedades del girasol en la Argentina : manual de reconocimiento. Instituto Nacional de Tecnología Agropecuaria.

Pereyra, V. and Escande, A. (2002). Mildiu o Enanismo del Girasol: Nuevos Problemas. Instituto Nacional de Tecnología Agropecuaria.

Pogoda, C.S., Reinert, S., Talukder, Z.I., Attia, Z., Collier-zans, E.C.E., Gulya, T.J., Kane, N.C. and Hulke, B.S. (2021). Genetic loci underlying quantitative resistance to necrotrophic pathogens Sclerotinia and Diaporthe (Phomopsis), and correlated resistance to both pathogens. Theoretical and Applied Genetics 134: 249–259.

Qi, L.L., Ma, G.J., Li, X.H. et al. (2019). Diversification of the downy mildew resistance gene pool by introgression of a new gene, Pl35, from wild *Helianthus argophyllus* into oilseed and confection sunflowers (*Helianthus annuus* L.). Theor Appl Genet 132, 2553–2565.

Qi, L.L., Ma, G.J. and Seiler, G.J. (2023). Registration of HA-DM9, HA-DM10, and HA-DM11 oilseed sunflower germplasms with dual resistance to sunflower downy mildew. Journal of Plant Registrations 17: 426–434.

Quiroz, F., Erreguerena, I., Clemente, G. and Escande, A. (2012). Impacto de la marchitez por *Verticillium dahliae* sobre el rendimiento de girasol In: XIV Jornadas Fitosanitarias Argentinas. UNSL – FICES., P 165.

ReTSaVe (Spanish acronym for Territorial Network for Plant Pathology Surveying) (2020). INTA ReTSaVe. Red Territorial de Sanidad Vegetal. Available at: https://retsave.com.ar/.

Ribone, A.I., Fass, M., Gonzalez, S., Lia, V., Paniego, N. and Rivarola, M. (2023). Co-Expression Networks in Sunflower: Harnessing the Power of Multi-Study Transcriptomic Public Data to Identify and Categorize Candidate Genes for Fungal Resistance. Plants. 12: 2767.

Ridao, A. (1994). Quebrado de tallo y otras afecciones provocadas por *Diaporthe helianthi* (Phomopsis helianthi Munt.) *In*: V. Pereyra and A. Escande, eds. Enfermedades del girasol en la Argentina, Manual de reconocimiento., 61–66.

Rivera, Y., Salgado-Salazar, C., Gulya, T.J. and Crouch, J.A. (2016). Newly emerged populations of *Plasmopara halstedii* infecting *Rudbeckia* exhibit unique genotypic profiles and are distinct from sunflower-infecting strains. Phytopathology 106: 752–761.

Rojo, R., Quiroz, F.J. and Escande, A. (2008). Effect of *Verticillium dahliae* microesclerotium levels in soil on Veticillium wilt of sunflower in Argentina. Journal of Plant Pathology 90(Suppl.2): 371–372.

Sackston, W.E. (1992). On a Treadmill: Breeding Sunflowers for Resistance to Disease. Annual Review of Phytopathology 30: 529–551.

Short, D.P.G., Gurung, S., Maruthachalam, K., Atallah, Z.K. and Subbarao, K. V. (2014). *Verticillium dahliae* race 2-Specific pcr reveals a high frequency of race 2 strains in commercial spinach seed lots and delineates race structure. Phytopathology 104: 779-85.

Singh, B.K., Delgado-Baquerizo, M., Egidi, E., Guirado, E., Leach, J.E., Liu, H. and Trivedi, P. (2023). Climate change impacts on plant pathogens, food security and paths forward. Nature Reviews Microbiology 21: 640–656.

Spring and Otmar (2019). Spreading and global pathogenic diversity of sunflower downy mildew - Review. 55: 149–158.

Talukder, Z.I., Hulke, B.S., Qi, L., Scheffler, B.E., Pegadaraju, V., McPhee, K. and Gulya, T.J. (2014). Candidate gene association mapping of Sclerotinia stalk rot resistance in sunflower (*Helianthus annuus* L.) uncovers the importance of COI1 homologs. Theoretical and Applied Genetics 127: 193–209.

Talukder, Z.I., Underwood, W., Ma, G., Seiler, G.J., Misar, C.G., Cai, X. and Qi, L. (2020). Genetic Dissection of Phomopsis Stem Canker Resistance in Cultivated Sunflower Using High Density SNP Linkage Map. Int. J. Mol. Sci. 21: 1497.

Teixeira, P.J.P., Colaianni, N.R., Fitzpatrick, C.R. and Dangl, J.L. (2019). Beyond pathogens: microbiota interactions with the plant immune system. Current opinion in microbiology 49: 7–17.

Thompson, S.M., Neate, S.M., Gulya, T.J. and Aitken, E.A.B. (2023). *Diaporthe gulyae* colonizes seeds of sunflower (*Helianthus annuus*) systematically through leaf, petiole and stem infection. European Journal of Plant Pathology 167: 41–58.

Thompson, S.M., Tan, Y.P., Shivas, R.G., Neate, S.M., Morin, L., Bissett, A. and Aitken, E.A.B. (2015). Green and brown bridges between weeds and crops reveal novel Diaporthe species in Australia. Persoonia : Molecular Phylogeny and Evolution of Fungi 35: 39.

Troglia, C., Corró Molas, A.E., Ghironi, E. and Astiz, V. (2022). Comportamiento de híbridos de girasol frente al Cancro del tallo y podredumbre del capítulo de girasol por Diaporthe sp In: XVII Jornadas Fitosanitarias. Pergamino, Argentina.

Usami, T., Itoh, M. and Amemiya, Y. (2009). Asexual fungus *Verticillium dahliae* is potentially heterothallic. Journal of General Plant Pathology 75: 422–427.

Vannier, N., Agler, M. and Hacquard, S. (2019). Microbiota-mediated disease resistance in plants. PLOS Pathogens 15: e1007740.

Vrandecic, K., Jurkovic, D., Riccioni, L., Cosic, J. and Duvnjak, T. (2010). *Xanthium italicum*, *Xanthium strumarium* and *Arctium lappa* as new hosts for *Diaporthe helianthi*. Mycopathologia 170: 51–60.

Wang, M., Roux, F., Bartoli, C., Huard-Chauveau, C., Meyer, C., Lee, H., Roby, D., McPeek, M.S. and Bergelson, J. (2018). Two-way mixed-effects methods for joint association analysis using both host and pathogen genomes. Proceedings of the National Academy of Sciences of the United States of America 115: E5440–E5449.

Zambelli, A., Mancebo, M.F., Bazzalo, M.E., Reid, R.J., Sanchez, M.C., Kontz, B.J. and Mathew, F.M. (2021). Six Species of *Diaporthe* Associated with Phomopsis Stem Canker of Sunflower in Southern Pampean Region of Argentina. Plant Health Progress 22: 136–142.

Zhang, F., Hu, Z., Wu, Z., Lu, J., Shi, Y., Xu, J., Wang, X., Wang, J., Zhang, F., Wang, M., et al. (2021). Reciprocal adaptation of rice and *Xanthomonas oryzae* pv. oryzae: cross-species 2D GWAS reveals the underlying genetics. The Plant Cell 33: 2538–2561.

Zubrzycki, J.E., Maringolo, C.A., Filippi, C.V., Quiróz, F.J., Nishinakamasu, V., Puebla, A.F., Di Rienzo, J.A., Escande, A., Lia, V.V., Heinz, R.A., et al. (2017). Main and epistatic QTL analyses for Sclerotinia Head Rot resistance in sunflower. PLOS ONE 12: e0189859.