Vitis versus *Erysiphe necator*

Hybridisation potential of *Vitis spp*. for powdery mildew resistance

Figure 1: Picture shows progeny of a *V. vinifera* x *V. romanetii* backcross. Hybrid on the left is highly susceptible to *E. necator* while the hybrid on the right is completely resistant through enhanced penetration resistance (Ramming *et al.*, 2011).

Name: Bob Leon Evenhuis
Student number: s2986191
Department: Ecophysiology of plants
Supervisor: Prof. dr. J.T.M. Elzenga
Date: 22-03-2021

Table of contents

List of abbreviations

PM:	Powdery mildew; grapevine PM: *Erysiphe necator*
DM:	Downy mildew; grapevine DM: *Plasmopara viticola*
NHR:	Nonhost resistance
HR:	Host resistance
PAMPs:	Pathogen associated molecular patterns
PTI:	PAMP triggered immunity
ETI:	Effector triggered immunity
PR genes:	Pathogenesis related genes
PRR:	Pathogen recognition receptors
RLK:	Receptor-like kinases
RLP:	Receptor-like proteins
RUN:	Resistance to *Uncinula necator* gene
REN:	Resistance to *Erysiphe necator* gene
R-genes:	Resistance genes
R-proteins:	Resistance proteins encoded by R-genes
NB-LRR:	Nucleotide binding leucine rich repeat receptor
LysM-RLK/RLP:	Lysine motive RLK/RLP

Abstract

One of the most cosmopolitan fruits is under threat of a fungal disease. The Eurasian grapevine, *Vitis vinifera,* is highly susceptible to the grapevine powdery mildew (PM), *Erysiphe necator*. Infection decreases yield and fruit quality. Therefore, enormous amounts of fungicides are used annually. Even though, vineyards only make up 3 percent of European agricultural land, two thirds of the total fungicide usage of the EU is applied in these vineyards. This is detrimental for the environment, brings enormous cost to farmers and is also harmful for farmworkers. It is therefore paramount to breed new grape varieties that combine the fruit qualities of *V. vinifera* with resistance to powdery mildew. To be able to do this, this thesis reviews the grapevine-PM pathosystem. In Chapter 1 the biology of the grapevine adapted obligate biotroph powdery mildew *E. necator* is discussed. *E. necator* is able to overcome the nonhost resistance of *V. vinifera.* Further, in this thesis multiple mechanisms of resistance against PM are reviewed. The mechanisms are divided according to the central dogma of plant pathology. Chapter 2 discusses nonhost resistance (NHR) and more specifically NHR in grapevines. NHR is based on recognising pathogen associated molecular patterns (PAMPs) with extracellular receptors, which will lead to PAMP triggered immunity (PTI). The mechanism of NHR is based on penetration resistance by callose deposition at site of fungal attack and on increased stilbene production. Two species of *Vitis*, *Vitis aestivalis* (North American) and *Vitis quinquangularis* (Chinese), show enhanced NHR to confer resistance against PM. Chapter 3 discusses host resistance (HR) and various *Vitis* species that are able to employ HR. HR works specifically against *E. necator*, which uses grapevines as its host. HR is based on recognising pathogen virulence factors, called effectors, by intracellular R-proteins, which leads to effector triggered immunity (ETI). The result of ETI is programmed cell death (PCD) of infected cells or penetration resistance. Multiple *Vitis* species, from North America and China and a landrace *V. vinifera,* have evolved R-proteins that recognise the effectors of the grapevine adapted PM and are thus able to confer HR by ETI. The insight in the various resistance mechanisms allow for recommendations on hybridisation of *Vitis* species with *V. vinifera* to get PM resistant varieties. These recommendations are discussed in Chapter 4. Primarily, it is important to look for a combination of different mechanisms from multiple *Vitis* species. These mechanisms should be pyramided in the new varieties to confer strong and resilient resistance against multiple *E. necator* strains. Secondly, it is necessary to use a polyculture of multiple varieties that have different mechanisms of resistance in vineyards. Lastly, it is needed to continuously search for new resistance mechanisms in wild and landrace grapevines to keep a backup of resistance mechanisms that can be bred into new varieties if resistance is broken. If all recommendations are used to breed new PM resistant varieties, there is a future for sustainable viticulture.

Introduction

Grapevines (*Vitis spp*.) and the Eurasian grapevine *Vitis vinifera* in particular have been cultivated for human consumption for over 7000 years. It is used for the production of table grapes, raisin, jelly and wine. Because of its long history of being grown next to man, few horticultural species have more social and cultural value. Over 1368 cultivars are being used for commercial wine growing around the world (Robinson *et al.*, 2012). Similarly, other uses require different varieties. Varieties are propagated by cloning. Such that the Pinot Noir you are drinking today has the exact same genetic information as one when it was developed by the Sistine monks in 1150. Unfortunately, this genotype together with all other *Vitis vinifera* genotypes, is susceptible to many diseases. Most notably to powdery mildew, which is caused by the fungus *Erysiphe necator*. There are many other pathogens that confer diseases to grapevine, like, grey mould and downy mildew. However, these diseases need specific environmental condition for successful infection. Whereas powdery mildew can always infect *Vitis* plant tissues. Currently, *E. necator* will destroy crops and reduce quality of the fruit if left untreated. Infection reduces photosynthesis capacity of the grapevine which results in: yield loss, lower fruit quality and declining sugar and acidity of the berries (Armijo *et al.*, 2016). Some closely related grapevine species in the *Vitis* genus have evolved resistance against *E. necator*. These species might provide interesting sources of resistance for grapevine breeders. Therefore, the research question of this thesis is: **What are the mechanisms of resistance against *Erysiphe necator* infections in the *Vitis* genus?** In the final chapter I will make recommendations about the potential of hybridisation of *Vitis spp*. for *E. necator* resistance.

Cultivated *Vitis vinifera* belong to the subspecies *vinifera* (or *sativa*) and they have descended from their wild counterpart *Vitis vinifera ssp. sylvestris*. The main difference is that the cultivated *ssp. vinifera* has perfect flowers as these flowers are synoecious. *Ssp. sylvestris* on the other hand is dioecious. A fruit plant with perfect flowers generally simplifies the cultivation of this fruit. Moreover, through selective breeding the berry quality of *V. vinifera* has been highly improved. It is important to notice that because all cultivated grapes have perfect flowers and this is a mutation from the wildtype, it is highly probable that that all cultivars from *V. vinifera* stem from only a handful of accessions. With low genetic variability as a consequence. In central Asia both subspecies of *V. vinifera* are able to crosspollinate each other (Riaz *et al.*, 2020). The *Vitis* genus consists of two subgenera: the *Euvitis* and the *Muscadinia*. The *Muscadinia* genus consists of one species: the *Muscadinia rotundifolia*, synonym: *Vitis rotundifolia*. This species is taxonomically different as it has 40 chromosomes (N=20) instead of 38 (N=19) for *Euvitis*. *Muscadinia rotundifolia* is taken as part of the *Vitis* genus even though it is not classified as a *Euvitis*, because several hybrids between *M. rotundifolia* and *V. vinifera* are bred successfully. The *Vitis* genus spans many species with a lot of diversity in North America and China. Previous success with hybridising *Vitis spp*. was attained by crossbreeding North American grapevines with *V. vinifera* to breed rootstocks with resistance to the root aphid *Phylloxera vastatrix*. When breeding new varieties, fruit quality is ultimately of the highest concern. For example, current successful hybrids of *V. vinifera* x *V. labrusca* have not been used in Europe for quality wine production because their so called “foxy” musk aromas.

It is interesting to notice that of all plant-microbe interactions only a very small percentage of plant-pathogen interaction results in diseased plants. This is because all plants possess the highly conserved innate immune system that, based on recognition of non-host pathogens, triggers an immune response. The immunity based on this system is called basal immunity or

nonhost resistance (NHR)[1], because the pathogens can normally not use the plant as a host. Some pathogens are able cause disease because they are specially adapted to infect a certain host plant. As a response, plants have evolved a second layer of the innate immune system called host resistance (HR). Similarly, the species in the *Vitis* genus use the two different layers of protection against infection of the grapevine powdery mildew *E. necator*. Chapter 2 of this document will discuss non-host resistance, while Chapter 3 will discuss host resistance in the *Vitis* genus.

To get a better insight in *Vitis-E. necator* pathosystem we will first look at the biology of the powdery mildew in Chapter 1. In this document we use the Arabidopsis-PM pathosystem to get a better understanding of the *Vitis*-PM pathosystem. Arabidopsis is a host to multiple species of powdery mildew. Moreover, we discuss the effect of the following non adapted powdery mildews: the grass powdery mildew *Blumeria graminis f. sp. hordei* and the cucurbit powdery mildew *Erysiphe cichoracearum* (Table 1). By studying these adapted powdery mildews and their host we will be able to reveal the mechanisms of PM resistance in the *Vitis* genus.

Table 1: Adapted powdery mildews that are discussed in this thesis and their host plants.

Host species	**Adapted powdery mildew**
Grapevines, *Vitis spp.*	*Erysiphe necator* (En)
Arabidopsis, *Arabidopsis thaliana*	Multiple species including: *Golovinomyces cichoracearum* (Gc), *G. orontii* (Go), *Oidium neolycopersici* (On) and *Erysiphe cruciferarum* (Ecr).
Barley, *Hordeum vulgare*	*Blumeria graminis f. sp. hordei* (Bgh)
Cucurbits, *Cucurbitaceae spp.*	*Erysiphe cichoracearum* (Eci)

[1]For a clear distinction between the two different layers of the plant innate immune system we use the term nonhost resistance (NHR) instead of basal immune system in this document.

Chapter 1

The biology of the adapted obligate biotroph pathogen *Erysiphe necator*

Erysiphe necator is the causal agent of grapevine powdery mildew. It is an obligate biotroph fungal pathogen, which means this fungus can only complete its lifecycle on life plant tissue. *E. necator* is parasitic on all species within the *Vitaceae* (Gadoury *et al.*, 2012). But the host with most economic value is *Vitis vinifera* or the Eurasian grapevine, which is highly susceptible to *E. necator*. In this chapter I will discuss the taxonomy and biology of *E. necator*. Moreover, we will dive into the consequences of *E. necator* on grape production worldwide and how this fungus has been combatted until now.

Taxonomy

E. necator is a fungus belonging to the *Erysiphaceae* family of powdery mildews. This family belongs to the phylum of *Ascomycota*, which is the largest phylum of fungi. Species belonging to this phylum generally form sexual structures called ascospores. A synonym for *Erysiphe necator* is *Uncinula necator*. Some 400 species of powdery mildew (PM) are known to science and they are able to infect around 10.000 host species of plants all of which belong to the angiosperms (Takamatsu, 2004). Including a lot of economically important crops, for example: apple (*Malus domestica*), roses (*Rosa hybrida*) and barley (*Hordeum vulgare*). Four species of PM are known to be able to infect Arabidopsis (*Arabidopsis thaliana*), however there are different ecotypes that show resistance to some of the PM species (Kuhn *et al.*, 2016). One species of powdery mildew can generally infect multiple closely related plant species. In the case of grapevine powdery mildew *E. necator*, this is most genera of the *Vitaceae* family, namely *Vitis*, *Cissus*, *Parthenocissus* and *Ampelopsis*. There is a lot of differences of susceptibility to *E. necator* between the genera. *Vitis* and *Ampelopsis* are more susceptible, while *Cissus* and *Parthenocissus* are resistant to *E. necator* (Feechan *et al.*, 2011). Some race specialisation on different host species has been found. *E. necator* isolates on Virginia Creeper (*Parthenocissus quinquefolia*) are not able to infect *Vitis spp.* Thus, *E. necator* colonies that grow on Virginia Creeper do not pose a threat to grape growing with *V. vinifera* (Gadoury *et al.*, 2012). Isolates of *E. necator* found on *Vitis spp.* are able to infect all of the *Vitis spp.* grapevines, with the exception of *Vitis rotundifolia*. *V. rotundifolia* is only affected by *E. necator* isolates found on *V. rotundifolia* (Gadoury *et al.*, 2012).

The biology of *E. necator*

The information in this paragraph is taken from Gadoury et al. *(2012) and Armijo* et al. *(2016).* *E. necator* overwinters in cleistothecium on the bark of the vines. Each cleistothecium contains four to six asci each of which usually bears four ascospores. With wet condition the asci burst open and the ascospores are released. At the start of the grapevine growing season the first ascospore colonies are already found on the first leaves. An ascospores germinates with one germ tube that later forms an appressorium. The appressorium forms a penetration peg that tries to penetrate the cuticle and plant cell wall. The peg is powered by pressure and infection is not established by the use of carbohydrate-active enzymes to weaken the cell wall like in other pathogenic fungi. When cell wall penetration is successful a haustorium is formed by invaginating the host cell membrane. The haustorium is the organelle that arranges the contact between pathogen and host plant. When a *E. necator* spore lands on a green part of the grapevine it emerges and forms a hypha. The hypha in turn forms an appressorium, this appressorium is always formed no matter the resistance of the *Vitis spp.* The resistance is about whether an haustorium is formed or not. The haustorium is the feeding organ of the biotroph fungus. Secondary hyphae are formed when the pathogen starts to take nutrients from the host, from which secondary appressoria may also be formed. Moreover, conidiophores are formed, these

give the pathogen its name, they appear as a grey-white powder on the affected plant tissue. Each conidiophore produces one conidia per 24-hour period. The optimal temperature for grapevine powdery mildew is between 23-30°C with an optimum at 26°C. Temperatures below 6°C inhibit disease development and with temperatures above 35°C the conidia cannot germinate. This is why in hot wine growing areas, like Spain and southern Italy, the disease pressure is much lower. When disease pressure is high there is hyphal contact between the two different mating types of *E. necator* and this will start the sexual reproduction by forming an ascocarp cleistothecium. The colony of *E. necator* will cease conidiospore production after hyphal contact between mating types. The difference between conidiospores and ascospores is that ascospores are from sexual reproduction while conidiospores are produced asexually. The germination of ascospores or conidia is favoured by humid condition however it is not necessary, this is in contrast to grapevine downy mildew (*Plasmopara viticola)*. Wet conditions are necessary for ascospore release from the cleistothecium.

Signs of *E. necator* infection

Powdery mildew infection is characterised a white-greyish powder on the infected tissue. PM is able to infect all green parts of the vine but mostly on leaves and stems, and also on berries when the vine is heavily infected. What seems a white-greyish powder is actually the conidia of the fungus (Gadoury *et al.*, 2012; Armijo *et al.*, 2016). When PM infected leaves are rubbed between thumb and fingers a distinctive fungal or champignon-like aroma can be perceived. This is in contrast to an infection with grapevine downy mildew, which does not give any perceivable aroma (Evenhuis, personal communication, 2020).

Current practices in disease management

Almost all grapes grown for production are *V. vinifera*, only in North- and South America some North American *Vitis spp.* are grown for their fruit. However, these have often been hybridised with *V. vinifera* for extra desirable fruit quality. This has however resulted in less disease resistance (Ramming, 2008). Thus, all grape production is under high pressure of grapevine powdery mildew *E. necator*. Since the introduction of *E. necator* to France in 1847 the disease has been treated with fungicides. Already six years after its introduction sulphur was being applied in most of the vineyards of France and where the pathogen went the use of sulphur sprays followed. In 1885 the Bordeaux mixture was developed by Pierre-Marie-Alexis Millardet and Ernest David. Which included copper(II) sulphate ($CuSO_4$) in the treatment of grapevine powdery mildew. Subsequently, Bourgogne vignerons started using Sodium Carbonate (Na_2CO_3) (baking soda ($NaHCO_3$) is also used). All these fungicides are mineral based and still widely used today, especially in organic viticulture. Later organic and inorganic fungicides were developed but these are mostly single-site modes of action fungicides. Because of gene repetitions, *E. necator* and other powdery mildew genomes are very large compared to other fungi. Unfortunately, this increases the chance of resistance against single-site mode of action fungicides like sterol demethylase inhibitors (Jones *et al.*, 2014). Currently, the total amount of annually applied fungicides in European vineyards is enormous. Even though vineyards cover only 3,3 percent of the total European agricultural land, 67% of the total amount fungicides in the EU is used in these vineyards (EUROSTAT EC., 2007).

Chapter 2

Nonhost resistance of *Vitis spp*. against fungal pathogens

Generally, plants are resistant to most pathogenic microorganisms through their innate immune system, and only a few can cause diseases (Lipka *et al.*, 2008). In the case of biotroph pathogens, like powdery mildews, the immune response is based on penetration resistance (Bent & Mackey, 2007; Gill *et al.*, 2015). As described extensively in the previous chapter, with successful infection biotroph pathogens draw nutrients from the host with an haustorium. The best way to stop pathogen proliferation is to resist penetration of the cell wall. This resistance can be accomplished by standard physical barriers or be triggered by the pathogen. The nonhost resistance (NHR) is triggered by recognising pathogen associated molecular patterns (PAMPs). In the case of fungal pathogens these PAMPs may be chitin, an important part of the fungal cell wall. *Vitis spp*. nonhost resistance is triggered by recognising PAMPs from non-adapted powdery mildew species (Feechan *et al.*, 2011). Through nonhost resistance they are able to inhibit growth of the non-adapted fungal pathogen and thereby stopping the sporulation and further spread of the pathogen. Some species of *Vitis* have an enhanced nonhost resistance and are thus also protected against adapted fungal pathogens by resisting penetration (See §2.1) (Fung *et al.*, 2008). This chapter discusses the mechanisms nonhost resistance in plants and from the *Vitis* genus is particular. This is important to understand why *E. necator* is a pathogen of *Vitis spp.*, because *E. necator* is able to disrupt NHR.

Table 1: The two layers of the plant innate immune system

Two different layers of the plant innate immune system can be described: (1) Nonhost- and (2) host resistance. They are differentiated by the type of pathogen-plant interaction. Nonhost resistance recognises PAMPs from non-adapted pathogens, resulting in PAMP triggered immunity (PTI). Adapted pathogens specialise in infecting a particular host. Host resistance recognises the effectors of these adapted pathogens, resulting in effector triggered immunity (ETI).

Defence mechanism:	**Pathogen type:**	**Recognises:**	**Response:**
Nonhost resistance (NHR), (Basal immune system)	Non-adapted pathogen	PAMPs	PAMP triggered immunity (PTI)
Host resistance (HR)	Adapted pathogen	Effectors	Effector triggered immunity (ETI)

Although they are thought of as two different layers of the immune system, nonhost and host resistance share more similarities than differences (Gill et al., 2015). Especially, the resistance mechanisms are thought to be similar, only the recognition of the pathogen justifies a separation (Table 2). Nonhost resistance is thought to be more durable (Gill et al., 2015). However, adapted pathogens are able to surpass nonhost resistance through the use of effectors (Bent & Mackey, 2007). In response to this, plants have developed R (resistance) genes, that recognise effectors from the adapted pathogen (Bent & Mackey, 2007). These R-genes and the immune response to adapted pathogens will be further discussed in Chapter 3. In this chapter we discuss physical barriers and plant toxins (§2.1) and nonhost resistance (§2.2) in the *Vitis* genus. To get a better insight in the mechanism of nonhost resistance we use the Arabidopsis-PM pathosystem and then translate this to the *Vitis* genus.

2.1 Nonhost resistance: Physical barriers and plant toxins that inhibit penetration

In American grapevines *Vitis labrusca* and hybrids of *V. labrusca* x *V. vinifera* a feature of physical resistance against grapevine downy mildew (DM), *Plasmopara viticola*, was identified. Resistance against DM was positively corelated with leaf hair density. DM needs

free water to infect the vines. The hydrophobic leaf hairs fasten the evaporation of water and thereby inhibit infection (Kono *et al.*, 2018). This is a great example of a physical barrier that inhibits infection. Kono *et al.*, (2018) have found a promising feature to breed into *V. vinifera* hybrids for DM resistance. However, no examples of physical barriers to inhibit powdery mildew infection in grapevines are known to date.

Plant toxins

Stilbene production is one of the ways plants can prevent fungal pathogens to infect plant tissues. Stilbenes are small molecules, like resveratrol, known as antifungal toxins. Stilbene synthase genes were upregulated in *V. vinifera* soon after PM inoculation (Fung *et al.*, 2008). However, in *Vitis aestivalis*, a PM resistant grapevine from North America, stilbene synthase genes were not upregulated. Pathogenesis related (PR) genes are normally activated after PAMP recognition. However, PR genes PR2 and PR3 were constitutively expressed more in *V. aestivalis* compared to *V. vinifera.* This is likely caused by constitutively higher levels of endogenous salicylic acid (SA) in *V. aestivalis* (Fung et al. 2008). *V. aestivalis* does not increase its SA levels when inoculated with the fungal pathogen *E. necator*. On the contrary, *V. vinifera* increases its endogenous SA after inoculation with *E. necator*, this is significant at 120 hours past inoculation (Fung et al 2008). Only three PM responsive transcripts were identified in *V. aestivalis* and this can possibly be explained by the constitutive high- or low-level gene expression in this species compared to *V. vinifera*. This in turn could be related to the higher levels of endogenous SA compared to *V. vinifera*. Expression levels of resistance genes can be tempered by reducing endogenous SA levels in overexpressing *Arabidopsis* mutants (Clarke *et al.*, 2001). Cell wall browning occurred in *V. aestivalis* in epidermal cells under appressoria of PM. However, still some secondary hyphae were formed and even secondary appressoria were observed. Cell wall browning occurred under secondary appressoria as well. The cell wall browning resulted in less pronounced *E. necator* colony development on *V. aestivalis* compared to *V. vinifera* (Fung *et al.*, 2008). Higher constitutive endogenous SA concentration and differentially expressed PR genes can be seen as an enhanced NHR defence mechanism against PM and are a possible explanation why *V. aestivalis* is resistant against PM and *V. vinifera* is not.

In Chinese *Vitis quinquangularis* stilbene production is higher than in *V. vinifera* and this is thought to be the source of resistance against *E. necator*. Overexpression of a *V. quinquangularis* stilbene synthase gene (VqSTS6) in a *V. vinifera* cv. Thomson Seedless, enhanced resveratrol production and increased resistance against *E. necator*. Interestingly, susceptible *V. vinifera* grown on overexpressing VqSTS6 rootstocks also showed increased resistance against *E. necator* (Liu *et al.*, 2019).

2.2 PAMP induced nonhost resistance

Nonhost resistance is induced when PAMPs are recognised by the plant cell. PAMPs are recognised by extracellular pathogen recognition receptors (PRRs). There are two types of PRRs: (1) receptor-like kinase (RLK), comprising of a ligand binding ectodomain and intracellular kinase domain, and (2) receptor-like proteins (RLP), that only have an extracellular ligand binding domain (Macho & Zipfel, 2014; Zipfel, 2014). RLPs are therefore thought to be part of multimer (Zipfel, 2014). For fungal pathogens a well-known PAMP is chitin, a part of the fungal cell wall. Chitin is recognised by lysine motive receptor-like kinases (LysM-RLK) and lysine motive receptor-like proteins (LysM-RLP). For Arabidopsis this is the AtCERK1 protein that codes for a LysM-RLK. PAMP recognition by RLK leads to activation of mitogen activated protein kinase cascades to upregulation of pathogenesis related genes to PTI (Bent & Mackey, 2007). Recently, two orthologue PRR proteins have been found in *V. vinifera: VvLYK1-1* and *VvLYK1-2*. These LysM-RPK's are homologous to the AtCERK1 of

Arabidopsis and confer resistance against non -adapted PM *E. necator* in Arabidopsis (Brulé *et al.*, 2019).

Arabidopsis vs non-adapted PM *Blumeria graminis hordei*

Arabidopsis successfully inhibits *Blumeria graminis f. sp. hordei* (*Bgh*) infection. In the majority (95%) of attempted penetrations by *Bgh*, the PM was not able to form a haustorium. In the remaining 5% the formation of an haustorium resulted in cell death (Lipka *et al.*, 2005). Thus, *Arabidopsis* is able to withstand invasion of the non-adapted PM *Bgh*. Pre-invasion defence is based on penetration resistance while post-invasion defence is based on a hypersensitive response resulting in cell death. Three different genes have been found to confer penetration resistance through mutant analysis: PENETRATION1 (PEN1), PEN2 and PEN3. PEN1 is a SNARE protein located in the cell membrane and associated with vesicle membrane fusion and secretion. Possibly related to callose depositions at attempted penetration site (Lipka *et al.*, 2008). PEN2 is associated with enzymatic activation of small molecules. It is localised to peroxisomes (Lipka *et al.*, 2005). PEN2 and PEN3 are together conferring resistance against non-adapted PM (Underwood & Somerville, 2008). PEN3 is another transmembrane protein and important in ATP dependent transport. PEN3 mutants are less susceptible to infection of adapted PM through a SA dependent hypersensitive response. This seems reminiscent of the mechanism described before in *Vitis aestivalis* (Fung *et al.*, 2008). Where high endogenous SA was related to resistance against adapted powdery mildew.

With our now better understanding on the mechanism of nonhost resistance we will look again at our focal species *V. vinifera*. Feechan *et al*. (2011) looked at nonhost resistance in a *V. vinifera*-non-adapted cucurbit pathosystem. Nonhost resistance is generally considered to be caused by penetration resistance. The non-adapted cucurbit PM *Erysiphe cichoracearum* only penetrated the *V. vinifera* in 25% ± 5% compared to 77% ± 10% by the adapted *E. necator*. Non-adapted powdery mildew can penetrate epidermal cells when vesicle transport and endocytosis is inhibited. This is potentially because PAMP receptor endocytosis is inhibited, and thus the alarm bells are not ringing. Another possibility is that the cell cannot deposit cell wall materials at the site of attempted penetration (Feechan *et al.*, 2011). Penetration resistance is considered to be dependent on the cytoskeleton polymerization and vesicle trafficking to the site of attempted infection (Underwood & Somerville, 2008). Inhibiting these mechanisms significantly increased penetration by a non-adapted powdery mildew in *V. vinifera* (Feechan *et al.*, 2011). Moreover, the PEN1 homologue VvPEN in *V. vinifera* also accumulates at the attempted site of fungal infection (Feechan *et al.*, 2013). These findings suggest that similar mechanisms of NHR penetration resistance are used in *V. vinifera* and Arabidopsis.

Proposed mechanism of NHR penetration resistance in *V. vinifera*:

1. Chitin recognition by PAMP recognition receptor VvLYK1-1/-2
2. VvPEN1 accumulation at site of fungal infection
3. Cytoskeleton rearrangements to position of attempted infection
4. Callose deposition at infection site
5. Penetration peg is not able to penetrate the cell wall

Host and nonhost resistance are sometimes using similar mechanisms, which leads to believe that host resistance occurs by reactivating the nonhost resistance (Gill *et al.*, 2015). Moreover, the mechanisms of nonhost resistance are probably highjacked for infection of the host cell by the adapted powdery mildew *E. necator* (Feechan et al., 2011). This brings us to the next chapter of this document. Where we will discuss the resistance of some *Vitis* species against the adapted mildew *E. necator*.

Chapter 3

Host resistance against adapted powdery mildew *Erysiphe necator* in the *Vitis* genus

The grapevine powdery mildew, *Erysiphe necator*, is an adapted pathogen that has evolved to use grapevines from the *Vitis* genus as its host. *E. necator* is able to disrupt the nonhost resistance of V*itis spp*. and thus it has the ability to penetrate epidermal cells. Through the formation of a haustorium nutrients are drawn from live plant tissue to complete its lifecycle. Some species within the genus *Vitis* have in response to this evolved resistance against the adapted powdery mildew. In contrast to the nonhost resistance explained in Chapter 2, a specific immunity has evolved in response to this pathogen. This host resistance is thought to be based on the recognition of virulence factors released by *E. necator*. Normally, these virulence factors allow *E. necator* to infect susceptible *Vitis spp*. In this chapter we dive in to the details of how some *Vitis spp*. resist *E. necator* infection.

Host adapted pathogens use effectors to bypass nonhost resistance

An evolutionary arms race between pathogen and host has resulted in the development of virulence mechanisms by the pathogen. Host-adapted pathogens have evolved proteins that act as virulence factors, called effectors, to actively suppress nonhost resistance in their host. Effectors were first discovered as avirulence factors, because they promoted plant resistance against a pathogen. However, pathogens evolved these effector proteins to be virulence factors, facilitating the infection of host tissue, by supressing nonhost resistance (Bent & Mackey, 2007). It is generally thought that effector proteins do not have a "housekeeping" function in the pathogen, and their only purpose is facilitating the infection (Bent & Mackey, 2007). Effector proteins inhibit nonhost resistance by blocking the mitogen activated protein cascades that is normally activated in PAMP triggered immunity (PTI) (see Chapter 2). *E. necator* can infect *V. vinifera* while non adapted cucurbit powdery mildew, *Erysiphe cichoracearum* cannot break the nonhost resistance of *V. vinifera* (Feechan *et al.*, 2011). This makes *E. necator* an adapted pathogen, that probably uses effector proteins to disrupt nonhost resistance of *Vitis spp*. It is not yet known, what the effector protein(s) of *E. necator* are. Some species of North American and Chinese *Vitis spp*. are known to be resistant against *E .necator* infection (Wan *et al.*, 2007; Dry *et al.*, 2010). Moreover, a few accessions of *V. vinifera ssp*. *sylvestris* and a few local *V. vinifera* cultivars have been found in central Asia (Hoffmann *et al.*, 2008). Host resistance against an adapted pathogen is thought to be conferred by resistance (R) genes (Bent & Mackey, 2007). R genes code for proteins that recognise the effector proteins directly or the changes in the cytosol caused by the effector proteins. These resistance proteins are intracellular receptors of which many have been found to consist of a nucleotide-binding (NB) part and a leucine-rich repeat (LRR) part, and are therefore called NB-LLR receptor-like kinases. When the effector or the effect thereof is recognised by the R-protein this results in effector triggered immune response (ETI). Resistant *Vitis* species can reinstate the nonhost resistance plus activate host resistance. Two main types of adapted PM resistance are observed: penetration resistance and programmed cell death induction (Feechan *et al.*, 2011).

Programmed cell death following penetration

One of the common mechanisms of resistance against adapted fungal pathogens is programmed cell death (PCD). The dead cell "traps" the feeding structure formed by the pathogen. This results in the stop of nutrient flow to the pathogen and thus the pathogen cannot complete its lifecycle. Some *Vitis spp*. that show resistance against adapted powdery mildew *E. necator* are known to use PCD. *Muscadinia rotundifolia* was one of the first grape species to be described using this mechanism of resistance. PCD is induced after the epidermal cells are penetrated by the powdery mildew pathogen (Feechan *et al.*, 2011). When *V. vinifera* was inoculated with *E. necator* spores, PCD was observed in only 2 percent of the penetrated epidermal cells. Two

American *Vitis* species: *Vitis riparia* and *Vitis rupestris*, are able to resist penetration of *E. necator*. Moreover, PCD was induced by *V. rupestris* and *V. riparia* in approximately 20% and 30% of penetrated cells respectively *M. rotundifolia* induces PCD in 75% of the penetrated cells by *E. necator*. Forward genetic screening allowed the isolation of the first adapted powdery mildew resistance gene in *M. rotundifolia.* The MrRUN1 gene confers complete resistance via the rapid induction of PCD in penetrated epidermal cells. Other *E. necator* resistance genes exhibit a lower frequency of PCD in penetrated cells. PCD occurs when effectors of the, adapted powdery mildew *E. necator*, are recognised by R-proteins.

Host resistance in a *Vitis vinifera* cultivar

Contrary to what pathogen-host evolution predicts, a *V. vinifera* cultivar has evolved host resistance against *E. necator*. The 'Kishmish vatkana' (VvKv) cultivar is probably only been used in grape growing after the *E. necator* was introduced into Eurasia. This cultivar has to be descendent from a wild *V. vinifera* population that was able to quickly evolve resistance against *E. necator*. The resistance to adapted powdery mildew in this cultivar is not complete as the fungus is still able to complete its lifecycle. Successful penetration is similar between VvKv and susceptible *V. vinifera* cultivar 'Nimrang' (VvNi). The progeny of the crossing of the aforementioned cultivars showed similar results. Although, hyphal development was not halted in VvKv, the growth of these hypha was restricted compared to the susceptible VvNi. This resulted in decreased conidiophore density at 120 hpi (Hoffmann *et al.*, 2008). This is a sign of decrease infection severity, slowing down the spread of the pathogenic fungus considerably. There was no significant difference conidiophore density between VvKv and *M. rotundifolia* descendant '01-1/867'. Cell wall browning occurred in cells contacted by the appressoria, in all genotypes. This browning gradually spread to the entire cell, sometimes browning in neighbouring cells occurred. Cell wall browning occurred more quickly and often in *M. rotundifolia*-derived genotype '01-1/867'. However, cell wall browning frequency in VvKv was not significantly different at 72 hpi from '01-1/867' (Hoffmann *et al.*, 2008). It is not sure whether the cell browning discussed here is the result of PCD. At least we can say that the PCD in VvKv was not as successful as PCD conveyed by RUN1. The dominant REN1 (Resistance Erysiphe Necator 1) loci is responsible for the resistance in VvKv. The REN1 locus is different from the RUN1 locus from *M. rotundifolia* as they can be found on chromosome 13 and 12, respectively. Similar features were found in the resistance loci, namely the nucleotide-binding site-leucine rich repeat (NB-LRR) genes. To conclude the 'Kishmish vatkana' cultivar shows partial resistance against the adapted powdery mildew *E. necator*. *E. necator* is able to penetrate the epidermal cell and draw nutrients from it. The PM is not able to develop visible colonies, because hyphal growth is significantly hampered by the host. As a result conidiophore development was significantly lower than susceptible control and not different compared to resistant *M. rotundifolia* progeny (Hoffmann *et al.*, 2008). REN1 cannot confer complete resistance against *E.* necator. Nonetheless, REN1 is a powerful tool in breeding PM resistant grapevines (Agurto *et al.*, 2017).

Chinese *Vitis* species show resistance against *E. necator*

China is thought to be the centre of origin of the *Vitis* genus. Interestingly, some Chinese grapevines show potential as sources of PM resistance. *Vitis piasezkii, Vitis pseudoreticulata* and *Vitis romanetii* are of special interest because they show strong resistance against *E. necator*. These are wild species and are not cultivated, although some species are grown for ornamental purposes. *V. piasezkii* is completely resistant with two resistance loci: REN6 and REN7 (Pap *et al.*, 2016). When these loci were crossed into a susceptible *V. vinifera* it became clear that REN6 is the cause of the complete resistance. The REN6 genotype quickly initiated PCD. Whereas, the REN7 genotype only initiated PCD under secondary appressoria. However,

REN7 was able to halt development of adapted PM compared to susceptible *V. vinifera*. Both *V. piasezkii* resistance genes recognise *E. necator* effectors from California and Australia. The difference between fast and slow resistance, with fast resulting in complete resistance, could be explained by the differently expressed R-genes. Moreover, different R-proteins potentially recognise different PM effectors. Different effectors might be released by the pathogen at different stages of infection. This might explain the observed PCD under secondary appressoria but not under primary in REN7 genotypic *Vitis* hybrids (Pap *et al.*, 2016). Similar to *V. piasezkii*, *V. pseudoreticulata* displayed high frequency of PCD at over 90% of penetrated epidermal cells at 5-7dpi. This resulted in less sporulation and characterised both species as resistant against *E. necator* (Hu *et al.*, 2019). In *V. romanetii* germinated conidia did not develop secondary hypha after appressorium formation thus it is argued that the fungus was not able to penetrate the cell wall. This allows us to predict that the resistance loci REN4 is based on penetration resistance (Ramming *et al.*, 2011). This a first resistance loci that displays host resistance through increased rapid penetration resistance instead of PCD. The actin cytoskeleton is required in both NHR and HR penetration resistance for callose deposition at the site of attempted penetration (Feechan *et al.*, 2011). Sporulation is completely halted by REN4 in multiple environments and against multiple strains of *E. necator*.

Microscopic images from after *E. necator* inoculation showed that in *V. piasezkii* and *V. pseudoreticulata* haustoria were not able to form normally and were poorly developed. Moreover, the deposition of cell wall like structures called papillae, were observed at attempted penetration site and within the vicinity of the haustoria. In *V. pseudoreticulata* no normal haustorial main bodies were found at 7dpi. Most of the haustoria were fully embedded into a cellular deposit with in the haustorial membrane, this resulted in the haustoria being encased. Moreover, massive multivesicular bodies accumulated in the cytosol of the penetrated epidermal cells. Plus, what is argued to be a lignified and thickened secondary cell wall developed around the neck of each haustorium. *V. piasezkii* was also found to have deposited a material within the extra-haustorial membrane, indicating that haustoria were encased. Defence associated genes responsible for biosynthesis of callose, chitinase and phytoalexins had higher transcript levels in Chinese *Vitis spp*. compared to *V. vinifera*, and were eventually downregulated 24hpi (Hu *et al.*, 2019). SA and conjugates were elevated at 0dpi and SA conjugates remained higher in *V. piasezkii* and *V. pseudoreticulata* compared to *V. vinifera*, indicating more active SA metabolism which is thought to be needed for rapid disease resistance (Fung *et al.*, 2008). In all three *Vitis spp.* SA levels peaked at 1-3 dpi and were back to previous levels at 7dpi. SA conjugates rapidly increased at 1dpi in both *V. pseudoreticulata* and *V. vinifera*, but *V. pseudoreticulata* had twice the levels of SA conjugates compared to *V. vinifera*. *V. piasezkii* reached peak levels at 5 dpi and decreased thereafter (Hu *et al.*, 2019).

Differences in resistance response between Chinese and North American *Vitis* species indicate that different cellular mechanisms might be used during their interaction with *E. necator* (Hu et al, 2019). Both encasement of haustoria and callose deposition, and post-penetration resistance, through NB-LRRs mediated PCD are expected to result in resistance in Chinese *Vitis* species. While only PCD resistance mechanisms are reported in North American *Vitis spp.*, with the exception of *V. aestivalis* (Fung *et al.*, 2008). In a comparison between multiple Chinese *Vitis spp.* and V. *riparia* and *V. labrusca*, the North American *Vitis* species show superior resistance to *E. necator* (Wan *et al.*, 2007). Moreover, more variability was found between different genotypes of the same species of Chinese *Vitis sp*. See Table 3 for an overview of resistance mechanisms in the resistant species of the *Vitis* genus.

Table 3: Overview of the resistant grapevine species from the *Vitis* genus discussed in this thesis. When known, resistance loci, resistance mechanism and origin of the species are included in the table.

Grapevine species	**Resistance Loci**	**Mechanism**	**Origin**	**Reference**
Vitis vinifera	REN1	Cell browning, haustorium encasement	Central Asia	(Hoffmann *et al.*, 2008)
Muscadinia rotundifolia	RUN1 RUN2	PCD PCD	North America	(Barker *et al.*, 2005) (Riaz *et al.*, 2011)
Vitis riparia	-	Enhanced NHR and PCD	North America	(Feechan *et al.*, 2011)
Vitis rupestris	-	Enhanced NHR and PCD	North America	(Feechan *et al.*, 2011)
Vitis labrusca	-	-	North America	(Wan *et al.*, 2007)
Vitis aestivalis	-	Enhanced NHR (stilbene production)	North America	(Fung *et al.*, 2008)
Vitis romanetii	REN4	Enhanced penetration resistance	China	(Ramming *et al.*, 2011)
Vitis piasezkii	REN6 REN7	PCD -	China	(Pap *et al.*, 2016)
Vitis pseudoreticulata	-	PCD	China	(Hu *et al.*, 2019)
Vitis quinquangularis	-	Stilbene production	China	(Liu *et al.*, 2019)

Chapter 4

Recommendations for breeding *Vitis* hybrids for *E. necator* resistance

Grapevine growing has economic and cultural value all over the world. Most of the 7.6 million hectares under vine is planted with a limited germplasm of the Eurasian grapevine *Vitis vinifera*. It is praised for her berry qualities for making wine, table grapes, raisins and jellies. However, this grapevine is highly susceptible to a range of pests, most notably the adapted powdery mildew *E. necator*. Today the crop is managed with extensive fungicide spraying regime. This has negative economic and health consequences for the farmers as well as negative consequences for the species living in and around the vineyards and the environment, because of greenhouse gas emissions during production and application of fungicides. Therefore, a new way of grapevine farming is needed. One of the ways this has been tried is by breeding new resistant grape varieties. One source of new grape varieties is through hybridisation. Many species in the *Vitis* genus are known to have a certain resistance against *E. necator* (Qiu *et al.*, 2015). In this thesis I have tried to give an overview of the resistance mechanisms of these species. Now I will provide recommendations on how to get to a more sustainable grape growing industry by using hybrid *Vitis* varieties. These varieties will combine the desired fruit qualities from *V. vinifera* with the resistance against *E. necator* found in landrace *V. vinifera*, North American and Chinese *Vitis* species. Until today, only a few resistant cultivars are commercially available, however this resistance is not complete.

Landrace *V. vinifera*

Unexpectedly, some *V. vinifera* grape varieties in Central Asia have been found to be resistant to *E. necator* (Hoffmann *et al.*, 2008). The grape variety ´Kishmish vatkana' has evolved a NB-LRR recognition protein inducing ETI. PCD of the penetrated cell restricted hyphal growth and sporulation following *E. necator* infection. Even though, the resistance is not complete the REN1 locus is an important source for breeding PM resistant grapevines. In the first place because it is found in a *V. vinifera* cultivar with already desirable fruit qualities. Moreover, this finding proves that *E. necator* resistance can quickly evolve in a wild population of grapevines. It is therefore recommended to keep looking for *E. necator* resistance in landrace *V. vinifera* and wild *V. vinifera ssp. sylvestris.*

North American *Vitis spp*.

Vitis species from North America have long been known to be resistant to *E. necator*. This is not surprising because this obligate biotroph originates from North America. Here it became an adapted fungus to grapevines from the family of *Vitaceae*. It has evolved still unknown effector proteins to suppress nonhost resistance in grapevines. As a response North American vines evolved receptors to recognise these effectors and trigger an immune response. That is based on PCD in *M. rotundifolia* and enhanced NHR in *V. aestivalis* and a combination of both mechanisms in *V. riparia* and *V. rupestris*.

Chinese *Vitis spp*.

Surprisingly, Chinese grapevines are also a source of *E. necator* resistance. The wild *Vitis* species confer resistance through PCD in *V. piasezkii* and *V. pseudoreticulata* and penetration resistance in *V. romanetii*. Chinese *Vitis spp*. have not been extensively studied so it will be interesting to see what other mechanisms of resistance will be found. Moreover, the breeding of interspecific hybrids has not been tried as much as with North American grapevines. Scientific trials show promising results.

E. necator is a highly adaptable fungus, because of its large and repetitive genome. This increases the risk of breaking the resistance by evolving new effector genes. Already, some *E.*

necator strains have been found to overcome single loci resistance. This is an important issue in breeding resistant varieties. When resistance genes are pyramided the resistance is more durable. Moreover, two or more complementing mechanisms of resistance will strengthen the plant defence response. It is recommended to breed resistant vines with multiple resistance mechanisms with for example both PCD and enhanced penetration resistance (Agurto *et al.*, 2017). It is highly probable that *E. necator* will adapt to resistant hybrid vines. Therefore, it is important to keep looking for resistance genes in wild *Vitis spp*. to make sure there is a diverse portfolio of sources of resistance. Pathogens, like *E. necator*, are able to overcome a single resistance loci quite easily when the cultivar is grown in a monoculture (Dangl *et al.*, 2013). It is therefore recommended to allow for more diversity in the vineyards. If there are multiple resistant genotypes in a vineyard there is less evolutionary pressure for *E. necator* to overcome the resistance.

Recommendations for breeding *Vitis* hybrids for *E. necator* resistance

- Combine sources of resistance from North American and Chinese species to prevent strain specific resistance
- Pyramid resistance genes to enhance resistance durability
- Combine resistance mechanisms to strengthen defence response
- Continue to search for resistance genes in wild *Vitis* species
- Change vineyard monocultures to polycultures of multiple varieties with different resistance genes and mechanisms

References

Agurto, M., Schlechter, R.O., Armijo, G., Solano, E., Serrano, C., Contreras, R.A., *et al.* 2017. RUN1 and REN1 pyramiding in grapevine (Vitis vinifera cv. crimson seedless) displays an improved defense response leading to enhanced resistance to powdery mildew (erysiphe necator). *Front. Plant Sci.* **8**: 1–15.

Armijo, G., Schlechter, R., Agurto, M., Muñoz, D., Nuñez, C. & Arce-Johnson, P. 2016. Grapevine pathogenic microorganisms: Understanding infection strategies and host response scenarios. *Front. Plant Sci.* **7**: 1–18.

Barker, C.L., Donald, T., Pauquet, J., Ratnaparkhe, M.B., Bouquet, A., Adam-Blondon, A.F., *et al.* 2005. Genetic and physical mapping of the grapevine powdery mildew resistance gene, Run1, using a bacterial artificial chromosome library. *Theor. Appl. Genet.* **111**: 370–377.

Bent, A.F. & Mackey, D. 2007. Elicitors, Effectors, and R Genes: The New Paradigm and a Lifetime Supply of Questions . *Annu. Rev. Phytopathol.* **45**: 399–436.

Brulé, D., Villano, C., Davies, L.J., Trdá, L., Claverie, J., Chiltz, A., *et al.* 2019. The grapevine (Vitis vinifera) LysM receptor kinases VvLYK1-1 and VvLYK1-2 mediate chitooligosaccharide-triggered immunity. 812–825.

Clarke, J.D., Aarts, N., Feys, B.J., Dong, X. & Parker, J.E. 2001. Constitutive disease resistance requires EDS1 in the Arabidopsis mutants cpr1 and cpr6 and is partially EDS1-dependent in cpr5. *Plant J.* **26**: 409–420.

Dangl, J.L., Horvath, D.M. & Staskawich, B.J. 2013. Pivoting the Plant Immune System. *Science (80-.).* **341**: 745–751.

Dry, I.B., Feechan, A., Anderson, C., Jermakow, A.M., Bouquet, A., Adam-Blondon, A.F., *et al.* 2010. Molecular strategies to enhance the genetic resistance of grapevines to powdery mildew. *Aust. J. Grape Wine Res.* **16**: 94–105.

EUROSTAT EC. 2007. *The use of plant protection products in the European Union. Data 1992-2003.* Office for Official Publications of the European Communities, Luxembourg.

Feechan, A., Jermakow, A.M., Ivancevic, A., Godfrey, D., Pak, H., Panstruga, R., *et al.* 2013. Host Cell Entry of Powdery Mildew Is Correlated with Endosomal Transport of Antagonistically Acting VvPEN1 and VvMLO to the Papilla. **26**: 1138–1150.

Feechan, A., Kabbara, S. & Dry, I.B. 2011. Mechanisms of powdery mildew resistance in the Vitaceae family. *Mol. Plant Pathol.* **12**: 263–274.

Fung, R.W.M., Gonzalo, M., Fekete, C., Kovacs, L.G., He, Y., Marsh, E., *et al.* 2008. Powdery mildew induces defense-oriented reprogramming of the transcriptome in a susceptible but not in a resistant grapevine. *Plant Physiol.* **146**: 236–249.

Gadoury, D.M., Cadle-Davidson, L., Wilcox, W.F., Dry, I.B., Seem, R.C. & Milgroom, M.G. 2012. Grapevine powdery mildew (Erysiphe necator): A fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. *Mol. Plant Pathol.* **13**: 1–16.

Gill, U.S., Lee, S. & Mysore, K.S. 2015. Host Versus Nonhost Resistance : Distinct Wars with Similar Arsenals.

Hoffmann, S., Di Gaspero, G., Kovács, L., Howard, S., Kiss, E., Galbács, Z., *et al.* 2008. Resistance to Erysiphe necator in the grapevine "Kishmish vatkana" is controlled by a single locus through restriction of hyphal growth. *Theor. Appl. Genet.* **116**: 427–438.

Hu, Y., Gao, Y.R., Yang, L.S., Wang, W., Wang, Y.J. & Wen, Y.Q. 2019. The cytological basis of powdery mildew resistance in wild Chinese Vitis species. *Plant Physiol. Biochem.* **144**: 244–253. Elsevier.

Jones, L., Riaz, S., Morales-Cruz, A., Amrine, K.C.H., McGuire, B., Gubler, W.D., *et al.* 2014. Adaptive genomic structural variation in the grape powdery mildew pathogen, Erysiphe necator. *BMC Genomics* **15**.

Kono, A., Ban, Y., Mitani, N., Fujii, H., Sato, S., Suzaki, K., *et al.* 2018. Development of SSR markers linked to QTL reducing leaf hair density and grapevine downy mildew resistance in Vitis vinifera. *Mol. Breed.* **38**. Molecular Breeding.

Kuhn, H., Kwaaitaal, M., Kusch, S., Acevedo-Garcia, J., Wu, H. & Panstruga, R. 2016. Biotrophy at

Its Best: Novel Findings and Unsolved Mysteries of the Arabidopsis-Powdery Mildew Pathosystem. *Arab. B.* **14**: e0184.

Lipka, U., Fuchs, R. & Lipka, V. 2008. Arabidopsis non-host resistance to powdery mildews. *Curr. Opin. Plant Biol.* **11**: 404–411.

Lipka, V., Dittgen, J., Bednarek, P., Bhat, R., Wiermer, M., Stein, M., *et al.* 2005. Plant science: Pre- and postinvasion defenses both contribute to nonhost resistance in Arabidopsis. *Science (80-.).* **310**: 1180–1183.

Liu, M., Ma, F., Wu, F., Jiang, C. & Wang, Y. 2019. Expression of stilbene synthase VqSTS6 from wild Chinese Vitis quinquangularis in grapevine enhances resveratrol production and powdery mildew resistance. *Planta* **250**: 1997–2007. Springer Berlin Heidelberg.

Macho, A.P. & Zipfel, C. 2014. Plant PRRs and the activation of innate immune signaling. *Mol. Cell* **54**: 263–272. Elsevier Inc.

Pap, D., Riaz, S., Dry, I.B., Jermakow, A., Tenscher, A.C., Cantu, D., *et al.* 2016. Identification of two novel powdery mildew resistance loci, Ren6 and Ren7, from the wild Chinese grape species Vitis piasezkii. *BMC Plant Biol.* **16**: 1–19. BMC Plant Biology.

Qiu, W., Feechan, A. & Dry, I. 2015. Current understanding of grapevine defense mechanisms against the biotrophic fungus (Erysiphe necator), the causal agent of powdery mildew disease. *Hortic. Res.* **2**: 1–9.

Ramming, D.W. 2008. "Thomcord" Grape. *HortScience* **43**: 945–946.

Ramming, D.W., Gabler, F., Smilanick, J., Cadle-Davidson, M., Barba, P., Mahanil, S., *et al.* 2011. A single dominant locus, Ren4, confers rapid non-race-specific resistance to grapevine powdery mildew. *Phytopathology* **101**: 502–508.

Riaz, S., Menéndez, C.M., Tenscher, A., Pap, D. & Walker, M.A. 2020. Genetic mapping and survey of powdery mildew resistance in the wild Central Asian ancestor of cultivated grapevines in Central Asia. *Hortic. Res.* **7**. Springer US.

Riaz, S., Tenscher, A.C., Ramming, D.W. & Walker, M.A. 2011. Using a limited mapping strategy to identify major QTLs for resistance to grapevine powdery mildew (Erysiphe necator) and their use in marker-assisted breeding. *Theor. Appl. Genet.* **122**: 1059–1073.

Robinson, J., Harding, J. & Vouillamoz, J. 2012. *Wine Grapes*. Allen Lane, Penguin Books Ltd, London.

Takamatsu, S. 2004. Phylogeny and evolution of the powdery mildew fungi (Erysiphales , Ascomycota) inferred from nuclear ribosomal DNA sequences. *Mycoscience* **45**: 147–157. Elsevier.

Underwood, W. & Somerville, S.C. 2008. Focal accumulation of defences at sites of fungal pathogen attack. *J. Exp. Bot.* **59**: 3501–3508.

Wan, Y., Schwaninger, H., He, P. & Wang, Y. 2007. Comparison of resistance to powdery mildew and downy mildew in Chinese wild grapes. *Vitis - J. Grapevine Res.* **46**: 132–136.

Zipfel, C. 2014. Plant pattern-recognition receptors. *Trends Immunol.* **35**: 345–351. Elsevier Ltd.