Elicitation of grapevine defense responses against *Plasmopara viticola*, the causal agent of downy mildew

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I. DEDICATION / WIDMUNG:

Für alle, die nach Wissen streben
Und ihren Horizont erweitern möchten
bereit sind, alles zu geben
Und das Unbekannte nicht fürchten

Für alle, die bereit sind, sich zu schlagen
In der Wissenschaftsschlacht
keine Angst haben
Wissen ist Macht

****

For all who seek knowledge
And want to expand their horizon
Who are ready to give everything
And do not fear the unknown

For all who are willing to fight
In the science battle
Who have no fear
Because Knowledge is power

II. DECLARATION

I hereby declare that the submitted work was made by myself. I also declare that I did not use any other auxiliary material than that indicated in this work and that work of others has been always cited. This work was not either as such or similarly submitted to any other academic authority.

ERKLÄRUNG

Hiermit erklare ich, dass ich die vorliegende Arbeit selbststandig angefertigt und nur die angegebenen Quellen and Hilfsmittel verwendet habe und die Arbeit der anderen wurde immer zitiert. Die Arbeit lag in gleicher oder ahnlicher Form noch keiner anderen Prufungsbehorde vor.

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IV. SUMMARY:

The aim of the present study was to investigate the mechanisms of induced resistance after the application of specific elicitors (plant activators) in *Vitis vinifera/Plasmopara viticola* pathosystem. For this purpose phosphonate-containing elicitors, whose solo substances and a fungicide from the strobilurin group were applied on leaves of potted vines (Riesling, Müller-Thurgau, Solaris, Regent). The elicitors were used protectively and curatively. The characterization of the effect of the elicitors *in planta*, and detection was carried out by microscopic molecular level (qPCR, microarray).

According to the assessment of the biological activity of the elicitors on leaves of potted vines, Frutogard®, algin Biovital®, phosphonate and phosphate showed the best results. The efficiencies were between 70% and 90%. The protective treatment was consistently more effective than curative. Autofluorescence measurements showed that tolerant varieties (cv. Regent; cv. Solaris) constitutively contain phenols. Induced and subsequently inoculated potted vines showed stronger autofluorescence than only inoculated plants.

Transcriptome studies showed that phosphate and phosphonate led to similar defense responses through activation of stress-related signaling pathways. In this context, PR proteins, secondary metabolites e.g. Phytoalexins and enhanced cell wall metabolism were induced. Generally, systemic acquired resistance by salicylic acid pathway and PR proteins and induced systemic resistance through jasmonic acid and ethylene pathways have been activated. Elicitation with Frutogard® (without subsequent inoculation) induced stress-related pathways; however, less than phosphate and phosphonate did. These included stress signaling pathways, secondary metabolites, and hormones such as gibberellins and cytokinins. In the case of protective treatment with Frutogard® it induced even less stress-related signaling pathways.

Based on the results obtained, specific instructions for the viticultural practices could be derived from this work, where the replacement of copper-containing pesticides for organic viticulture is in focus in this study.

V. ZUSAMMENFASSUNG:

Ziel der vorliegenden Arbeit war die Untersuchung von Mechanismen der Induzierten Resistenz nach Applikation spezifischer Elicitoren (Pflanzenstärkungsmittel) im Pathosystem *Vitis vinifera*/*Plasmopara viticola*. Hierzu wurden phosphonathaltige Pflanzenstärkungsmittel, deren Solo-Substanzen sowie ein Fungizid aus der Gruppe der Strobilurine auf Blätter von Topfreben (Sorten Riesling, Müller-Thurgau, Solaris, Regent) appliziert. Die Elicitoren wurden protektiv und kurativ eingesetzt. Die Charakterisierung der Wirkungsweise der Elicitoren *in planta* erfolgte mittels mikroskopischem Nachweis sowie auf molekularer Ebene (qPCR; MicroArray).
Bei der Bewertung der biologischen Wirksamkeit der Elicitoren an Blättern der Topfreben zeigten Frutogard®, Algin Biovital®, Phosphonat und Phosphat die besten Ergebnisse. Die Wirkungsgrade lagen bei durchschnittlich 70% bis 90%. Die protektive Behandlung war durchweg effizienter als die kurative. Autofluoreszenz-Messungen zeigten, dass tolerante Sorten (cv. Regent; cv. Solaris) konstitutiv Phenole beinhalten. Die induzierten und anschließend inokulierten Topfreben wiesen eine stärkere Autofluoreszenz als die ausschließlich inokulierten Pflanzen auf. Transkriptom-Studien ergaben, dass Phosphat und Phosphonat zu ähnlichen Abwehrreaktionen führten, nämlich durch induzierte Aktivierung stressbezogener Signalwege. In diesem Zusammenhang dominierten PR-Proteine, sekundäre Metaboliten e.g. Phytoalexine und ein erhöhter Zellwandstoffwechsel. Allgemein wurde die systemisch aktivierte Resistenz durch den Salicylsäureweg und PR-Proteine sowie die induzierte systemische Resistenz durch den Jasmonsäureweg und Ethylen aktiviert. Ohne Einfluss des Schadenerregers wurden bei Applikation von Frutogard® im Vergleich zu Phosphat und Phosphonat deutlich weniger Signalwege induziert. Dazu gehörten Stresssignalwege, sekundäre Metaboliten und Hormone wie Cytokinine und Gibberelline. Im Falle des protektiven Einsatzes von Frutogard® wurden noch weniger stressbezogene Signalwege aktiviert.
Anhand der hier erarbeiteten Ergebnisse lassen sich spezifische Handlungsanweisungen für die weinbauliche Praxis ableiten. Im Fokus steht dabei der Ersatz kupferhaltiger Pflanzenschutzmittel für den ökologischen Weinbau.

1. INTRODUCTION

1.1 General introduction

Grapevine (*Vitis* v*inifera* L.) is a major horticultural crop with the area dedicated to viticulture exceeding 7.5 million ha (Table 1.1) (OIV, 2007). It is the most widely cultivated and economically important fruit crop worldwide (Vivier and Pretorius, 2002). It is believed that grapevines have evolved in several different areas of the world, which led to many different varieties. The origin of cultivation of the *V. vinifera* grape now planted throughout the world is probably in southern Caucasia, now north-west Turkey, northern Iraq, Azerbaijan and Georgia (Mullins et al., 1992). Viticulture is a very old agricultural practice. It existed in Egypt 3500 BC (Kliewe, 1981) and pictures showing vines growing on structures date back to around 1500 BC (Janick, 2002). It is assumed that the Chinese started cultivating *V. vinifera* vines by 2000 BC (Huang, 2000) while people in Transcaucasia/Mesopotamia started it around 4000 BC (Olmo, 1996). The European Union is ranked number 1 all over the world for viticultural area (4.139.975 ha = 55%), table grape production (29.050.923 t = 43%) and wine production (191.015.000 hl = 67%) (Delrot, 2010).

Table 1.1: Surface area of the vineyards in major wine producing countries (ha)

Country	2000	2001	2002	2005	2006	2007
Spain	1.180.800	1.235.000	1.228.000	1.161.411	1.200.000	1.200.000
France	915.000	914.000	912.000	854.824	842.026	830.000
Italy	830.000	825.000	848.000	754.987	786.300	770.000
USA	356.500	415.000	412.000	378.320	379.271	380.000
Germany	104.724	103.605	104.000	98.875	99.172	99.500
Luxembourg*	1.344	1.342	1.309	1.300	1.299	1.279
Total World	7.913.000	7.918.000	7.950.000	7.340.758	7.520.595	7.501.872

Source: Organisation Internationale de la Vigne et du Vin (OIV).
* Das Weinjahr (2000 – 2007): Institute of Viticulture, Remich (Luxembourg).

Unfortunately, most of the cultivars used for winemaking – including the widely used European *Vitis vinifera* cultivars that account for about 90% of worldwide grape production for winemaking – are highly susceptible to several pathogens (Gomès and Coutos-Thévenot, 2009). Many of these pathogens have been introduced from distant wild grape varieties in North America that are tolerant to these pathogens. Chemical control is the most effective measure currently used to control diseases especially in viticulture. Since fungal infections are one of the major reasons for penalties in grape quality and yield losses, most common pesticides in viticulture are

fungicides (Rossberg, 2009). Therefore, grapevine disease control requires intensive use of fungicides (Costa et al., 2010). They are predominantly used to control downy mildew (causal agent: *Plasmopara viticola*), powdery mildew (causal agent: *Erysiphe necator*, formerly *Uncinula necator*) and grey mould (causal agent: *Botrytis cinerea*) (González-Álvarez et al., 2012). For example, in 2006, 54 % of the global sales of downy mildew fungicides were against *P. viticola* in grapevine (Gisi, 2008). However, the inadequate use of pesticides in viticulture can cause increased concentrations of their residues in vineyards soils (and other environmental compartments) and in the wine (Flores-Vélez et al., 1996; Ribolzi et al., 2002; Hildebrandt et al., 2008) raising public concern (Jacobson et al., 2005). Therefore, one of the major goals of sustainable viticulture is the reduction of fungicide input in vineyards. Thus, grape growers face increasing pressure to reduce those treatments (Jacobson et al., 2005, Komárek et al., 2010).

Induced resistance that relies on the plant's own resistance mechanisms seems to be a promising alternative to currently used strategies (Walling, 2001; Gozzo, 2003; Vallad and Goodman, 2004). There have been increasing evidences in the literature that induced resistance using plant activators is environmentally friendly in disease control (Iriti et al., 2011). Induced resistance is based on the notion that plants can be 'immunized' against future infection by pathogens (Hunt et al., 1996). Adrian et al. (2004) have suggested that stimulation of the synthesis of phytoalexins, which are antimicrobial compounds, could be a strategy to limit the use of pesticides in vineyards. Moreover, it is assumed that pathogens cannot easily develop resistance against host based defense responses such as production of pathogenesis-related (PR) proteins and phytoalexins (Gisi, 2002), which are produced during induced resistance.

1.2 Grapevine downy mildew

1.2.1 History

Downy mildew of grapevine has both economic and historic importance (Viennot-Bourgin, 1981; Hewitt and Pearson, 1988). The story of downy mildew in Europe started with grapevine phylloxera, the root insect native to eastern North America, when it was first introduced around 1863. The French government offered a reward of 300.000 francs in 1873 to anyone who could find a way to stop phylloxera. Though attempts have been made, the prize was never claimed (Campbell, 2004). Therefore, grafting the European *V. vinifera* grapes to American rootstocks was the best solution at that time. Unfortunately, with the introduction of phylloxera-resistant rootstocks, another North American native pathogen (*Plasmopara viticola, Class: Oomycota*), was unintentionally introduced. Since that time, downy mildew caused by *P. viticola* became the most important grapevine "fungal" disease in middle Europe (Müller and Sleumer 1934; Mohr 2005; Agrios, 2005). Downy mildew was first reported in France in 1878 and within four years it had spread to all regions of France (Pearson et al., 1988).

Like the introduction of the pathogen to Europe, its first control method was also discovered unintentionally. It started when a vine-grower outside the town of Bordeaux painted grapevine clusters near the road with a greenish blue paste made by mixing copper sulphate and lime that gave leaves and fruits an unappetizing taste to stop pedestrians from stealing grapes from his vineyard (Prial, 1987). One day a passing scientist (Pierre-Marie Alexis Millardet) observed that these grapes did not develop downy mildew. In 1885, he introduced his famous bouillie bordelaise (Bordeaux mixture) composed of copper sulphate and lime. Since then copper containing fungicides are still used around the world for control of members the oomycetes, especially in organic agriculture (e.g. *P. viticola*, *Phytophthora infestans*, *Pseudoperonospora humili, Peronospora tabacina*).

1.2.2 Economic importance

Plasmopara viticola (Berk. and Curtis) Berl. and De Toni (1888), the causal agent of downy mildew, is native to the North American wild grapevine species. Although it attacks native grapevines, it does not affect them very seriously because they have co-evolved with the pathogen and developed a tolerance against it. However, it is a devastating disease on the V*itis vinifera* species. Since the grapevine *Vitis vinifera* –

the European branch – had evolved in the absence of the downy mildew pathogen, it is extremely susceptible to it. Downy mildew is still most destructive in Europe and in the eastern half of the United States, particularly in regions with warm, wet climate during the growing season (Wong et al., 2001), while dry areas are usually free of the disease.

Downy mildew affects leaves, fruits, and shoots of grapevines. It causes losses through killing parts of the leaf tissues or total defoliation, through production of low-quality or entirely destroyed grapes, and through weakening, dwarfing, and killing of young shoots. When the weather is optimal for the pathogen and no protection is provided, downy mildew can easily destroy 50 to 75% of the crop in one season (Agrios, 2005). Only after the epidemiology of the disease was uncovered by Müller and Sleumer (1934), the use of efficient plant protection products was optimized (Claus, 1979). However, in every season downy mildew requires substantial chemical treatments to ensure high quality grape production (Schmitt et al., 2010). If weather conditions are favorable for disease development (wet, moderate temperatures) and heavy disease pressure will arise, eight to ten applications may be necessary to control the disease. In organic viticulture this problem is even more severe due to the lack of efficient control agents and their generally minor efficiency period.

1.2.3 Biology and life style of *Plasmopara viticola*

The causal agent of grape downy mildew belongs to the class of the Oomycetes. Due to their morphological, physiological and ecological similarities to fungi, the Oomycetes, known also as water molds, were traditionally treated within mycology and have previously been included within the kingdom of Fungi; however, recent findings concerning their evolutionary phylogeny have led to their re-classification into the new domain Chromista (Stramenopiles) (Van der Auwera et al., 1995) (Table 1.2), which also includes chromistan (heterokont) algae (Cavalier-Smith, 1986; Dick, 2001, 2002; Kirk et al., 2001). *P. viticola* belongs to the family of Peronosporaceae (Gams et al., 1998, Dick, 2002). Although they share a similar lifestyle with true fungi, they are more closely related to algae (Harsham, 2007). Unlike true fungi, they contain ß-1,3- and ß-1,6-glucan, cellulose (ß-1,4-glucan) and recently it was shown that small amounts of chitin are present their cell walls (Agrios, 2005; Kortekamp, 2008).

Peronosporales, an order of Oomycetes, are obligate biotrophs. They establish intimate relations with their hosts by inducing a complex reorganization of host and pathogen cellular membranes forming special structures (haustoria) for obtaining nutrients from the host through redirecting host metabolism and suppressing host defense (Hahn and Mendgen, 2001; Voegele and Mendgen, 2003; Hückelhoven, 2005, 2007; O'Connell and Panstruga, 2006). *P. viticola* is a heterothallic (self-sterile) diploid oomycete consisting of two mating types that must be present for sexual reproduction to occur (Agrios 2005; Wong et al., 2001). It is also a holomorphic oomycete that has both sexual (teleomorphic) and asexual (anamorphic) stages, the former represented by oospores, while the latter by zoospores.

Table 1.2: Taxonomic classification of *P. viticola* (Gams et al., 1998, Dick 2002)

Kingdom	Chromista (Synonyme: Stramenopila)
Phylum	Oomycota (Pseudofungi)
Class	Oomycetes
Order	Peronosporales
Family	Peronosporaceae
Genus	Plasmopara
Species	*Plasmopara viticola*

1.2.4 Disease development and symptoms of downy mildew

P. viticola survives the winter (overwintering) as oospores, first in dead leaf lesions and shoots, after litter degradation spare in the soil. In certain areas mycelium overwinters in infected, but not killed, twigs (Agrios, 2005). Infection cycle (primary infection) of *P. viticola* starts with the oospores that germinate during rainy periods in the spring when environmental conditions are suitable (sufficient soil moisture, > 95 % relative humidity and temperature above 12 °C) for growth. Normally, sexual propagation exists prior to overwintering of infectious spores, which provide the first source of inoculum in spring (Kiefer et al., 2002) and for further soil-borne infections throughout the growing period (Loskill et al., 2006). However, the rapid sequence of asexual propagation is responsible for the efficient spread (secondary infection) of this oomycete during the growing season (Gobbin et al., 2005; Kennelly et al., 2007; Vercesi et al., 1999). Overwintered oospores germinate forming macrosporangia that, under the aforementioned conditions, release zoospores (swarm spores) that are motile asexual spores utilizing flagella for locomotion. This process is more efficient

in the dark, since light can interrupt it (Brook, 1979; Rumbolz et al., 2002). Zoospores are splashed with rain drops into the canopy as “infested” water falls onto the leaves where they initiate the first infection cycle (Figure 1.1). After reaching the abaxial side of the leaf, they swim in the water film until they encounter a stoma, where they shed their flagella and encyst. Subsequently, a germ tube emerges from each spore and reaches into the substomatal cavity (Gindro et al., 2003; Kiefer et al., 2002), where it expands forming an infection vesicle. Eventually, a primary hypha emerges (opposite to the site of the spore), and develops into a mycelium that spreads within the leaf tissue forming haustoria that penetrate into the cells of the host (Kiefer et al., 2002). In later stages of infection, further hyphae are developed, allowing for a further spread of *P. viticola* in the intercellular spaces (Kortekamp et al., 1998). Since *P. viticola* is heterothallic, the existence and meeting of two different mating types in the colonized tissues can lead to the formation of oogonium and antheridium (Wong et al., 2001; Scherer and Gisi, 2006). After mating of female and male gametes in the oogonium, an oospore is formed (Agrios, 2005) that encyst in the leaf litter. After overwintering, the infection in the next season is initiated by this robust dormant body.

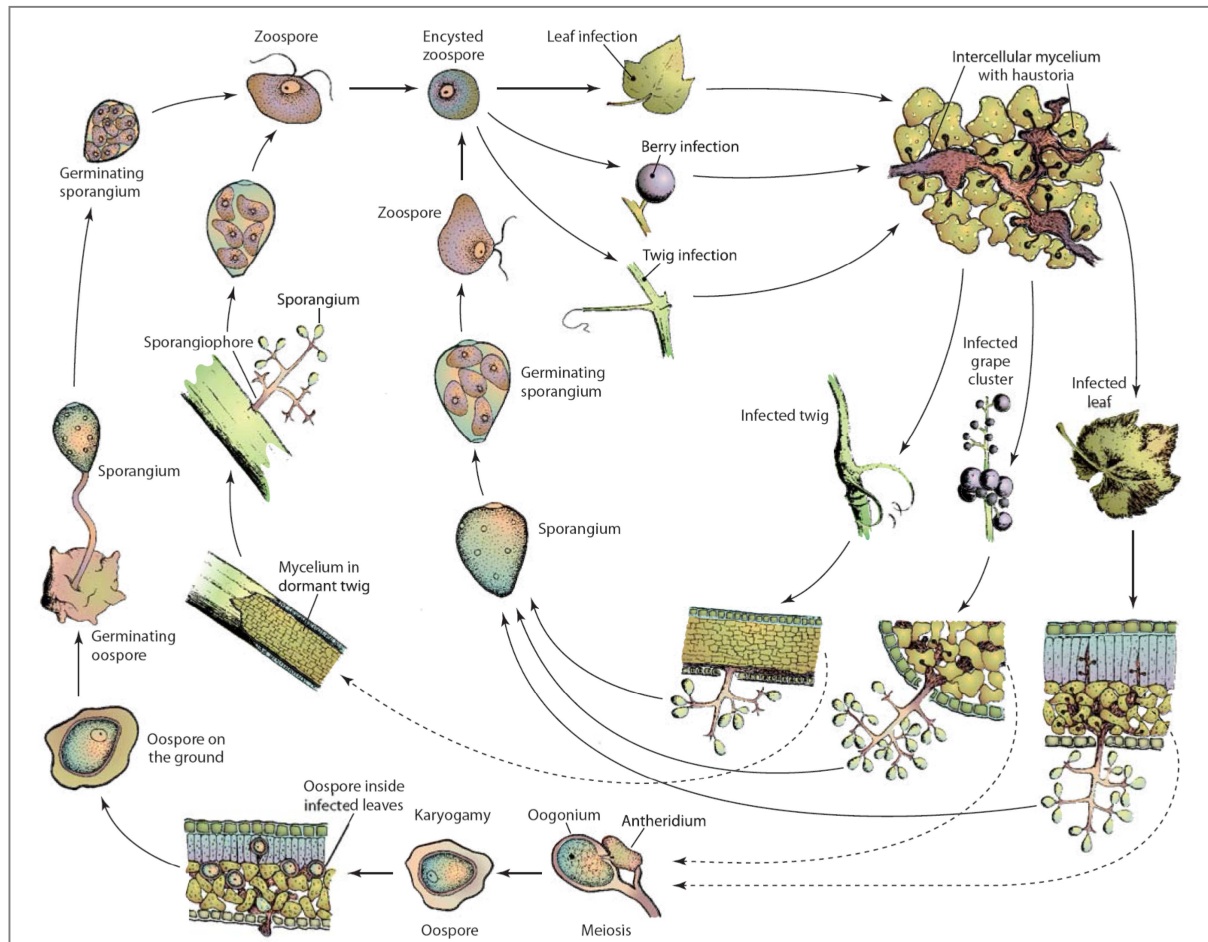


Figure 1.1: Disease cycle of downy mildew of grapes caused by *Plasmopara viticola*. Agrios, 2005

The speed of incubation time and resulting oomycete spreading depend on temperature and relative humidity. Under optimal conditions (22-25 °C) first symptoms (oilspots) are visible after four to five days. However, under natural conditions average incubation periods range between 5-18 days (Mueller and Sleumer, 1934). Subsequent to incubation sporulation may occur if relative humidity is high (> 97 %), temperatures are favorable (12.5 °C at the beginning of the wet period; average temperature 11 °C) and darkness for a minimum of four hours is provided. After sporulation, sporangiophores with eggshaped sporangia containing asexually produced zoospores emerge on the abaxial side of the leaf from the stomatal cavity. Those zoospores are the initial cells for the first secondary disease cycle. Under favorable conditions (humid, warm, dark), secondary infection can be repeated five to eight times a year (Schlösser, 1997).

Symptoms start first as small, pale yellow irregular spots appearing on the upper surface of the leaves. Later, “oily spots” are seen on the upper leaf surface due to the colonization of the parenchymal cells and absorption of the nutrients from these cells

through haustoria (Keil, 2007). Subsequently, a white downy growth of the sporangiophores may appear on the underside of those oilspots (Figure 1.1). Eventually, the infected leaf areas are killed and turn brown in the centre of each lesion, while the sporangiophores of the oomycete turn grey. The spots often enlarge to form expanded dead areas and frequently lead to premature defoliation (Agrios, 2005). In infected half grown berries, the fungus can grow completely inside with no visible white sporangiophore growth, but the berries would dry out and turn into so-called leather berries. In late or localized infections of shoots, the shoots usually are not killed but show various degrees of distortion (Agrios, 2005).

1.2.5 Chemical control of *P. viticola*

The copper-containing Bordeaux mixture represented the first milestone in the control of *P. viticola*. Thus, it is considered the first oomycete fungicide in the history of phytomedicine (Heitefuss, 2000). Until today, copper is still used in viticulture as copper oxychloride, copper hydroxide and copper octanoate, respectively. In organic viticulture, the use of copper-based products is still very important, being the most reliable method to control grape downy mildew (Berkelmann-Löhnertz et al., 2008, 2012). Copper accumulates in target pathogens and forms complexes with enzymes possessing sulphydryl-, hydroxyl-, amino-, or carboxyl-groups; as a consequence, the enzymes are inactivated. This leads to a general disruption of metabolism and breakdown of cell integrity, which interfere with the germination of oomycete spores. Therefore, copper-containing fungicides need to be applied protectively. In addition to the direct toxic effect, copper also causes a retardation of plant growth and a hardening of foliage and berries, which has additional benefit such as protection against secondary pathogens and climatic stress (Gisi, 2002). Copper-containing fungicides could also lead to some desirable physiological effects such as improving the maturity and strength of the wood (Winkler 1980; Brendel, 1984). The era of copper-free fungicides confirmed the efficacy of zinc dimethyldithiocarbamate against *P. viticola* (Morel, 1946). After World War II copper-free fungicides were successful because they are stable and cost effective (Zobrist, 1954). Moreover, fewer problems with phytotoxicity were observed than in the case of copper-based fungicides (Kundert, 1956). Later, many organic, copper-free fungicides were developed to control *P. viticola* such as cymoxanil (Serres and Carraro, 1976), acylalanine metalaxyl (Murolo and Stanich, 1980; Wicks, 1980; Cesari et al., 1981; Marais and

Tromp, 1981), phenylamide oxadixyl (Gisi et al., 1983) and the strobilurin-based fungicides (Godet et al., 1997).
Quinone outside inhibitors (QoIs) e.g. strobilurins, phenylamides e.g. mefenoxam, carboxylic acid amides e.g. dimethomorph, mandipropamid and cyano-acetamide oximes (cymoxanil) (Gessler et al., 2011) inhibit mitochondrial respiration, while phenylamides inhibit rRNA polymerization, whereas the modes of action of the other two fungicides are unknown (Gisi and Sierotzki, 2008). As for phenylamides, quinone outside inhibitors strobilurins and cyano-acetamide oximes, their curative effect is limited, therefore, protective treatments should dominate the spraying programme or treatments with curative acting agents should be applied strictly in the first quarter of the incubation period (Kassemeyer, 2008). In viticulture, the latter strategy requires a sensitive forecast model with high accuracy and validity (Berkelmann-Löhnertz, 2012).
Products with phosphorous acid and phosphonates are used to control *P. viticola*; however, they are not considered as true fungicides since they do not directly kill the pathogen and some of them are commercialized as fertilizers (Gessler et al., 2011). Nevertheless, they provide good protection against *P. viticola* (Magarey et al., 1990; Magarey et al., 1991a). Phosphonate has both preventive as well as post-infection activity (Gessler et al., 2011). When applied up to 13 days post infection, it even reduced sporulation (Wicks et al., 1991).
However, some of the mentioned fungicides have been removed from the market according to the guidelines for plant protection products in the European Union (council directive no. 414/91). Recently, new active ingredients have been developed (Egger, 2008) such as Iprovalicarb (Stenzel et al., 1998), Famoxadone (Andrieu et al., 2001), Fenamidone (Latorse et al., 1998; Mercer et al., 1998) and Benzamide Fluopicolide (Gouot, 2006; Latorse et al., 2006).

1.2.5.1 Side effects of chemical control

Copper-based products may be associated with many side effects but most importantly is the contamination of the soil with high concentration of copper. Since copper is a heavy metal, it is not easily degraded in the soil and, therefore, the long term use of these products leads to soils with high concentration of copper (Kühne et al., 2011). In a French study, for example, some vineyard soils were found to contain between 100 and 1500 mg Cu/kg soil (Flores-Veles et al., 1996; Besnard et al.,

1999). Those concentrations are many times higher than normal (~ 60 mg/kg) (Pietrzak and McPhail, 2004). Copper-based products may be associated other side effects such as phytotoxicity visible as burning of young shoots and leaves (Claus, 1979). Some copper products such as copper sulfate (blue vitriol) can inhibit the germination of pollen grain and/or the growth of pollen tube (Gärtel, 1961). Furthermore, the long-term use of copper-containing fungicides in vineyards resulted in its accumulation in the soil (Parat et al., 2002; Pietrzak and McPhail, 2004). More seriously, the exposure to some pesticides presents a toxicological risk for workers in agriculture (Remor et al., 2009) if precautions are not taken. It was reported that vineyard workers might develop serious acute and chronic respiratory problems, including lung carcinoma, due to the inhalation of Cu-containing fungicides (especially the Bordeaux mixture) (Pimentel and Marques, 1969; Zuskin et al., 1997; Santić et al., 2005).

1.2.5.2 Resistance of *P. viticola* to some fungicides

Due to the non-specific mode of action of Cu-containing fungicides, no resistant strains of *P. viticola* occurred. However, risks associated with the use of synthetic organic fungicides are mainly concerned with the emergence of resistant strains of *P. viticola* (Table 1.3) (Chen et al., 2007). In 1980, the first resistant isolates of *P. viticola* were observed against phenylamides in France (Clerjeau and Simone, 1982; Moreau et al., 1987). Later, other resistant strains were found against metalaxyl, ofurace and milfuram (Gay-Bellile et al., 1983), anilide (Clerjeau et al., 1984), cymoxanil (Gullino et al., 1997) and QoI fungicides (Wong and Wilcox, 2000). To avoid resistance against specific single-site fungicides, products with multi-site activity are recommended to be used in alteration with single-site fungicides. Some of those multi-site fungicides used in mixtures or in alternation with site-specific fungicides are, for example, Mancozeb, Folpet, Chlorothalonil and copper formulations (Gessler et al., 2011). However, in a growth chamber study it was found that 10 % of *P. viticola* populations developed resistance after 2-4 pathogen cycles after treatment with one spray of Oxadixyl + Mancozeb mixture (Samoucha and Gisi, 1987c). Thus, to prevent emerging of resistance strains, fungicides that share the same active ingredients should be used repeteadly in during vegetation period.

Table 1.3: List of fungicides against which some strains of *P. viticola* have developed resistance. From Fungicide Resistance Action Committee (FRAC), 2011

Fungicides	Remark	Reference
Phenyl-amides	In the field	Staub et al., (1981); Bosshard et al., ,(1983); Leroux et al., (1985)
Quinone outside Inhibitors QoIs (e.g. Strobilurins)	In the field	Heaney et al., (2000); Gullino et al., (2004); Sierotzki et al., (2005) ; Wong and Wilcox, (2000)
Cyanoacetamide oximes	In the field	Gullino et al., (1997)
Phosphonates	In the field	Khilare et al., (2003)
Metalaxyl, ofurace and milfuram	In the field	Gay-Bellile et al., (1983)
Anilide	In the field	Clerjeau et al., (1984)
Cymoxanil	In the field	Gullino et al., (1997)
Carboxylic acid amides	Inheritance of resistance	Gisi et al., (2007); Blum et al., (2010)

1.2.5.3 Integration of the agro-ecosystem to reduce the use of fungicides

In order to reduce negative impact of chemical control in viticulture, the use of pesticides – particularly fungicides – has to be limited. This demand is in line with general recommendations of integrated control and verbalized in several pesticide reduction programmes. To this end, several computer-based forecasting models that predict the onset and progress of diseases are used (Bleyer, 2008). The goal of such forecasting systems is the optimal timing of fungicide applications. Several simulation models have been developed in different countries for forecasting *P. viticola*: in Germany with “Geisenheimer Prognosemodell” (Berkelmann-Löhnertz et al., 2011) and “Freiburger Prognosemodell” (Kassemeyer, 1996); in Switzerland with the help of computer programs and warning systems (Blaise and Gessler, 1990; Siegfried et al., 1992); in Italy with PLASMO (Orlandini et al., 1993); in France with MILVIT (Muckensturm, 1995) and EPIcure (Raynal et al., 2007). In those models, the input parameters are local weather conditions such as temperature, rainfall and leaf wetness (determining infection events by sporangia releasing zoospores) as well as relative humidity and temperature (for the length of the incubation period). Some of them additionally predict the end of the ripening period and the germination potential of oospores representing one essential condition for primary infection (starting point of the epidemiology) (Berkelmann-Löhnertz et al., 2011). The output parameters are

the prediction of the primary infection and following soil borne infections, infections of the secondary disease cycle (leaf borne), individual incubation periods and sporulation, as well as the progress of resulting disease epidemics. Those parameters can be used by public consulters or directly by the grape growers as decision support tools for the timing of treatments and the appropriate choice of products (Gisi, 2002). Bleyer et al. (2008) and Berkelmann-Löhnertz et al. (2011) both integrated a system for the timing of applying the protective fungicides against downy mildew considering the amount of unprotected leaf area emerged since the last spray based on models that describe the rate leaf area development (Schultz, 1992).

1.3 Induced resistance

The activation of the plant’s defense system, known as induced resistance, has been the focus of many researchers (Sticher et al., 1997). Its specific activation is considered to be an alternative method to protect plants against pathogens (Walters et al., 2005). In viticulture, induced resistance has gained increasing attention (Gindro et al., 2012), especially since the risks associated with the use of pesticides have become evident (Flores-Vélez et al., 1996; Ribolzi et al., 2002; Hildebrandt et al., 2008). Although susceptible grapevine varieties fail to activate an effective defense response against *P. viticola* – probably because they did not co-evolve with the pathogen (Di Gaspero et al., 2007) – it has been shown that susceptible grapevine is able to defend itself against it (Gessler et al., 2011) if defense responses are activated. However, these defense responses are not activated in response to infection with *P. viticola* (Polesani et al., 2010) and require external elicitation.

1.3.1 History of induced resistance

Induced resistance has been known for a long time (Chester, 1933; Gäumann, 1946). This phenomenon earned increased relevance in crop protection since the studies of Frank Ross (Ross, 1966) and Joseph Kuć (Kuc, 1982; Madamanchi and Kuc, 1991; Hammerschmidt and Kuc, 1995). They demonstrated that this type of resistance could be linked to an activation of defense mechanisms such as the accumulation of pathogenesis-related (PR) proteins (Hammerschmidt and Kuc, 1995). Those biochemical evidences for inducible defenses were first reported in the 1950s (Kuc, 1957; Allen, 1959; Müller, 1959). Since that time many different

inorganic and organic compounds have been shown to induce resistance in plants (Kuc, 2001). For example, D- or DL-phenylalanine was found to induce resistance in apple (Kuc et al., 1959) while phenylserine induced resistance in cucumber (Hijwegen, 1963). By the end of the 1970s, salicylic acid was shown to be an inducer of resistance (White, 1979) in many plants. Moreover, some synthetic compounds have been demonstrated to induce resistance (Kessmann et al., 1994; Cohen, 2002). The first synthetic resistance activator (acibenzolar-S-methyl “ASM” also known as BTH) was commercialized in the 1990s, and many other chemicals with potential to induce resistance have been identified (Hammerschmidt, 2007). Induced resistance in crop protection exploits the phenomenon, where chemical compounds (abiogenic elicitors) or living organisms-derived compounds (biogenic elicitors) can mimic pathogen- or microbe-derived molecular patterns (PAMPs/MAMPs) or trigger downstream signaling and evoke the plant defense responses. The use of the term ‘activator’ in the context of systemic acquired resistance (SAR) was coined by Kessmann et al., (1994). During the last decade many resistance inducers have been tested for their ability to induce defense responses of the susceptible *V. vinifera* against *P. viticola* such as chitosan (Aziz et al., 2006), laminarin (Aziz et al., 2003), sulfated laminarin (Trouvelot et al., 2008a; Allègre et al., 2009), oligogalacturonide (Allègre et al., 2009), β-aminobutyric acid (BABA) (Reuveni et al., 2001; Hamiduzzaman et al., 2005; Dubreuil-Maurizi et al., 2010), Frutogard® (Harm et al., 2011), BTH (Perazzolli et al., 2008) and plant extracts (Godard et al., 2009).

1.3.2 Concept of induced resistance

During their whole life plants are exposed to a wide range of potential pathogens. Therefore, they have developed a number of (defense) resistance mechanisms to protect themselves. These defense mechanisms have been classified broadly as avoidance, tolerance and resistance (Parlevliet, 1992).

Resistance can be subdivided into two classes, (a) preformed (constitutive or passive) and (b) acquired (induced or active) resistance (Hammerschmidt and Nicholson, 1999). Preformed resistance has many types. The broadest type is the non-host resistance, where plants are resistant to most putative pathogens, to which the plant is not a host (incompatible interaction/avirulent pathogen). It is the most common (Heath, 2000) and the most effective form of resistance in higher plants (Mauch-Mani, 2002). The second type is general resistance. It is usually not race-

specific and commonly assumed to be polygenic. It is also known as field resistance, partial resistance, quantitative, or horizontal resistance. The third type is race-specific resistance, also called gene-for-gene resistance or vertical resistance. It is based on the specific interaction between the products of avirulence (avr) genes in the pathogen and resistance (R) genes in the host (Flor, 1971). The molecular basis for this type of resistance is explained by an elicitor-receptor model (Gabriel and Rolfe, 1990), where avr genes code for products that are recognized by the corresponding R gene products.

Induced resistance is the phenomenon by which a susceptible plant can be “induced” to defend itself against a broad range of virulent pathogens. It is a non-specific form of resistance that may act against a wide range of taxonomically unrelated pathogens (Ryals et al., 1994; Hunt et al., 1996; Hammerschmidt and Smith-Becker, 1999; Durrant and Dong, 2004; Bostock, 2005; Lyon, 2007). It depends on recognition of pathogen- or microbe-derived molecular patterns (PAMPs/MAMPs), also known as elicitors, via plant pattern-recognition receptors (PRRs) that induces plant resistance known as PAMP-triggered immunity (PTI) (Zipfel and Felix, 2005). These PAMPs could be bacterial flagellin, fungal chitin or oomycete glucans (Ausubel, 2005). PAMPs are in fact a new term for the previously so-called general elicitors (Aziz et al., 2007).

PTI represents the first set of defense responses activated by the plant under attack. However, these defense responses must integrate with other defense pathways such as salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) pathways (Glazebrook, 2005; Wang et al., 2008a) to provide protection.

Some pathogens, however, have evolved and acquired mechanisms to help them to escape PTI via pathogen Avr-proteins (effectors), causing effector-triggered susceptibility (ETS), that is assumed to be the key for successful pathogens to grow and multiply in a potentially hostile plant environment (Alfano and Collmer, 2004; Chisholm et al., 2006; Jones and Dangl, 2006). Nevertheless, plants respond by activating a second set of defense responses (Pieterse and Dicke, 2007) that relies on R-gene products (R-proteins) that recognize these effectors initiating the so-called effector-triggered immunity (ETI).

This could be done via direct interaction between R-protein and effector (receptor-ligand-model) (Deslandes et al., 2003; Dodds et al., 2006; Jia et al., 2000; Ueda et al., 2006), via monitoring their cellular targets (guard model) (Dangl and Jones, 2001;

Van der Biezen and Jones, 1998), or via the so-called decoy model, in which many effector targets mimic real targets to serve recognizing the effector, which then can be detected by the R-protein to activate defense (Nandi et al., 2003; Van der Hoorn and Kamoun, 2008; Zhou and Chai, 2008). However, ETI could be suppressed by modifying R-proteins using different strategies, ranging from host-protein ubiquitination, phosphorylation and dephosphorylation to alteration of the RNA metabolism (Block et al., 2008; Da Cunha et al., 2007) leading to effector-triggered susceptibility (ETS) (Jones and Dangl, 2006).

1.3.3 Local cellular events

In the past few years, several studies tried to decipher defense-related early signaling events in grapevine using model cell suspension cultures or entire plants (Repka, 2006; Vandelle et al., 2006). Plant responses to infection starts at membranes through pathogen recognition and signal transduction (Mathieu et al., 1991). Indeed, one of the early events after exposure to a pathogen is changing the membrane permeability. This leads to ions fluxes, such as K^+, H^+ and Ca^{2+} (Mathieu et al., 1991; Thuleau et al., 1994), which in turn leads to gene activation and triggering of defense responses. Another early reaction is related to changes and reinforcement of structural barriers, cell wall appositions, the so-called "papillae", which are formed at the infection site. They contain callose and other polysaccharides, phenolic compounds, reactive oxygen species (ROS) and various proteins (Flor et al., 2005). In tolerant grapevine varieties such as Solaris, callose was deposited at the stomata after infection with *P. viticola* (Gindro et al., 2003).

Reactive oxygen species (ROS) are forms of oxygen that are energetically more reactive than molecular oxygen. Typically ROS (sometimes also referred to as AOS, active oxygen species, or ROI, reactive oxygen intermediates) are molecular species that have undergone electron addition(s) and thus can be reduced or excited forms of oxygen. ROS are generated in plants in many ways in several cellular compartments (Mittler, 2002; Desikan et al., 2004b). Host plants can increase the concentration of ROS such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) in a process known as oxidative burst (Hammerschmidt and Nicholson, 1999). This oxidative burst can coordinate a set of defense responses in plants (Aziz et al., 2004). They can trigger signals that affect gene expression (Lamb and Dixon, 1997), strengthen plant cell walls through cross-linking reactions (Thordal-Christensen et al.,

1997) and they also initiate later defense responses (Hammerschmidt and Nicholson, 1999). ROS may regulate the expression of stress related genes directly through modification of transcription factor activity, or indirectly, through signal transduction such as the Mitogen Activated Protein Kinase (MAPK) cascade. Moreover, it is also possible that ROS act as transmissible signals, mediating long-distance effects (Alvarez et al., 1998). ROS at the site of infection may also be produced in quantities capable of killing micro-organisms directly (Apostol et al., 1989; Legendre et al., 1993; Walters, 2003; Custers et al., 2004).

The hypersensitive response, a form of programmed cell death (Kamoun et al., 1999), is localized and rapid cell death of one or a few host plant cells in response to invasion by a pathogen. The response can be very effective against obligate biotrophic parasites, as they require living host cells for nutrition (Hammerschmidt and Nicholson, 1999). ROS affect establishment of infection, enable redox signal transduction e.g. hydrogen peroxide together with nitric oxide (NO) and salicylic acid (SA), amplify resistance responses (Delledonne et al., 2003) and trigger programmed cell death (Kamoun et al., 1999).

Another set of pathogen-responsive molecules are synthesized during induced resistance. These are high molecular weight compounds known as pathogenesis-related proteins (PR-proteins). 17 different classes of these proteins have been identified in plants. Some of them have unknown functions (PR-1 and PR-17) (Van Loon and Strien, 1999; Van Loon et al., 2006b). β-(1,3)-glucanases (PR-2) and chitinases (PR-3, -4, -8 and -11) target the fungal cell wall. Thaumatin (PR-5), proteinase inhibitors (PR-6), peroxidases (PR-9), endoproteinases (PR-7), ribonucleases (PR-10), defensins (PR-12), thionins (PR-13), lipid transfer proteins (PR-14), oxalate oxidases or germin and germin-like proteins (PR-15 and PR-16) are also considered among the PR proteins (van Loon et al., 2006b).

Representatives of each class (with the exception of PR-13) have been found and identified by sequence similarity search in grapevine (Gomes and Thévenot, 2009). A putative sequence of a *V. vinifera* PR-1 protein was identified and cloned by Bertsch et al. (2003). The expression of PR-1 is strongly dependent on the nature of elicitor used (Repka, 2001b). The PR-2, -3, -4 and -5 classes are well documented in grapevine, while PR-15 and PR-16 (germin and germin-like proteins) were only lately described in grapevine. Seven members of the grapevine germin-like multigenic family were cloned in *V. vinifera* (Godfrey et al., 2007).

Other antimicrobial low molecular weight secondary metabolites, known as phytoalexins, may be produced locally at the site of infection (Hammerschmidt, 1999). Stilbenes, the predominant phytoalexins in grapevine, possess antifungal activity and thus enable plants to cope with pathogen attack (Bavaresco, 2009). Grapevine stilbenes have been identified as resveratrol (trans- and cis-isomers, 3,4',5-trihydroxystilbene) (Langcake and Pryce, 1977a), resveratrol glucosides including piceid (trans- and cis-resveratrol- 3-O-β-D-glucopyranoside) (Waterhouse and Lamuela Raventòs, 1994; Mattivi et al., 1995; Romero-Pérez et al., 1999) and resveratroloside (resveratrol- 4'-O-β-D-glucopyranoside), viniferins (Langcake and Pryce, 1977b; Bavaresco et al., 1997; Pezet et al., 2003), and pterostilbene (trans-3,5-dimethoxy-4'-hydroxystilbene) (Langcake et al., 1979). Resveratrol, the most studied and best known stilbene (Bavaresco, 2009), is mainly found in grape skin and therefore present in wine (Siemann and Creasy, 1992). It is claimed to play a role in the reduction of risk for cardio-vascular diseases, cancer and other diseases (Aggarwal and Shishodia, 2006). Grapevine contains constitutively produced stilbenes. They are also present in small amounts in lignified organs such as stems and canes, in seeds and in roots (Bavaresco and Fregoni, 2001). The role of downy mildew infection on stilbene synthesis has been extensively investigated (Bavaresco and Fregoni, 2001). A more recent study pointed out that resveratrol dehydrodimers, the δ-viniferin and ε-viniferin, are the major dimers to be synthesized under stress from *P. viticola* (Pezet et al., 2003; Gindro et al., 2006). According to Pezet et al. (2004b) resistance is associated with the conversion of resveratrol to viniferins, while susceptibility is associated with the formation of piceid from resveratrol, which indicates that resveratrol metabolism may play an important role in connection with resistance or susceptibility of vines.

1.3.4 Systemic cellular events

1.3.4.1 Systemic acquired resistance (SAR)

The two forms of induced resistance that have been so far best characterized are (a) systemic acquired resistance (SAR) and (b) induced systemic resistance (ISR) (Van Loon et al., 1998). SAR results from limited primary infection by a pathogen, whereas ISR can be triggered by nonpathogenic organisms that colonize root or leaf surfaces. Resistance is induced at the whole plant level by a localized pathogen inoculation

(Durrant and Dong, 2004). However, the two forms are mechanistically different in being based on different molecular mechanisms (Hammerschmidt, 2009).

SAR can be broadly defined as a form of induced resistance that is activated throughout a plant typically following infection by a pathogen that causes localized necrotic lesions. The necrosis can be the result of disease induced by a pathogen or a hypersensitive response (HR) (Kuc, 1982; Kuc et al., 1975; Ross, 1961b). SAR is dependent on salicylic acid (SA) signaling (Gaffney et al., 1993). However, the role of SA as a mobile signal for SAR is still debatable (Rasmussen et al., 1991; Shulaev et al., 1995; Vernooij et al., 1994; Dempsey and Klessig, 2012; Fu et al., 2013). The development of SAR takes several days and is accompanied by the systemic expression of genes encoding PR-proteins and their protein products (Hammerschmidt, 1999a; Van Loon, 1997; Van Loon and Van Strien, 1999; Van Loon et al., 2006). Plants expressing SAR are ‘‘primed’’ to respond to subsequent infections by expression of additional defenses, such as the oxidative burst, cell wall alterations at the site of attempted penetration, and phytoalexin production (Conrath et al., 2000, 2002, 2006). Moreover, SAR is effective against a broad range of pathogens that include bacteria, true fungi, oomycetes and viruses (Deverall, 1995; Hammerschmidt and Kuc, 1995; Kuc, 1982). However, SAR is most effective against biotrophic and hemibiotrophic pathogens and not against necrotrophs (Glazebrook, 2005; Oliver and Ipcho, 2004).

1.3.4.2 Induced systemic resistance (ISR)

Unlike SAR, ISR induction is associated with the interaction with certain plant growth promoting rhizobacteria that do not induce a necrotic response or cause any type of visible symptoms. ISR is dependent on jasmonic acid (JA) and ethylene (ET) signaling pathways, and its induction does not result in systemic expression of PR genes (Van Loon et al., 1998). Unlike SAR, it has been suggested that ISR is most effective against necrotrophic pathogens (Glazebrook, 2005). Although no defense mechanisms are activated in aboveground plant tissues upon perception of the resistance-inducing signal from growth promoting rhizobacteria, plant expressing ISR are primed (sensitized) to express basal defense responses faster and/or more strongly in response to subseuent pathogen attack (Conrath et al., 2002). This leads to broad-spectrum resistance with minimal impact on seed set and plant growth (Van Hulten et al., 2006), since it offers a cost-efficient resistance strategy, enabling the

plant to react more effectively to any invader encountered by boosting infection-induced cellular defense responses (Beckers and Conrath, 2007; Conrath et al., 2006).

Although SAR and ISR work through different pathways, there is a cross-talk between SA- and JA/ET-pathways. They can act antagonistically, additively, or synergistically depending on the intensity and duration of the signals provided to the host plant (Mur et al., 2006). Moreover, these pathways are interacting with other defense signals (ABA, auxin, GA, H_2O_2, and NO) known to enhance or antagonize SA- and/or JA-defense signaling (Lopez et al., 2008; Robert-Seilaniantz et al., 2007; Spoel and Dong, 2008; Lindermayr et al., 2010). However, this cross-talk is necessary for the plant to fine-tune its defense responses (Bostock, 2005; Pieterse et al., 2006).

1.4 Induced resistance in grapevine

Since the study of defense responses in plants is time consuming, cell cultures/suspensions are often used due to their simplicity and fast results. Grapevine cell suspension has been shown to be a good model system of reduced complexity for studying the defense mechanisms (Bru et al., 2006). This system has been used to study defense related responses using either chemical compounds or cell wall extracts of micro-organisms (Liswidowati et al., 1991; Melchior et al., 1991; Morales et al., 1998; Tassoni et al., 2005). Most of the elicitors investigated in cell culture systems are oligosaccharides (Côte et al., 1998; Ridley et al., 2001; Aziz et al., 2004). Nevertheless, there are also many studies that are made on plants in greenhouse and field trials.

Various molecules, such as laminarin (Aziz et al., 2003), β-aminobutyric acid (BABA) (Slaughter et al., 2008), BcPG1 (Poinssot et al., 2003), cyclodextrin (Bru et al., 2006), sulfated laminarin (PS3) (Trouvelot et al., 2008) and salicylic acid (Wen et al., 2005) have been shown to induce defense responses in grape vine (Table 1.4). Some plant extracts have also been shown to possess direct antifungal properties against pathogenic fungi, while others could indirectly inhibit fungal development by eliciting endogenous mechanisms of defense against *P. viticola* (Gindro et al., 2007). However, it is necessary to further evaluate the activities of new natural products as fungitoxic compounds or as elicitors to enhance crop protection (Fung et al., 2008; Copping et al., 2007) in the field.

Two scenarios are expected after treating plants with resistance inducers. Either defense responses will be induced and lead to physiological changes even before the infection occurs or no or few defense responses occur until plants are challenged by the pathogen and then a faster and/or greater defense responses will be activated. This later phenomenon is known as 'priming' or sensitizing (Zimmerli et al., 2000; Conrath et al., 2001, 2002). BABA is considered as a priming agent, where pretreatment with low concentrations BABA primes the cells to react more quickly and efficiently to subsequent elicitor treatment or pathogen attack (Conrath et al., 2002). Table 1.4 shows resistance inducers used to elicit the grapevine defense responses against *P. viticola* in cell cultures and cell suspensions as well as in greenhouse and field.

Table 1.4: Examples of resistance inducers suitable for application or even used in grapevine production

Elicitors (plant activators)	Target pathogen	References
BABA-Cu complex	*Plasmopara viticola*	Reuveni et al., (2001)
Cellulose from *Trichoderma viride* (in cell culture)	Several pathogens	Calderón et al., (1993)
BABA (β-aminobutyric acid)	*Plasmopara viticola*	Cohen et al., (1999); Slaughter et al., (2008)
BTH Bion® (in Europe) and Actigard® (in the USA)	*Botrytis cinerea* and *Plasmopara viticola*	Iriti et al., (2004); Harm et al., (2011)
Oxycom™ (5% v/v stabilized solution of peracetic acid)	Several pathogens	Kim et al., (2001)
Milsana® (extract of giant knotweed *Reynoutria sachalinensis*)	Several pathogens	Schilder et al., (2002); Schmitt et al., (2002)
Chitosan-based activator called Elexa®	*Plasmopara viticola* and *Erysiphe necator*	Schilder et al., (2002)
Methyl jasmonate (MeJA) (suspension culture)	Several pathogens	Repka et al., (2001; 2004), Hamiduzzaman et al., (2005)
Laminarin (β-1,3-glucan)	*Plasmopara viticola*	Aziz et al., (2003)
Frutogard®	*Plasmopara viticola*	Harm et al., (2011)
LIN (Linoleic acid)	*Plasmopara viticola*	Harm et al., (2011)
Algin Biovital®	*Plasmopara viticola*	Tilco Biochemie
Myco-Sin VIN®	*Plasmopara viticola*	Tilco Biochemie

1.4.1 Allocation (fitness) cost of induced resistance

Costs in evolutionary terms are defined as any trade-off between resistance and another fitness-relevant process (Heil, 2002). Thus, allocation costs are the impacts that result due to the distribution of metabolic resources to resistance pathways rather than to other processes such as growth or reproduction (Heil, 2002). Induced resistance is expressed only after pathogen challenge or chemical elicitation to save the metabolic effort and/or avoid possible negative effects of defense responses when they are not required (under pathogen-free condition) (Heil, 1999, 2001a). Negative impacts of resistance might result from autotoxicity, since some resistance traits are toxic to the plant, and their constitutive expression might impose a significant metabolic burden (Baldwin and Callahan, 1993). It has also been shown that constitutive expression of induced resistance under pathogen-free conditions can have negative effects on plant growth and reproduction (Durrant and Dong, 2004) and – in the case of grapevine even more important – possibly on quality. Therefore, it is advisable to conduct further studies with each resistance inducer, under pathogen-free conditions, to check if there is any allocation cost.

1.4.2 Resistance inducers in combination with fungicides

Unfortunately, application of induced resistance in the vineyard still often suffers from inconsistency and provides only limited disease control (Adrian et al., 2012) presumably because of the influence of environmental conditions, genotype and crop nutrition on the expression of induced resistance (Regnault-Roger, 2012). Therefore, it was suggested that resistance inducers should be considered as an additional option within the framework of an integrated crop management strategy rather than as direct replacements for fungicides (Lyon and Newton, 1999). Indeed, they may complement them synergistically when used in combination (Harm et al., 2011; Baider et al., 2003), thereby reducing the input of fungicides in viticulture. It has been reported that a combination between fungicides and elicitors provides effective protection. In some cases the combination between the fungicide and resistance inducers resulted in a reduction of the required fungicide amount (e.g. copper containing fungicides). Another way to reduce fungicide input is to replace one or more of the application dates with resistance inducers. Table 1.5 lists fungicides that have been used in combination with plant activators. For example, a combination of Oxycom™ and Microthiol® fungicide was more effective than Microthiol® alone in

protecting grapes (berry clusters) against powdery mildew caused by *Erysiphe necator* (Reglinski et al., 2007). Also BABA enhanced the activity of Fosetyl aluminium (Fosetyl-Al) and N-(trichloromethylthio) Phthalamide (Folpet) for controlling *Plasmopara viticola* (Reuveni et al., 2001). In vineyard trials, application of Milsana® every 7-10 days reduced the incidence of powdery mildew and bunch rot (*Botrytis cinerea*) on grape berries to the same degree or better than sulfur and the copper containing agent FW 450 (Dow AgroSciences) (Schmitt et al., 2002). Tank mixes containing BABA + Fosetyl-Al and BABA + Folpet, at reduced rates, were as efficacious as metalaxyl + Cu (Ridomil®-Cu) or Dimethomorph + Mancozeb (Acrobat® Plus) (Reglinski et al., 2007). A disease management program was proposed that integrated BABA with other fungicides in order to reduce intensive use of site-specific fungicides against *P. viticola*. This is of particular interest, since, as previously mentioned, fungicide resistance is a concern for the control of downy mildew in vineyards (Leroux and Clerjeau, 1985). Some plant activators are able to provide full protection against one disease at least. For example, in the greenhouse and field trials Frutogard® completely controlled grape downy mildew (no disease symptoms appeared) (Harm et al., 2011).

Table 1.5: List of plant activators (elicitors) used in combination with fungicides in grapevine

Elicitors in combination with fungicides	Target pathogen	References
BABA in combination with fungicides (BABA + fosetyl-Al and BABA + Folpet)	*Plasmopara viticola*	Reuveni et al., (2001)
Milsana® and Myco-Sin® in combination with the bacterial antagonist (*Brevibacillus brevis*)	*Plasmopara viticola* and *Erysiphe necator* & *Botrytis cinerea*	Schmitt et al., (2002)
5-chlorosalicylic acid (5CSA) in combination with the fungal antagonist *Ulocladium oudemansii*	*Botrytis cinerea*	Reglinski et al., (2005)
Oxycom™ in combination with fungicide (Microthiol®)	*Erysiphe necator*	Reglinski et al., (2007)

1.5 Aim of the work

Application of fungicides is the most effective method to control downy mildew and other pathogens in viticulture. This is true for integrated viticulture as well as for organic viticulture. Considering the aforementioned problems/risks associated with chemical methods, inducing grapevine resistance would represent an interesting sustainable alternative, where the dependence on fungicides in general and copper-based fungicides in particular to control downy mildew disease in viticulture could be reduced or even replaced with plant activators (resistance inducers). Therefore, the overall objective of this work was to evaluate the ability of some resistance inducers to induce grapevine defense mechanisms against *P. viticola*, the causal agent of downy mildew, under greenhouse conditions through the characterization of mode of action of these resistance inducers morphologically as well as *in planta* on microscopic, metabolic and molecular level.

To achieve this goal, several steps had to be followed:

1- Testing the efficacy of different resistance inducers against *P. viticola* in greenhouse.

2- Determining the best timing of application by using two different strategies:
   a. Protective treatment, where the plants are first treated with the resistance inducers then inoculated with the pathogen (24h later).
   b. Curative treatment where the plants are first inoculated with the pathogen and then treated with the resistance inducers (24h later).

3- Understanding the mode of action of the resistance inducers at different investigation levels.
   a. Microscopic investigation to check the cytological responses such as callose deposition.
   b. Assessment of induced-metabolites e.g. stilbenes (in tolerant varieties) by noncontact leaf autofluorescence measurements.
   c. Measuring the gene expression of some stress responsive genes by qPCR.
   d. Whole transcriptome microarray study to get a global overview on the differentially expressed genes that may play a role in this pathosystem.

2. MATERIAL AND METHODS

2.1 Greenhouse experiments

2.1.1 Grapevine varieties

Four grapevine varieties have been used in this study (Table 2.1). The first variety is Riesling, a white grape variety (*Vitis vinifera*) that originated in the Rhine region of Germany since 15th century. Its grape is aromatic and displays high acidity. It is used to make dry, semi-sweet, sweet and sparkling white wines. It is susceptible to *P. viticola*. The second variety is Müller-Thurgau also known as Rivaner, a variety of white grape (*Vitis vinifera*). It was created by Hermann Müller (Geisenheim, 1882) by crossing Riesling with Madeleine Royale. It is also used to make white wine. It is highly susceptible to *P. viticola*. The third variety is Solaris, a variety of white grape used for wine. It was created by Norbert Becker (grape breeding institute in Freiburg, 1975) by crossing the variety Merzling with Gm 6493 (which is Zarya Severa x Muscat Ottonel). It shows a broad resistance (tolerance) against the most significant fungal diseases which affect grapes, such as downy mildew. The fourth variety is Regent, a dark-skinned inter-specific hybrid grape variety, used for making red wine. It has both European (*Vitis vinifera*) and American vine species in its pedigree. Regent was created by Professor Alleweldt (Geilweilerhof, Institute for Grape Breeding, 1967) by crossing Diana with the interspecific hybrid Chambourcin. It also shows a broad resistance (tolerance) against the most significant fungal diseases which affect grapes, such as downy mildew.

Table 2.1: Grapevine varieties used in this study

Variety	**Origin**	**Susceptibility to *P. viticola***
Riesling	---------------------	Susceptible
Müller-Thurgau	Riesling x Madeleine Royale	Highly susceptible
Solaris	Merzling x Gm 6493	Tolerant
Regent	Diana x Chambourcin	Tolerant

2.1.2 Plant cultivation

Two-eye cuttings of *Vitis vinifera* cv. Riesling and Müller-Thurgau were collected from the mature shoots after the first frost, i.e. after being stratified. They were disinfected by soaking them in 0.5% Chinoplant® solution (Stähler, active ingredient: 8-hydroxychinoline) for 12 h. Thereafter, they were stored at 4ºC and 95% rel. humidity until use. Before cultivation, cuttings were soaked in lukewarm water for half an hour

in order to gain sufficient moisture. Cuttings were then reduced (by cutting their edge) at both ends by about an inch to remove the dried edges. The lower eye was removed and the cuttings were put in boxes filled with a mixture of 50% perlite and 50% standard soil mixture. Cuttings were cultivated for 10-12 weeks at an average temperature of 24:22ºC day:night and irrigated twice a week. Plantlets were fertilized during irrigation (fertigation) once a week (after the emergence of the first leaf) with 1 g/l Flory® 3 Mega (18 g N, 12 g P, 18 g K, 2 g Mg). Thereafter plantlets were potted in MCI-17 pots filled with standard soil ED 73. Plantlets were fertilized once a week. Young vine plants are best suited for inoculation trials when they have six to eight leaves unfolded.

2.1.3 Preparing the pathogen (*Plasmopara viticola*)

2.1.3.1 Maintaining (storing) and preparation of the pathogen (inoculum)

Because *P. viticola* is an obligate biotroph oomycete, it is maintained on living plants (*in vivo*). This is done by repeatedly inoculating susceptible grapevines with the pathogen. One week after inoculation, infected leaves with visible symptoms, represented by a white growth (sporangiophores) on the abaxial side of the leaves, were collected; two leaves were put together, with the abaxial (lower) surface facing each other, to avoid losing some sporangiophores. The leaves were frozen at -20°C until use.

One week before inoculation, the frozen leaves were taken out of the freezer and let to thaw at room temperature then carefully washed, by spraying de-ionized/tap water mix (50:50) at the abaxial side, to collect the sporangia that contain the zoospores. The density of the sporangial solution was counted and adjusted using a Haemocytometer (VWR, International GmbH, Darmstadt) to a concentration of 10^5-10^6/ml.

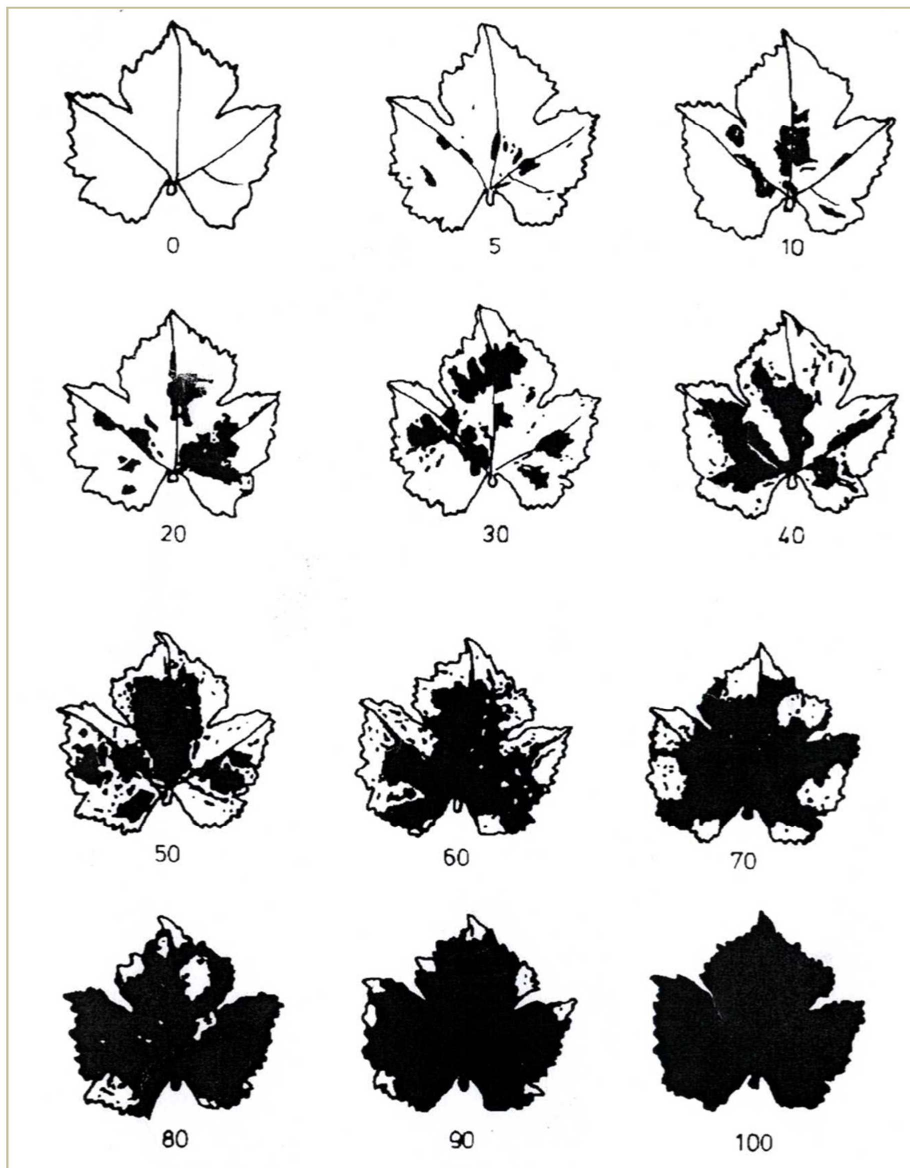
2.1.3.2 Inoculation

Sporangia suspension was sprayed, using a household sprayer, on the abaxial leaf surface. After inoculation, potted vines were immediately covered with a dark plastic wrap, previously moistened with tap water, for 24 h to create an ideal microclimate (liquid water conditions and a very high relative humidity) for the infection process and disease development. Inoculation was carried out on three biological replicates. After 24 h the plastic wrap was removed.

2.1.3.3 Assessment of the disease severity

In order to induce sporulation, vines were wrapped again at the end of the incubation period for twelve hours overnight. Considering average summer temperatures, that was done on day six after inoculation. The disease severity scheme from the European Plant Protection Organization (EPPO) was used (figure 2.1) to assess disease severity. However, instead of twelve symptom grades, only seven were applied to simplify the evaluation. Table 2.2 shows degrees of disease symptoms and their conversion from 12 into 7 grades.

Fig 2.1: Disease severity assessment scheme according to EPPO



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Scheme with twelve grades for disease severity assessment (according to EPPO).
1 = 0 symptoms, 2 = 5%, 3 = 10%, 4 = 20%, 5 = 30%, 6 = 40%, 7 = 50%, 8 = 60%, 9 = 70%, 10 = 80%, 11 =90%, 12 = 100%

Table 2.2: Disease severity assessment scheme using 7 classes

Infection intensity (%)	Leaf symptoms
no disease	Healthy (no symptoms)
< 5%	Light mycelial growth and individual shiny spots
5-10%	Obvious mycelial growth or individual shiny spots
10-25%	Obvious mycelial growth and individual shiny spots
25-50%	Strong mycelial growth and individual shiny spots
70-75%	Strong mycelial growth, leaf discoloration
> 75%	Leaf completely infected

To get a better understanding of the efficiency of the elicitors, elicitors' efficacy (EE) was calculated from the disease severity using the following formula:

$$EE = \frac{\text{Disease severity (control)} - \text{Disease severity (treatment)}}{\text{Disease severity (control)}} \times 100$$

2.1.4 Resistance inducers and elicitation

Six resistance inducers (biogenic and abiogenic) plus a fungicide were used in this work (Table 2.3). Frutogard® (Tilco Biochemie) contains brown algae extract (*Ascophyllum nodosum*, Laminaria spp.), amino acids and phosphonate, while Algin Biovital® (Tilco Biochemie) contains algae extract (*Ascophyllum nodosum*, Laminaria spp.), sugar beet extract and phosphate. ß-1, 3-Glucan (Sigma) is a solo-compound derived from the cell wall of yeast. Myco-Sin® VIN (Biofa AG) is a rock-flour based compound mixed with sulphuric acid. Phosphonate solo (Tilco Biochemie) (a constituent of Frutogard®) and phosphate solo (Tilco Biochemie) (a constituent of Algin Biovital®) were also tested. Pyraclostrobin (BASF) , a strobilurin, was included to see if it has protective or curative activity. It has a fungicidal activity due to inhibition of fungal growth through inhibiting the respiratory chain by blocking the electron transfer. Water was used as a control. The elicitors were prepared (by dissolving in water) according to the manufacturer recommendations (Table 2.4).

Table 2.3: Compounds used for elicitation

Elicitor name	Type of elicitor	Active ingredient(s)	%
Frutogard® (Alginure Bioschutz)	Biogenic	24% Brown algae (Laminaria Spp.), 7% plant amino acids, phosphonate, phosphate	1%
Algin Biovital (Alginure Pilzfrei)®	Biogenic	51% Algae, 8% sugar beet extracts, 41% P-K fertilizer	1%
ß-1, 3-Glucan	Biogenic	Constituent of the yeast cell wall	0.25%
Myco-Sin® VIN	Biogenic	Based on rock flour and sulphuric acid and plant extract	0.5%
Phosphonate	Abiogenic	Phosphonate	1%
Phosphate	Abiogenic	Phosphate	1%
Strobilurin (Cabrio®; without Metiram)	Fungicide	Pyraclostrobin	0.24%
Water	Control	- - -	- - -

Biogenic elicitors: molecules originating from living organism.
Abiogenic elicitors: pure chemicals.

Table 2.4: Concentrations of the elicitors

Elicitor	%	For 2 liter
Frutogard	1.00 %	20 g
Algin Biovital	1.00 %	20 g
Myco-Sin Vin	0.50 %	10 g
ß-1,3-Glucan	0.25 %	5 g
Phosphonate	1.00 %	20 g
Phosphate	1.00 %	20 g
Strobilurin	0.24 %	4,8 g

Elicitors were applied (sprayed) separately on potted vines at the concentrations indicated in table 2.4. For each treatment, 2L of the elicitor were prepared. Spraying was carried out using an airbrush gun under pressure (3 bar). Leaves were sprayed evenly on both, abaxial (lower) and adaxial (upper) surfaces of the leaves until the dripping wet point. The sprayer was thoroughly washed with water between treatments (elicitors) to avoid elicitor carry over. Plants were left ca. 30 min to dry before they were placed in the greenhouse. Each treatment was carried out in three biological replicates.

2.1.5 Application of the elicitors (protective – curative)

2.1.5.1 Greenhouse experiments (2009)

Elicitors were applied on vines (cv. Riesling) using two different methods in order to determine the best way (time) of application. In the protective method, plants were first treated with the elicitors and then inoculated 24 h later, while in the curative method, plants were first inoculated then treated with the elicitors 24 h later. Some plants were only elicited or only inoculated. Control plants were only sprayed with water or not sprayed at all (Table 2.5). Table 2.6 a, b shows an overview of the whole experiment. To assure randomization, plants were distributed randomly according to a randomization plan in the greenhouse chamber.

2.1.5.2 Greenhouse experiments (2010 and 2011)

The same experiments using the same elicitors and procedures were repeated again using Müller-Thurgau in the second season (2010). In the third season (2011), the same experiments were made using both varieties (Riesling and Müller-Thurgau) but using only four elicitors (best performing elicitors). These elicitors were Frutogard®, Algin Biovital®, phosphonate alone (constituent of Frutogard®) and phosphate alone (constituent of Algin Biovital®).

2.1.5.3 Greenhouse experiments (2012)

Greenhouse experiment on Regent and Solaris (protective)

In order to investigate the effect of the elicitors on tolerant grape varieties, the best performing elicitors Frutogard®, Algin Biovital®, phosphonate and phosphate, were applied on the varieties Regent and Solaris that are tolerant to downy mildew. Plant cultivation, inoculum preparation, elicitor concentrations and application method (protective) were the same as previously described.

Container plants experiment on Regent and Solaris (protective)

In this experiment, one-year old potted grapevines were used. The same elicitation method (protective) and inoculation procedures were applied on container plants. The defense response in these plants was assessed by using a hand-held multi-parameter optical sensor (Multiplex® 3.6; company FORCE-A) based on noncontact leaf and fruit autofluorescence measurements. Several real-time optical signatures

such as chlorophyll content, content of constitutive and induced polyphenols, mainly flavonols and anthocyanins, were obtained as outputs.

Table 2.5: Treatment combinations (elicitation and/or inoculation)

	Treatment	Protective*	Curative*
1	Frutogard® (Alginure Bioschutz)	Only elicited	Only elicited
2		Elicited and inoculated	Inoculated and elicited
3	Algin Biovital® (Alginure Pilzfrei)	Only elicited	Only elicited
4		Elicited and inoculated	Inoculated and elicited
5	Glucan	Only elicited	Only elicited
6		Elicited and inoculated	Inoculated and elicited
7	Mycosin®-VIN	Only elicited	Only elicited
8		Elicited and inoculated	Inoculated and elicited
9	Phosphonate from Frutogard	Only elicited	Only elicited
10		Elicited and inoculated	Inoculated and elicited
11	Phosphate from Alginure	Only elicited	Only elicited
12		Elicited and inoculated	Inoculated and elicited
13	Strobilurin (Cabrio®)	Only elicited	Only elicited
14		Elicited and inoculated	Inoculated and elicited
15	Water	Treated with water only	Treated with water only
16	Inoculation	Inoculated only	Inoculated only
17	Non-treatment	Not treated, not inoculated	Not treated, not inoculated

*In protective treatment: plants were first treated then inoculated, hence elicited and inoculated
*In curative treatment: plants were first inoculated then treated, hence inoculated and elicited

Table 2.6a: An overview on the protective treatment plan (Riesling, 2009)

Treatment	0	1 dat	2 dat	3 dat	4 dat	_ _ _	6 dat	_ _ _	8 dat	9 dat
Inoculation		0 dai	I dai	II dai	III dai	_ _ _	V dai	_ _ _	VII dai	VIII dai
Time point of sampling	1	2	3	4	5	_ _ _	6	_ _ _	7	_ _ _
Application of the elicitors	**X**									
Inoculation		**X**								
Incubation									**X**	
Disease severity assessment										**X**
No. of plants for sampling	**3**	**27**	**51**	**51**	**51**		**51**		**51**	
Elicited and Inoculated plants			24	24	24		24		24	
Only elicited plants	3	27	27	27	27		27		27	

dat = day after treatment dai = day after inoculation

Table 2.6b: An overview on the curative treatment plan (Riesling, 2009)

Treatment		0	1 dat	2 dat	3 dat	_ _ _	5 dat	_ _ _	_ _ _	8 dat
Inoculation	0 dai	I dai	II dai	III dai	IV dai	_ _ _	VI dai	_ _ _	VIII dai	IX dai
Time point of sampling	1	2	3	4	5	_ _ _	6	_ _ _	7	_ _ _
Application of the elicitors		**X**								
Inoculation	**X**									
Incubation									**X**	
Disease severity assessment										**X**
No. of plants for sampling	**3**	**6**	**51**	**51**	**51**		**51**		**51**	
Inoculated and elicited plants		3	24	24	24		24		24	
Only elicited plants	3	3	27	27	27		27		27	

dat = day after treatment dai = day after inoculation

Table 2.7a: Sampling plan during protective treatment

Sampling day	Total no. of plants	No. of plants in each treatment
Day 1	3	3 Control (neither treated nor inoculated)
Day 2	27	(7 elicitors x 3) + (3 water) + 3 (Control)
Day 3	51	27 = (7 elicited) x 3 + 3 (water) + 3 (Control)
		24 = (7 elicited and inoculated) x 3 + 3 (inoculated)
Day 4	51	27 = (7 elicited) x 3 + 3 (water) + 3 (Control)
		24 = (7 elicited and inoculated) x 3 + 3 (inoculated)
Day 5	51	27 = (7 elicited) x 3 + 3 (water) + 3 (Control)
		24 = (7 elicited and inoculated) x 3 + 3 (inoculated)
Day 6	51	27 = (7 elicited) x 3 + 3 (water) + 3 (Control)
		24 = (7 elicited and inoculated) x 3 + 3 (inoculated)
Day 7	51	27 = (7 elicited) x 3 + 3 (water) + 3 (Control)
		24 = (7 elicited and inoculated) x 3 + 3 (inoculated)

Table 2.7b: Sampling plan during curative treatment

Sampling day	Total no. of plants	No. of plants in each treatment
Day 1	3	3 Control (neither treated nor inoculated)
Day 2	6	3 (Control) + 3 (inoculated)
Day 3	51	27 = (7 elicited) x 3 + 3 (water) + 3 (Control)
		24 = (7 inoculated and elicited) x 3 + 3 (inoculated)
Day 4	51	27 = (7 elicited) x 3 + 3 (water) + 3 (Control)
		24 = (7 inoculated and elicited) x 3 + 3 (inoculated)
Day 5	51	27 = (7 elicited) x 3 + 3 (water) + 3 (Control)
		24 = (7 inoculated and elicited) x 3 + 3 (inoculated)
Day 6	51	27 = (7 elicited) x 3 + 3 (water) + 3 (Control)
		24 = (7 inoculated and elicited) x 3 + 3 (inoculated)
Day 7	51	27 = (7 elicited) x 3 + 3 (water) + 3 (Control)
		24= (7 inoculated and elicited) x 3 + 3 (inoculated)

2.2 Microscopical investigation

Leaf disc sampling and bleaching

Leaf discs (1 cm diameter) were obtained by punching out the discs using a cork borer, from the 4th unfolded leaf. Leaf discs were bleached by placing them immediately in discoloring solution [trichloroacetic acid (0,15%, w/v) – ethanol/chloroform (4/1, v/v)] for 24h. After bleaching, leaf discs were transferred into fixing i.e. microscopic solution [glycerol/distilled water (1:1, v/v)].

2.2.1 Leaf disc staining

It is necessary to stain the intercellular parasitic structures specifically to distinguish them from host cells especially cell walls because oomycetes are cellulosic micro-organisms and their cell walls contain mostly cellulose. Aniline blue was used to stain the oomycete inside the tissues. It binds selectively to the ß-glucoside-linked polysaccharides of oomycete mycelium. To do this, bleached leaf discs were stained using 0.05% w/v aniline blue (Sigma). The aniline fluorescence was analyzed by an epifluorescence microscope (Axioplan; Carl Zeiss) using excitation at 395-440 nm.

2.3 Assessment of stilbene content (in tolerant varieties “Regent and Solaris”)

The defense related secondary metabolites phytoalexins mainly stilbenes were measured based on noncontact leaf autofluorescence measurements using an optical sensor (Multiplex® 3.6, ForceA). Stilbene level was represented in real-time optical signatures by the content of constitutive and induced flavonols stilbenes. This experiment used only plant treated protectively and was limited to only four elicitors (best performing elicitors); namely Frutogard®, Algin Biovital®, phosphonate and phosphate, where each treatment had three biological replicates. Readings were taken 0, 3, 6 and 24 hours after elicitor treatment in the first days and 0, 3, 6 and 24 hours after inoculation in the second day. 10 leaves from each plant were measured using the hand-held optical sensor.

Leaves (bigger than 10 cm diameter) were held in front of the UV-light source for 3 seconds in order to enable the epidermal UV absorbance by Fluorescence Excitation Ratio (FER) method to measure the flavonol content.

2.4 Gene expression analysis using real-time RT-PCR

Sampling was carried out early in the morning, where young leaves (the first apical three leaves) were collected at intervals of 24 hours for seven days according to the plan shown in table 2.7a,b. Each plant was sampled only once to avoid the activation of wound-responsive genes. Leaves were cut with a sharp razor blade and wrapped in aluminum foil (Roth) then immediately flash-frozen in liquid nitrogen and stored in the freezer at -80°C. Three replicates corresponding to leaves from three individual plants were sampled for all treatments and controls.

2.4.1 Total RNA extraction for real-time RT-PCR

Total RNA was extracted according to Chang et al. (1993) and modified by Wielgoss and Kortekamp (2006). 100 mg of frozen leaves were ground in liquid nitrogen in a pre-cooled mortar. The leaf powder was immediately transferred in a pre-cooled 2ml eppendorf tube. 700 µl of extraction buffer (Table 2.8a) was added to the powder and immediately vortexed to homogenize the sample. Samples were then incubated during 3 min. in a water bath at 65°C and subsequently cooled at room temperature. For the RNA purification from proteins and polysaccharides, chloroform-isoamyl alcohol (24:1 v / v) was added at a ratio of 1:1 and mixed during 10 min. To form the three phases (upper (aqueous), middle (protein) and chloroform phase (organic)), centrifugation was performed at 7.000 rpm at 4°C for 10 min. The upper phase was carefully transferred into a new tube. The chloroform-isoamyl alcohol separation step was repeated to remove the remaining proteins and DNA. Total RNA was precipitated overnight using ¼ volume of 10 M LiCl at 4°C. To pellet RNA, the tubes were centrifuged at 10.000 rpm at 4°C for 30 min. Then, LiCl was decanted and the pellet was washed with 0.5 ml SDS (0.5%) at room temperature for 10 min. Again, the resolved RNA was mixed with an equal volume of chloroform-isoamyl alcohol to separate it into phases. RNA was precipitated using 98% ethanol at -20°C for 120 min. Finally, to pellet the RNA, tubes were centrifuged at 10.000 rpm at 4°C for 30 min. Ethanol was decanted and the pellet was dried at room temperature for 10 min. The RNA pellet was resuspended in 50 µl RNase free water. RNA was purified and treated with DNase I (Qiagen, RNeasy cleanup kit) according manufacturer's instructions to remove any DNA contamination (Table 2.8b).

Table 2.8a: RNA extraction buffer

Ingredients	Concentration	1 M	1 Liter
CTAB	2 %	364.46 g/mol	20.0 g
PVP (30)	2 %	30.000 g/mol	20.0 g
Tris-HCl; pH 8.0	100 mM	121.14 g/mol	12.1 g
EDTA; pH 8.0	25 mM	372.24 g/mol	9.3 g
NaCl	2 M	58.44 g/mol	116.8 g
Spermidine	0.5 g/L	145.25 g/mol	0.5 g
β-mercaptoethanol	2 ml/100ml	Added after autoclaving	

Table 2.8b: RNA purification using Qiagen RNeasy cleanup kit

Steps	Buffer/kit	Description
Protein digestion	RLT	Denaturing proteins (RNase and DNase)
RNA binding	Spin column	Binding the RNA to the filter
1st wash	RW I	Washing digested proteins
DNA digestion	DNaseI	Removing the rest of DNA
2nd wash	RW II	Removing the DNA and DNase buffer
Elution	RNase free water	In 50 µl RNase free water

RNA purity and concentration were determined by measuring the absorbance at 230, 260 and 280 nm using the NanoDrop-1000 spectrophotometer (Thermo Scientific). The following criteria were targeted: A260/A280 = 1.8 – 2.1 and A260/A230 = 1.8 – 2.2. RNA integrity was assessed using an Agilent 2100 Bioanalyzer RNA 6000 Nano LabChip (Agilent, Diegem, Belgium). RIN (RNA Integrity Number) was calculated using an algorithm adapted for plant RNA profiles. All RIN values were between 7.0 and 7.8 indicating good RNA quality (non-degraded) for gene expression experiments.

2.4.2 cDNA synthesis

cDNA was synthesized from 1 µg of total RNA using MultiScribe® Reverse Transcriptase (Applied Biosystems, USA). Random primers (hexamers) were used in order to have transcripts as long as possible that cover the transcriptome. The final volume of the reaction was 50 µl (Table 2.9). Reverse transcription was performed according to the parameters displayed in table 2.10. The cDNA produced had a concentration of 20 ng/µl equivalent RNA. cDNAs were stored at -20°C for short period.

Table 2.9: Reagents used for cDNA synthesis

Master mix	1x
Buffer (10x)	5 µl
$MgCl_2$ (25 mM)	11 µl
dNTPs (2.5 mM each)	10 µl
Random hexamer (50 µM)	2,5 µl
RNase inhibitor (20 U/µl)	1 µl
RT enzyme (50 U/µl)	1,25 µl
RNA diluted in water (1 µg)	19,25 µl
Total volume	50 µl

Table 2.10: Reverse transcription protocol

Step	Temp (°C)	Time
Denaturation	25°C	10 min
Elongation	48°C	30 min
*Inactivation	95°C	5 min

*Reverse transcriptase inactivation

2.4.3 Real-time RT-PCR (qPCR)

qPCR reactions were performed on an ABI PRISM® 7500 Fast Real-Time PCR System (Applied Biosystems, USA) using SYBRgreen®. Reactions were performed in 25 µl containing 100 nM of primers (forward and reverse), 5 µl cDNA (corresponding to 10 ng), and 12.5 µl 2× SYBR MESA GREEN MasterMix Plus, Low ROX (Eurogentec, Liège, Belgium). To ensure reliable results, PCR efficiency was determined for each gene (Radonic et al., 2004). To this end, 5 µl were taken from each cDNA sample and pooled. Five serial ten-fold dilutions of pooled cDNAs were made starting from 10 to 0,001 ng/µl using DNase free water. The raw Ct values were plotted against log-transformed concentrations to obtain the PCR efficiency (E) from the following equation ($E=10^{(-1/slope)} - 1$). All PCRs displayed efficiencies between 92% and 105% (Table 2.11). Primer sequences are indicated in table 2.11.

Reactions, performed on three biological replicates, were run in duplicates using the manufacturer's recommended cycling parameters (Table 2.12 and 2.13). No-template controls were included for each primer pair. Specificity of the primer pairs was assessed by the presence of a single peak during the final dissociation curve step. Six genes were investigated: chitinase (CHIT-1b), lipoxygenase (LOX) and stilbene synthase (STS) (Trouvelot et al., 2008) were used as genes of interest, while actin

and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Heibertshausen, personal communication) and elongation factor 1 (EF1) (Trouvelot et al., 2008) were used as reference genes for normalization during gene expression analysis.

Table 2.11: Primer sequences used for real-time RT-PCR

Gene	Acc. no. *	Primer	Sequence 5'-3'	Amp. length Eff. (%)
STS	X76892	For	AGG AAG CAG CAT TGA AGG CTC	101 bp 105%
		Rev	TGC ACC AGG CAT TTC TAC ACC	
LOX	AY159556	For	CCC TTC TTG GCA TCT CCC TTA	101 bp 106%
		Rev	TGT TGT GTC CAG GGT CCA TTC	
CHIT_1b	Z54234	For	CCC AAG CCT TCC TGC CAT A	96 bp 92%
		Rev	TGT GAT AAC ACC AAA ACC GGG	
Actin	AY847627	For	GCC TGA TGG GCA AGT CAT	244 bp 97%
		Rev	GCT GGG AGC AAG AGC AGT	
GAPDH	EF192466	For	TCA AGG TCA AGG ACT CTA ACA CC	226 bp 90%
		Rev	CCA ACA ACG AAC ATA GGA GCA	
EF1	EC959059	For	GAA CTG GGT GCT TGA TAG GC	164 bp 97%
		Rev	AAC CAA AAT ATC CGG AGT AAA AGA	

* Accession numbers in NCBI or TC TIGR number.

STS: Stilbene synthase, LOX: 9-lipoxygenase , CHIT1b: class I chitinase, Actin, GAPDH: Glyceraldehyde 3-phosphate dehydrogenase, EF1: elongation factor 1 alpha

Table 2.12: Reagents used for real-time RT-PCR

Master mix	**1x**
Water (H_2O)	5.5 µl
Forward primer (2.5 mM)	1 µl
Reverse primer (2.5 mM)	1 µl
RT mix	12.5 µl
RNA (2 ng/µl)	5 µl
Total volume	20 µl

Table 2.13: Real-time RT-PCR protocol

Step	**Time**	**Cycles**	**Time**
Holding	10 min	95°C	10 min
Denaturation	40 cycles	95°C	15 sec
Extension		60°C	1 min
Melting curve	To check primers specificity		

2.5 Microarray experiment (in Verona, Italy)

2.5.1 Total RNA extraction

RNA was extracted from 100 mg frozen leaves using Spectrum^{TM} Plant Total RNA extraction kit following the manufacturer's protocol (Sigma) (Table 2.14). DNA was removed during RNA extraction using On-column DNase digestion (Sigma). Around 20 µg total RNA were eluted in elution buffer. RNA purity, concentration and integrity were evaluated as mentioned in 2.3.1.

Table 2.14: RNA isolation steps using Spectrum^{TM} Plant Total RNA extraction kit

Steps	Buffer	Description
Cell lysis	Lysis buffer	Cell lysis and freeing the RNA
RNA binding	Binding buffer	Binding the RNA to the filter
1st wash	Washing buffer I	Washing cell debris and proteins
DNA digestion	DNase I	Removing remaining DNA
2nd wash	Washing buffer II	Removing the DNase buffer
Elution	RNase free water	In 50 µl RNase free water

2.5.2 cDNA synthesis and labeling

This part of work was done in collaboration with Prof. Delledonne lab at the Biotechnology Department, Verona University, Italy. First-strand cDNA was synthesized from 10 µg total RNA using SuperScript II RT and oligo dT primers according to the manufacturer's instructions (Table 2.15a). Samples were incubated at 42°C for 1 hour. The first-strand cDNA was then used as a template for the second-strand cDNA synthesis using DNA polymerase I and dNTP Mix and incubation at 16°C for 2 hours (Table 2.15b). Samples were cleaned-up with RNaseA and precipitated using phenol:chloroform:isoamyl alcohol and 7.5 M ammonium acetate. cDNA purity and integrity was evaluated according to the criteria mentioned in 2.3.1.

Table 2.15a: First strand cDNA synthesis using Invitrogen SuperScript II cDNA synthesis kit

Components	Volume
RNA	10 µg
oligo dT Primer	1 µl
Total	11 µl
Incubation at 70°C for 10 min.	
5X First Strand Buffer	4 µl
0.1 M DTT	2 µl
10 mM dNTP Mix	1 µl
Incubation at 42°C for 2 min	
SuperScript II RT	2 µl
Incubation at 42°C for 60 min	
Total	20 µl

Table 2.15b: Second strand cDNA synthesis using Invitrogen SuperScript II cDNA Synthesis Kit

Components	Volume
cDNA from table 2.15b	20 µl
DEPC Water	91 µl
5x Second Strand Buffer	30 µl
10 mM dNTP Mix	3 µl
10 U/µl DNA Ligase	1 µl
10 U/µl DNA Polymerase I	4 µl
2 U/µl RNase H	1 µl
Total	150 µl
Incubation at 16°C for 120 min	

cDNA was labeled using NimbleGen® one-color DNA Labeling Kit that contains Klenow fragment (DNA polymerase I without 5' → 3' exonuclease activity). Labeling was done using 1 µg cDNA, Cy3-Random Nonamers and dNTP/Klenow master mix. Samples were incubated at 37°C for 2 hours. Cy3-labeled cDNA samples were precipitated using isopropanol and 5 M NaCl. cDNA quality control was performed as previously mentioned. Labeling was performed according to the NimbleGen® user guide (http://www.nimblegen.com/products/lit/expression_userguide_v5p0.pdf)

2.5.3 Hybridization and scanning

The chips used for this experiment were NimbleGen® arrays. They were designed using the V1 prediction from CRIBI (http://genomics.cribi.unipd.it/Download/VitisMTA) that is based on the 12X genome assembly produced by the French-Italian consortium. The chip contains 12 sub-arrays (12Plex, 090918_Vitus_exp_HX12), where twelve samples can be simultaneously hybridized. Each array contains 135,000 features (60mer oligonucleotide probes) including multiple probes per target, allowing simultaneous monitoring of the expression of 29,582 grapevine genes.

All hybridizations, staining, and processing of arrays were performed using components from the NimbleGen® Hybridization Kit according to the NimbleGen® user guide (http://www.nimblegen.com/products/lit/expression_userguide_v5p0.pdf. 4 µg of each cDNA sample was resuspended in a different Sample Tracking Controls (STCs), a Cy3-labeled 48mer oligonucleotide, to mark which sample is hybridized to which array. cDNA samples were then added to hybridization solution master mix that contained alignment oligo, a mixture of Cy3 and Cy5 labeled 48mer oligonucleotides that hybridize to alignment features on NimbleGen® arrays and required for proper extraction of array data from the scanned image. Samples were then incubated at 95°C for 5 minutes for denaturation, protected from light, then at 42°C until sample loading. The slide was put in the Precision Mixer Alignment Tool (PMAT) with HX12 Mixer. Each sample was loaded into the corresponding fill port and then placed in NimbleGen® Hybridization System and let to hybridize at 42°C for 16 hours. The slide was washed sequentially using 3 washing solutions (I, II, and III) supplied with the kits. It was vigorously agitated for 10 - 15 seconds in warm Wash I to quickly remove the hybridization buffer and then in wash II for 1 minute with vigorous, constant agitation and finally in wash III and for 1 minute with vigorous, constant agitation. The slide was spin dried in a NimbleGen® Microarray Dryer for 2 minutes and directly scanned.

Scanning, data extraction, and array calibrations were performed by NimbleGen® Systems. Arrays were scanned using an Axon GenePix 4400A scanner and data were extracted using the NimbleScan software. Scanner settings were set according to user guide http://www.nimblegen.com/products/lit/expression_userguide_v5p0.pdf.

2.6 Optimization of the choice of reference genes for qPCR for microarray validation

To assure an accurate measuring of the gene expression, normalization using reference genes was used. Knowing that there is no ideal universal reference gene that could be used for all experiments, six putative genes were investigated under different experimental conditions (elicited, inoculated, control ”neither elicited nor inoculated”). These genes were cyclophilin, ubiquitin conjugating enzyme (UBQ) and SAND family protein (SAND) (Reid et al., 2006), GAPDH and actin (Heibertshausen, personal communication) and elongation factor 1 (EF1) (Trouvelot et al., 2008) (table 2.16). Moreover, the optimal number of reference genes to be used in normalization was determined, since it has been emphasized in the literature that using multiple reference genes increases accuracy. RNA extraction was made according to Chang et al. (1993) and modified by Wielgoss and Kortekamp (2006) (table 2.8a). RNA was purified and treated with DNase I (Qiagen, RNeasy cleanup kit) (table 2.8b). RNA quality control was made as previously explained. Two different software programs, GeNorm (Vandesompele et al., 2002) and Normfinder (Andersen et al., 2004), were used for selection of most stable reference genes under the aforementioned conditions.

Table 2.16: Primer sequences used for real-time RT-PCR

Gene	Acc. no. *	Primer	Sequence 5'-3'	Amp. Length Eff. (%)
Cyclo-pihilin	EC969926	For	GGA GCC TGA GCC TAC CTT CTC	66 bp 94%
		Rev	GTG TTC GGC CAG GTG GTA GA	
UBQ	EC922622	For	GAG GGT CGT CAG GAT TTG GA	75 bp 92%
		Rev	CTT AAA GAT GGT AAG TGC AGG GC	
SAND	CF405409	For	CAA CAT CCT TTA CCC ATT GAC AGA	76 bp 99%
		Rev	CTT ATC TGC AAG TGG ATC AAA TGC	
Actin	AY847627	For	GCC TGA TGG GCA AGT CAT	244 bp 97%
		Rev	GCT GGG AGC AAG AGC AGT	
GAPDH	EF192466	For	TCA AGG TCA AGG ACT CTA ACA CC	226 bp 90%
		Rev	CCA ACA ACG AAC ATA GGA GCA	
EF1	EC959059	For	GAA CTG GGT GCT TGA TAG GC	164 bp 97%
		Rev	AAC CAA AAT ATC CGG AGT AAA AGA	

2.7 Statistical analyses

Statistical analysis of the results obtained from greenhouse experiments (disease severity assessment) was done using SPSS (PASW 18) software. Tukey's HSD test, with $\alpha = 0.05$, was applied, where means and standard deviations of biological replicates were used as input. For accurate relative expression calculation, normalization was done using geometrical averaging of multiple reference genes as recommended by Vandesompele et al. (2002).

To determine the stability of expression of the housekeeping genes as well as the optimal number of reference genes for normalization, the geNorm software (Vandesompele et al., 2002) and Normfinder (Andersen et al., 2004) was used in which the relative expression values were used as an input to the software. For statistical analysis of these data, a 2-tailed T-test with unequal variance was performed on Log-transformed datasets using SPSS (PASW 18 software). Graphs were made by Sigma Plot 7.101.

Microarray data were extracted using the NimbleScan software. Gene calls were generated with RMA (Robust Multichip Analysis; Irizarry et al., 2003). Variation within and across arrays was removed during data normalization using the RMA at the probe level (Irizarry, 2003; Bolstad, 2003). Differential expression analysis was performed with LIMMA (Linear Models Microarray Analysis; Smyth, 2005) R package. P values were adjusted for multiplicity with Benjamini Hochberg (BH) method (Benjamini et al., 1995). For identification of differentially expressed genes, a fold change of -0.5 and 0.5 in $\log_2$ ratios [$\log_2$ (inoculated - control)] and p values adjusted for false discovery rate (FDR) less than 0.05 were considered.

Microarray data were visualized using MapMan (version 3.5.1, Gabi Primary Database). It has been adapted for *Vitis* by adding three selected pathways to cope with the grapevine physiology: carotenoid pathway, terpenoid pathway and phenylpropanoid pathway. To date, 13,145 grapevine genes have been assigned to 219 networks, including networks for metabolic, hormone, transport, and transcriptional pathways (Schlauch et al., 2010).

2.8 Chemicals, devices and softwares

Chemicals and kits	Manufacturer
Chinoplant®	Stähler, Stade
ß-1, 3-Glucan	Sigma, Steinheim, Germany
Myco-Sin® VIN	Biofa AG, Münsingen, Germany
Alginure Pilzfrei®	Tilco Biochemie, Reinfeld, Germany
Frutogard® (Alginure Bioschutz)	Tilco Biochemie, Reinfeld, Germany
Phosphonate from Frutogard®	Tilco Biochemie, Reinfeld, Germany
Phosphate from Alginure Pilzfrei®	Tilco Biochemie, Reinfeld, Germany
Strobilurin Cabrio® (fungicide)	BASF, Ludwigshafen, Germany
EDTA	Biowhittaker, Maryland, USA
Tris HCl	Sigma, Steinheim, Germany
LiCl	Merck, Leuven, Belgium
NaCl	Merck, Darmstadt, Germany
β –mercaptoethanol	Biorad, CA, USA
Spermidine	Sigma, Steinheim, Germany
CTAB	Sigma, Steinheim, Germany
PVP	Sigma, Steinheim, Germany
glycerol	Sigma, Steinheim, Germany
DNase/RNase free water	Invitrogen, Carlsberg, USA
DEPC-treated water	Invitrogen, Carlsberg, USA
Spectrum™ Plant Total RNA extraction Kit	Sigma-Aldrich, Steinheim, Germany
DNA digestion kit DNase	Sigma-Aldrich, Steinheim, Germany
Aniline blue	Sigma-Aldrich, Steinheim, Germany
RNeasy clean-up kit	Qiagen, Hilden, Germany
SuperScript II	Invitrogen, Carlsberg, USA
cDNA labeling kits	Roche NimbleGen Inc, Madison, USA
RNA 6000 Nano LabChip	Agilent, Diegem, Belgium
cDNA kit, MultiScribe® RT	Applied Biosystems, USA
SYBR MESA GREEN MasterMix Plus	Eurogentec, Liège, Belgium
Primers for Real-time RT-PCR	Eurogentec, Liège, Belgium
Devices and equipments	**Manufacturer**
Epifluorescence microscope (Axioplan)	Carl Zeiss, Göttingen, Germany
Aluminium foil	Roth, Karlsruhe
Hemocytometer	VWR International GmbH, Darmstadt
Centrifuge	Beckmann Coulter, California, USA
Water bath	Grant, Vel, Leuven, Belgium
T-Professional Thermocycler	Biometra Analytik, Neatherland
7500 fast Real-time PCR System	Applied Biosystems, USA
2100 Bioanalyzer	Agilent, Diegem, Belgium
NanoDrop-1000	Thermo Scientific, Villebon sur Yvette, France
2100 Bioanalyzer RNA 6000	Agilent, Belgium
Aluminium foil	Roth, Karlsruhe
Precision Mixer Alignment Tool (PMAT)	Roche, NimbleGen Inc., Madison, USA
Vitis vinifera NimbleGen microarray	Roche, NimbleGen Inc., Madison, USA
Axon GenePix 4400A scanner	Molecular Devices, Sunnyvale, USA
Hybridization System	NimbleGen
Multiplex® 3.6	ForceA, Paris, France
Softwares and guidelines	**Manufacturer**
Sigma Plot 7.101	SPSS, Inc., Chicago, USA
EPPO (disease severity scheme)	European plant protection organization
Mapman (3.5.1)	Gabi Primary Database
GeNorm	Vandesompele et al., 2002
Normfinder	Andersen et al., 2004
Linear Models Microarray Analysis (LIMMA)	Smyth, 2005
Software (v2.5) for microarray analysis	NimbleScan
Excel 2000	Microsoft Office, Inc., USA

3. RESULTS:

3.1 Disease severity assessment and efficiency of the elicitors

Four grapevine varieties were used for the cultivation of potted vines serving as test plants. Riesling and Müller-Thurgau are susceptible varieties to *P. viticola*, whereas Regent and Solaris are tolerant to *P. viticola*. For a better comparability between the experiments, the efficiencies of the elicitors are presented. Additionally, disease severity data are listed in tables to get an idea of the disease level in the control plot.

3.1.1 Efficiency of the elicitors on susceptible varieties (2009 – 2011)

Efficiency of the elicitors in cv. Riesling (2009)

The efficiencies against *P. viticola* of most of the tested elicitors were higher in the protective treatment than in the curative treatment. The efficiency of the Strobilurin containing fungicide was 82 % in the protective treatment compared to 5 % in the curative treatment. The best efficiencies in both protective treatment (100 %) and curative treatment (98 %) were observed for phosphonate. The efficiency of phosphate in the protective treatment was 80 %, whereas it was 41 % in the curative treatment. Similar results were obtained for Algin Biovital®: 71 % in the protective treatment and 18% in the curative treatment. The efficiency of Frutogard® was 56 % in the protective treatment and 74 % in the curative treatment, thus being the only elicitor with higher efficiency when applied curatively. The lowest efficiencies were observed for Myco-Sin® VIN (46 % in the protective treatment and 33 % in the curative treatment) and ß-1, 3-glucan (6 % in the protective treatment and 14 % in the curative treatment) (Figure 3.1). Disease severity for protective and curative treatments is represented as mean values in table 3.1.

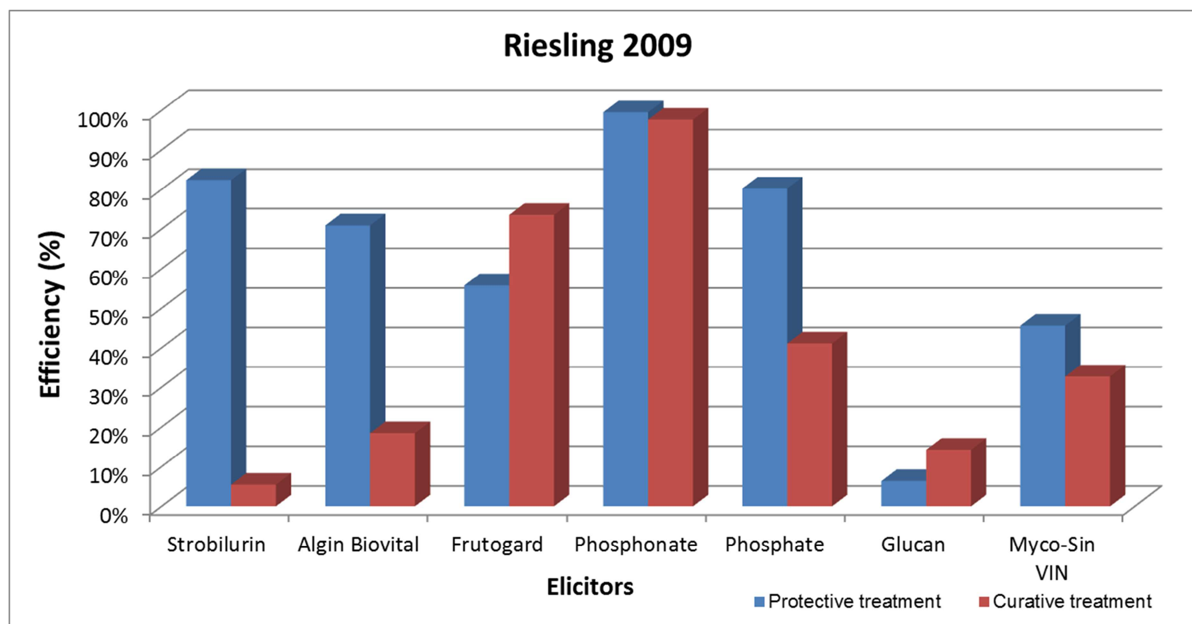

Figure 3.1: Assessment of the efficiency of the elicitors in protective and curative treatments against *P. viticola* calculated from the disease severity (Riesling, 2009), statistics are in table 3.1

Efficiency of the elicitors in cv. Müller-Thurgau (2010)

The efficiencies in 2010 (Table 3.2) were different compared to 2009 (Figure 3.1). For example, the efficiency of Strobilurin was 100 % in both treatments (protective and curative), unlike in 2009, were it had a very high efficiency in the protective treatment and very low efficiency in the curative treatment. Phosphonate had a moderate efficiency, however, the protective treatment had slightly higher efficiency than the curative treatment, unlike in 2009, where phosphonate had a very high efficiency in both treatments. The best efficiencies in both the protective (96 %) and the curative treatment (98 %) were observed for phosphate. Algin Biovital® showed moderate efficiency in both treatments (42 % in the protective treatment and 39 % in the curative treatment). Frutogard® showed high efficiency: 82 % in the protective treatment and 99 % in the curative treatment. In case of Myco-Sin® VIN, the application instant of time regarding the date of inoculation (protective or curative) did not affect its efficiency. Myco-Sin® VIN had 11 % efficiency in the protective treatment, while it had 10 % efficiency in the curative treatment. On the contrary, for ß-1, 3-glucan, the application point in time impacted on the efficiency, since an efficiency of 80 % was observed in the protective treatment, compared to 4 % in the curative treatment. Disease severity for protective and curative treatments is represented as mean values in table 3.2.

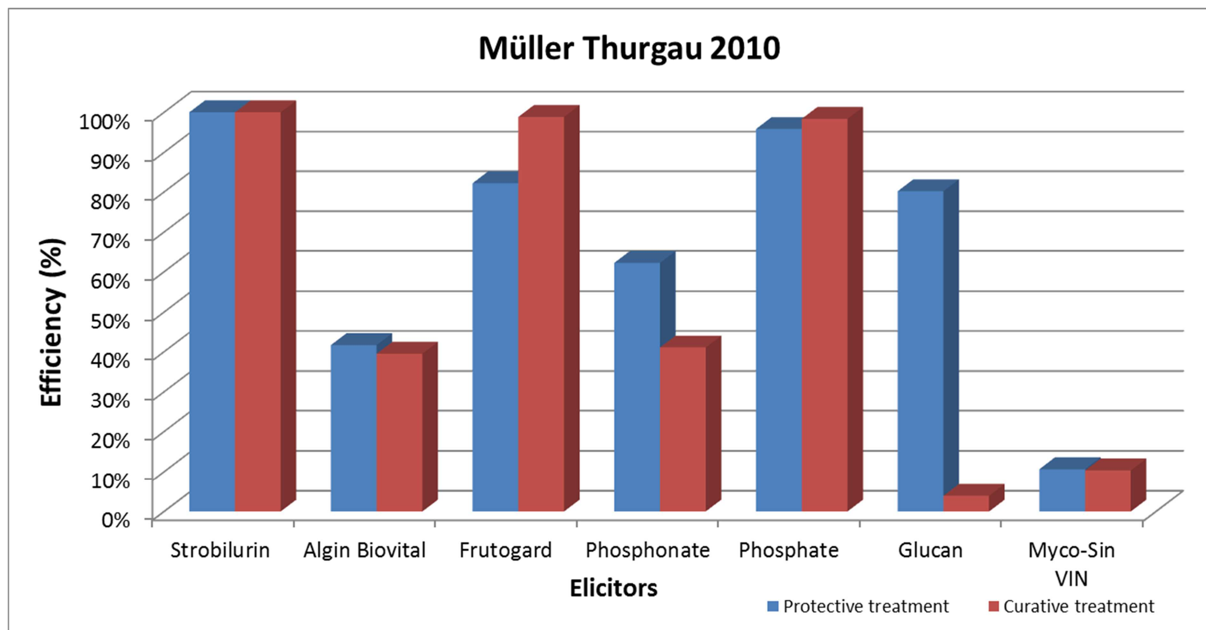

Figure 3.2: Assessment of the efficiency of the elicitors in protective and curative treatments against *P. viticola* calculated from the disease severity (Müller-Thurgau, 2010), statistics are in table 3.2

Efficiency of the elicitors (Riesling and Müller-Thurgau, 2011)

Efficiency of the elicitors (Riesling)

The best performing elicitors in 2009 and 2010 were applied in 2011 on Riesling. These elicitors were phosphonate, phosphate, Algin Biovital® and Frutogard®. In general, the protective treatment was more efficient than the curative treatment (Figure 3.3). For phosphonate and Algin Biovital®, the protective treatment was much more efficient (88 % and 56 %, respectively) than the curative treatment (8 % and 3 %, respectively). Phosphate showed high efficiency in both treatments with a higher efficiency in the protective treatment (91 %) than in the curative treatment (64 %). Frutogard® was efficient in both protective and curative treatments (87% and 93 %, respectively) (Figure 3.3). Disease severity for protective and curative treatments is represented as mean values in table 3.3.

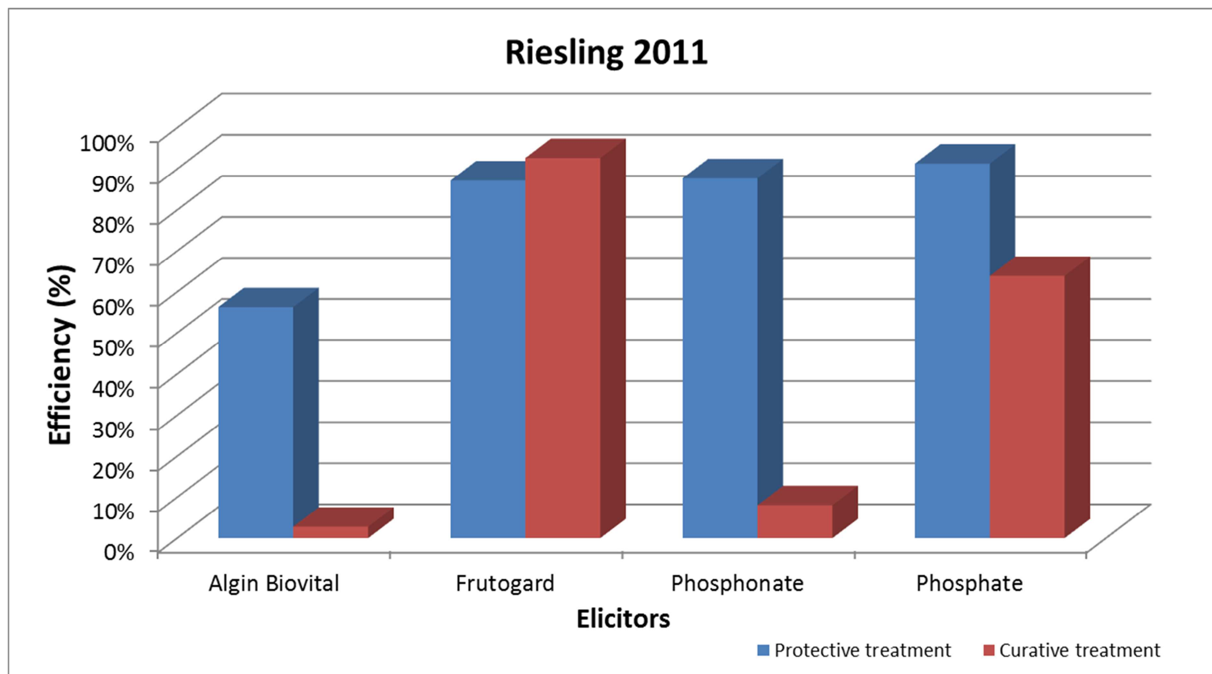

Figure 3.3: Assessment of the efficiency of the elicitors in protective and curative treatments against *P. viticola* calculated from the disease severity (Riesling, 2011), statistics are in table 3.3

Efficiency of the elicitors (Müller-Thurgau)

The same four elicitors (phosphonate, phosphate, Algin Biovital® and Frutogard®) were applied on Müller-Thurgau. In general, all elicitors except phosphonate showed similar efficiency in both protective and curative treatments. Phosphate had the same efficiency in both treatments (94 %), while Frutogard® had a very high efficiency with a slight increase in case of curative treatment (96 %) compared to the protective treatment (90 %). Algin Biovital® showed low efficiency in the protective treatment (41 %) and in the curative treatment (29 %). As for phosphonate, the efficiency was 72 % in the protective treatment and 22 % in the curative treatment (Figure 3.4). Disease severity for protective and curative treatments is represented as mean values in table 3.4.

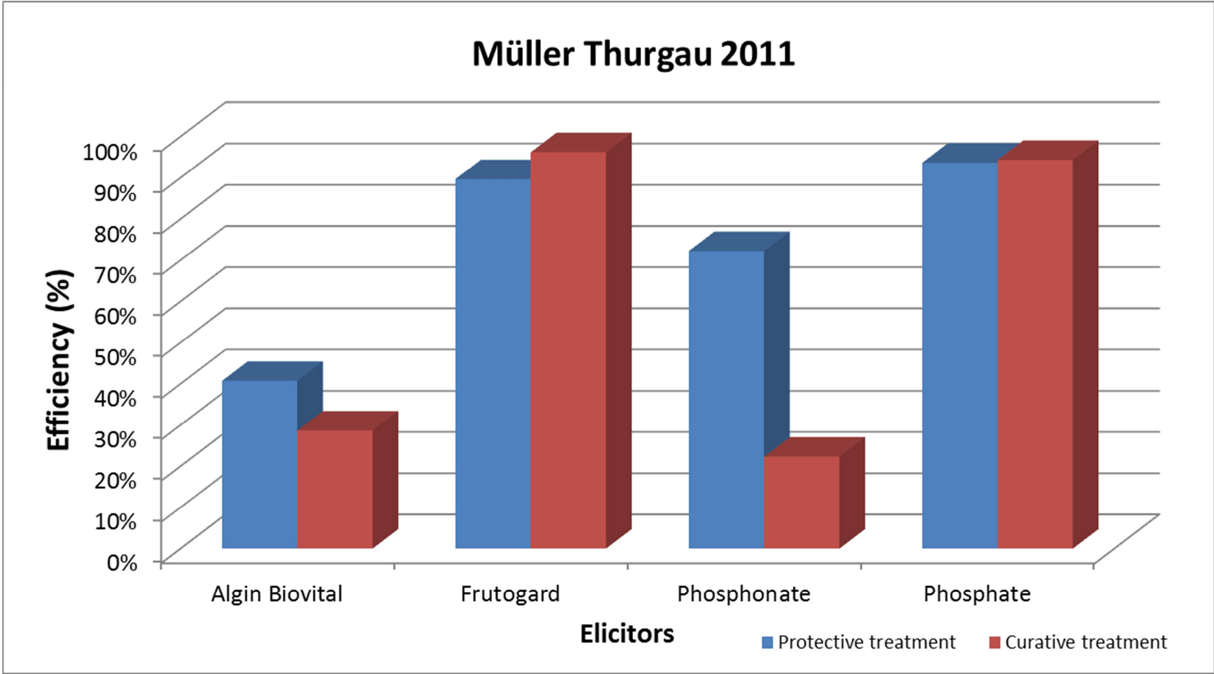

Figure 3.4: Assessment of the efficiency of the elicitors in protective and curative treatments against *P. viticola* calculated from the disease severity (Müller-Thurgau, 2011), statistics are in table 3.4

Table 3.1: Disease severity assessment (Riesling, 2009). Disease severity for protective and curative treatments is represented as mean values (MV) with their standard deviations (Std). Significant differences (Sig) between treatments were assessed by applying an ANOVA test followed by a Tukey HSD Test α = 0,05.

Riesling (2009)		**Inoculated (Control)**	**Strobilurin 0.24%**	**ß-1, 3-glucan 0.25%**	**Phosphonate 1%**	**Phosphate 1%**	**Myco-SIN 0.5%**	**Algin 1%**	**Frutogard 1%**
Protective Treatment	**MV**	25,1	4,4	23,5	0,1	5	13,7	7,3	11,1
	Std	18,2	3,4	10,5	0,4	3,7	6,5	7,8	8,6
	Sig (0.05%)	a	c,d	a,b	d	c,d	b,c	c,d	c
Curative Treatment	**MV**	75,1	71	64,4	1,7	44,3	50,6	61,3	19,9
	Std	9,5	13,7	30,6	1,7	13,8	11,9	10,7	7,3
	Sig (0.05%)	a	a	a,b	e	c	b,c	a,b	d

Different letters (a, b, c, d and e) indicate significantly different values

Table 3.2: Disease severity assessment (Müller-Thurgau, 2010). Disease severity for protective and curative treatments is represented as mean values (MV) with their standard deviations (Std). Significant differences (Sig) between treatments were assessed by applying an ANOVA test followed by a Tukey HSD Test α = 0,05.

Müller-Thurgau (2010)		**Inoculated (Control)**	**Strobilurin 0.24%**	**ß-1, 3-glucan 0.25%**	**Phosphonate 1%**	**Phosphate 1%**	**Myco-SIN 0.5%**	**Algin 1%**	**Frutogard 1%**
Protective Treatment	**MV**	18,5	0,0	3,7	7,0	0,8	16,5	10,8	3,3
	Std	7,3	0,0	4,0	4,3	1,3	9,6	5,9	3,4
	Sig (0.05%)	a	d	c,d	b,c	c,d	a	a,b	c,d
Curative Treatment	**MV**	40,3	0,0	38,8	23,8	0,7	36,2	24,4	0,5
	Std	17,4	0,0	12,0	12,2	1,4	9,5	8,0	0,8
	Sig (0.05%)	a	c	a	b	c	a	b	c

Different letters (a, b, c and d) indicate significantly different values

Table 3.3: Disease severity assessment (Riesling, 2011). Disease severity for protective and curative treatments is represented as mean values (MV) with their standard deviations (Std). Significant differences (Sig) between treatments were assessed by applying an ANOVA test followed by a Tukey HSD Test α = 0,05.

Riesling (2011)		Inoculated (Control)	Phosphonate 1%	Phosphate 1%	Algin 1%	Frutogard 1%
Protective Treatment	MV	85,4	10,5	7,4	37,3	10,9
	Std	6,6	9,4	6,2	11,9	8,1
	Sig (0.05%)	a	c	c	b	c
Curative Treatment	MV	86,0	79,0	31,0	83,5	6,3
	Std	13,2	19,6	13,4	12,6	7,6
	Sig (0.05%)	a	a	b	a	c

Different letters (a, b, c and d) indicate significantly different values

Table 3.4: Disease severity assessment (Müller-Thurgau, 2011). Disease severity for protective and curative treatments is represented as mean values (MV) with their standard deviations (Std). Significant differences (Sig) between treatments were assessed by applying an ANOVA test followed by a Tukey HSD Test α = 0,05.

Müller-Thurgau (2011)		Inoculated (Control)	Phosphonate 1%	Phosphate 1%	Algin 1%	Frutogard 1%
Protective Treatment	MV	80,8	22,4	5,1	47,9	8,3
	Std	10,3	12,7	4,9	12,1	4,5
	Sig (0.05%)	a	c	d	b	d
Curative Treatment	MV	90,6	70,4	5,1	64,6	3,5
	Std	12,0	14,0	5,1	19,2	4,2
	Sig (0.05%)	a	b	c	b	c

Different letters (a, b, c and d) indicate significantly different values

3.1.2 Efficiency of the elicitors on tolerant varieties

Efficiency of the elicitors in cv Regent and Solaris (2012)

After testing the elicitors on susceptible varieties such as Riesling and Müller-Thurgau, in 2012, their efficiency was investigated in tolerant varieties. Therefore, elicitors were applied, only protectively, on Regent and Solaris as tolerant varieties at two different growth stages: the potted vines were at the age of three months, while container vines were one year old. In the greenhouse experiment on potted vines cv. Regent and potted vines cv. Solaris, no disease symptoms were observed, while a disease severity of 100 % was observed in simultaneously inoculated potted vines cv. Riesling (Figure 3.5), which indicates that the absence of the symptoms on Regent and Solaris is due to their tolerance. Similar results were observed on container plants from Regent and Solaris. However, disease severity on Riesling plants was lower in this experiment (55 %). Only-inoculated Regent plants and Algin Biovital® treated Regent plants showed relatively low (20 %) and very low (5 %) symptoms, respectively (Figure 3.6).

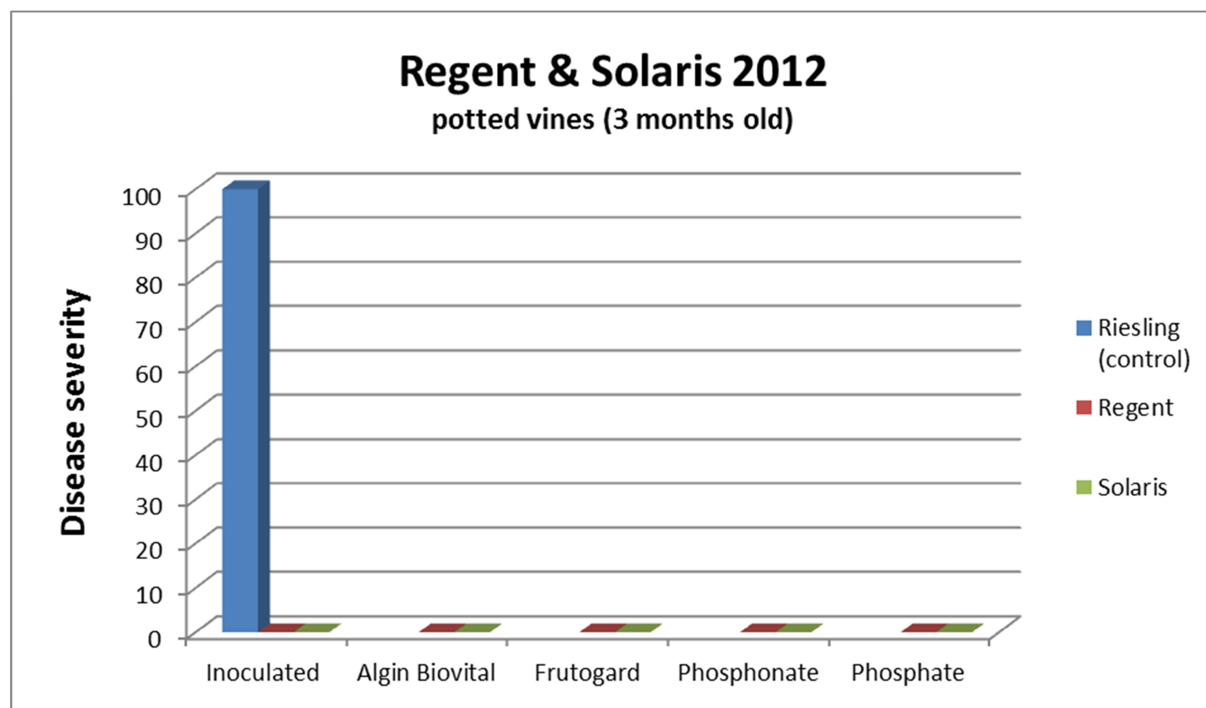

Figure 3.5: Assessment of disease severity during the protective treatment in the potted grapevine varieties Riesling (control), Regent and Solaris (2012)

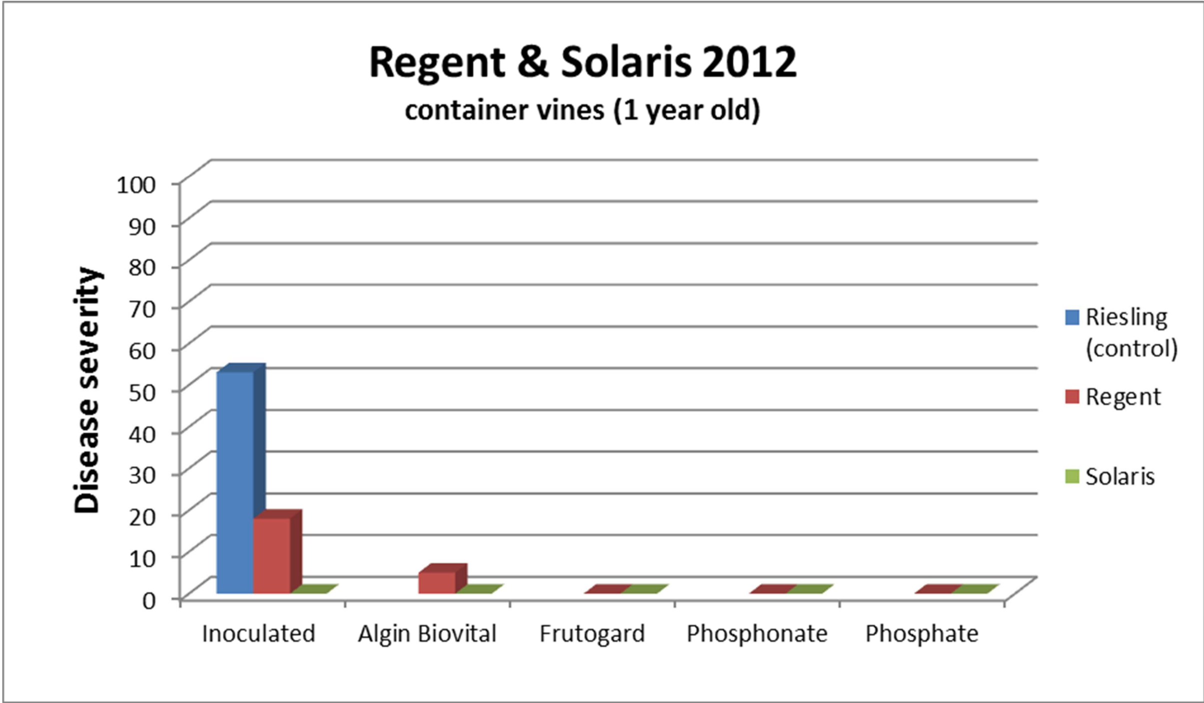

Figure 3.6: Assessment of disease severity during the protective treatment in the container grapevines (1 year old) varieties Riesling (control), Regent and Solaris (2012)

3.2 Accumulation of stilbenes in response to inoculation and/or elicitation (Regent and Solaris, 2012)

Some of the defense related secondary metabolites (stilbenes) were measured using an optical sensor based on noncontact leaf autofluorescence measurements. Stilbenes are one of the major induced polyphenols produced in stressed grapevine. In general, Regent produced more stilbenes (between 90 and 150 Multiplex unit) than Solaris (between 80 and 120 Multiplex unit) after elicitation and infection (Figure 3.7 a and b). Control plants (neither treated nor elicited) from Regent and Solaris contained stilbenes but in low amounts, which indicates that stilbene production is constitutive in these tolerant varieties. Moreover, it was clear that there was a fluctuation in stilbene content during the day. It was observed that stilbene content increased at 3 hat (hour after treatment) then reduced at 6 hat and then increased again to reach its maximum at 24 hat (Figure 3.7 a and b). In the second day it followed the same pattern, however, in Solaris, stilbene content suddenly increased to reach its maximum at 24 hai (hour after inoculation) or 48 hat (Figure 3.7, b).

Inoculated Regent plants showed a slight increase after inoculation (between 105 and 115 Multiplex unit), while inoculated Solaris plants showed a strong increase in stilbene content that reached its maximum at 24 hai (between 95 and 125 Multiplex unit). Water treated plants exhibited the same trend in both varieties. However, stilbene content in water treated Regent plants was higher (105 to 120 Multiplex unit), whereas in water treated Solaris plants the content was between (85 to 105 Multiplex unit) (Figure 3.7 a, b). Plants treated with Frutogard® and Algin showed the highest content of stilbenes in both varieties. The stilbene content showed a steady increase over time in case of Regent plants, while in Solaris there was a strong fluctuation over the time (Figure 3.7 b). Regent and Solaris plants treated with phosphate had the third highest stilbene content after plants treated with Frutogard® and Algin Biovital (Figure 3.7 a, b). However, plants treated with phosphonate reached its maximum at 6 hai then decreased afterwards (Figure 3.7 b).

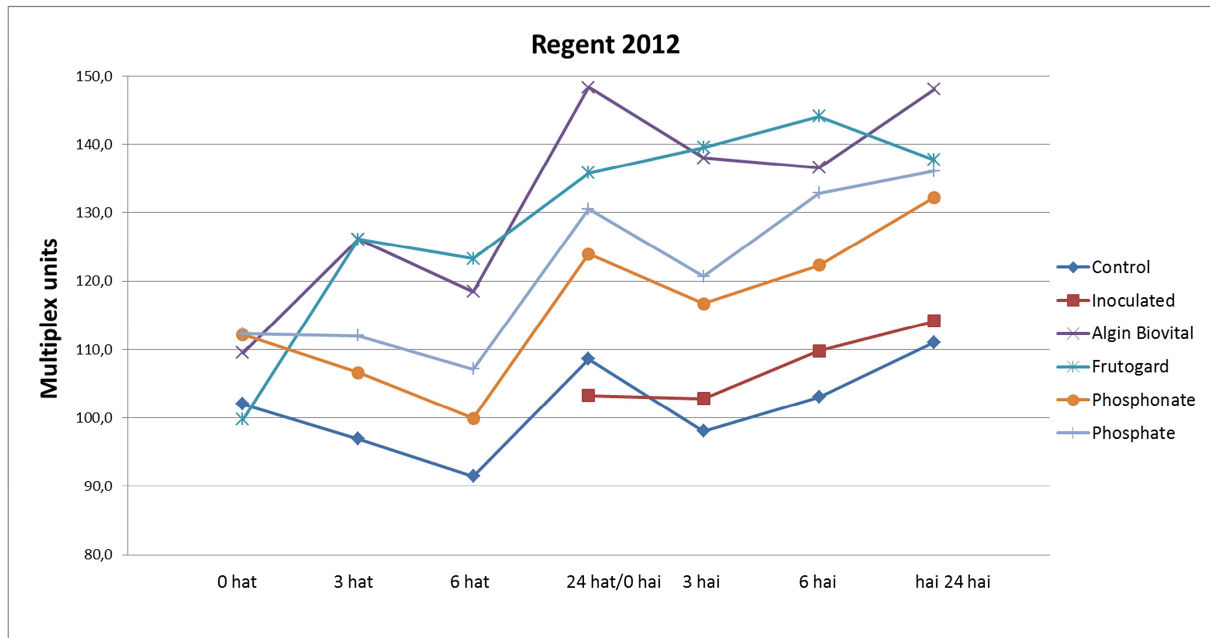

Figure 3.7a: Stilbene content (in Multiplex units) in Regent during the protective treatment (2012) in 1-year-old container grapevines. hat = hour after treatment, hai = hour after inoculation

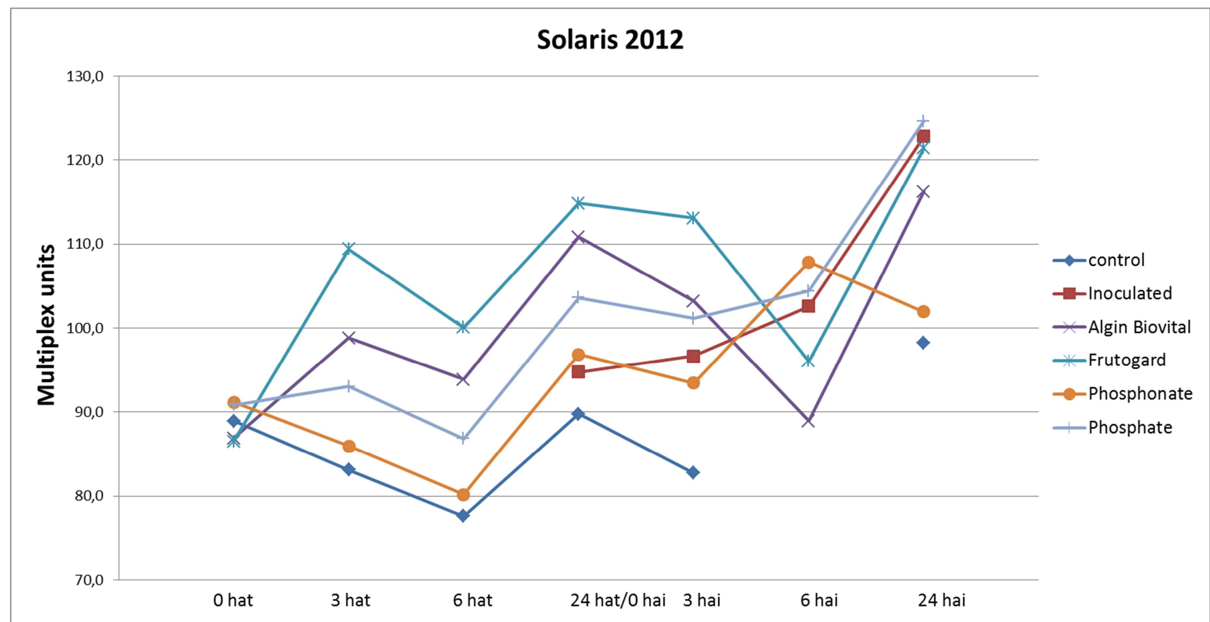

Figure 3.7b: Stilbene content (in Multiplex units) in Solaris during the protective treatment (2012) in 1-year-old container grapevines. hat = hour after treatment, hai = hour after inoculation

3.3 Epifluorescence microscopy observations

To visualize intercellular infection structures of *P. viticola* inside the leaf tissue, observations with epifluorescence microscopy were conducted after aniline blue and trypan blue staining. Both dyes were specific to pathogen structures.

3.3.1 Pathogen development (Formation of infection structures)

Presence of sporangia and zoospores on the lower leaf surface

Sporangia were efficiently stained with trypan blue (Figure 3.8, A and B), while the zoospores could not be observed. However, aniline blue stained the encysted zoospores (Figure 3.8, D and C). The reason for this may be that the first samples for microscopy were taken one day after infection and normally once the zoospores are released from the sporangia, they shed their flagella and encyst at the stomatal cavity.

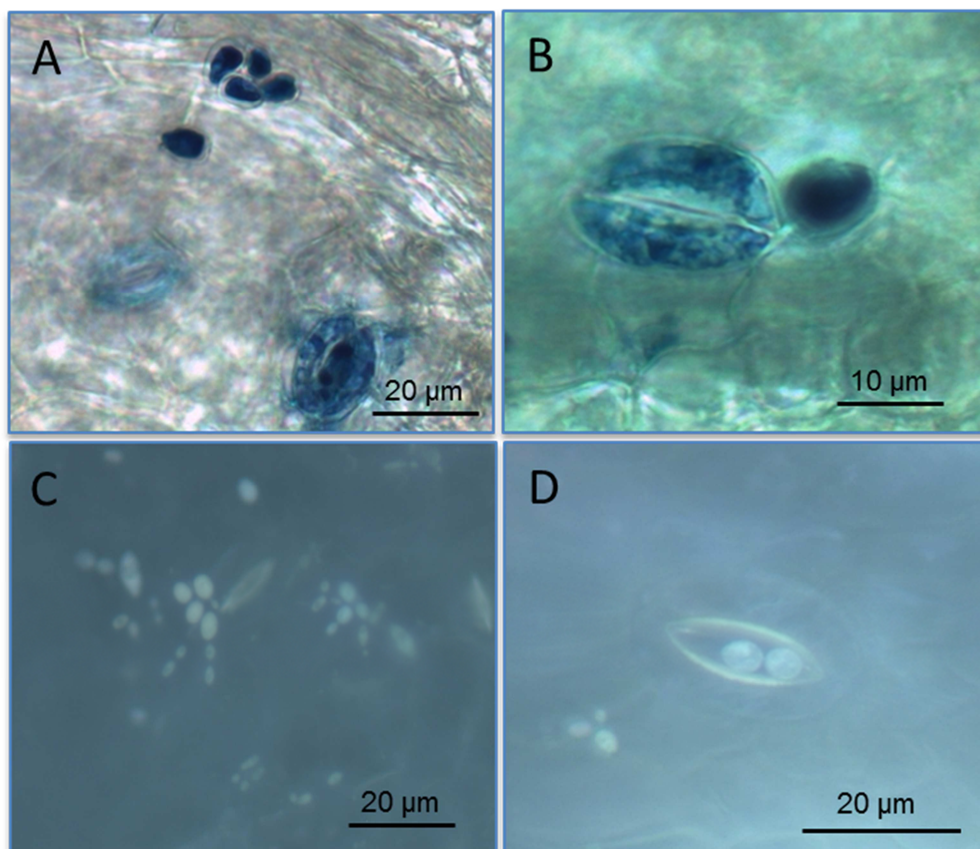

Figure 3.8: Observation with epifluorescence microscope of the abaxial side of the leaf of the non-treated susceptible grapevine cultivar (Riesling) infected with *P. viticola* 24 h post inoculation after trypan blue and aniline blue staining
(A) Sporangia (egg-shaped) on the abaxial side of the leaf (trypan blue)
(B) Sporangia approaching the stomata (trypan blue)
(C) Sporangia releasing zoospores (aniline blue)
(D) Encysted zoospores at the stomatal cavity (aniline blue)

Hyphal growth and haustoria inside the mesophyll

After zoospores germinate, a primary hypha emerges and enters the mesophyll to grow endophytically. After few days of inoculation, a complex mycelium was observed inside the mesophyll tissues of the inoculated, susceptible grapevine cultivar (Riesling) (Figure 3.9). Inoculated plants showed the highest rate of infection represented in massive mycelial growth (Figure 3.9, A). Plants treated with elicitors and inoculated showed less mycelial growth (Figure 3.9, B) that was in some cases restricted to some areas (Figure 3.9, C) as in the case of the plants treated with Myco-Sin VIN®. Control plants (non-inoculated) and elicited plants (non-inoculated) did not show any mycelial growth (Figure 3.9, D). At later stages of infection, further hyphae are developed, allowing P*. viticola* to spread into the intercellular spaces. Haustoria were seen under the epifluorescence microscope as shiny spots spreading along the mycelia (Figure 3.10). Haustorium formation is very important in the establishment of successful biotrophy. It plays a pivotal role in exchanging of nutrients and signal molecules with the host plant. It was difficult to quantify the mycelial growth in the mesophyll. However, infection rate represented by hyphal growth in the mesophyll was different between protective and curative treatment in only inoculated plants, since the disease severity was lower in the protective treatment compared to the curative treatment (Table 3.5). Treated plants showed different infection rates which indicates that different elicitors acted differently on *P. viticola* and its life cycle. In general, inoculated plants that were treated protectively showed less hyphal growth than those treated curatively. The mycelial growth (Table 3.5) between different treatments was in relation to the efficiency of the elicitors (refer to the corresponding table in the previous chapter). Indeed, plants treated with elicitors that showed higher efficiency in concern of symptom formation (sporulation) had less mycelial growth in the mesophyll band and vice versa.

Table 3.5: Average of hyphal growth (in %) of *P. viticola* in the mesophyll of Riesling 2009

	Infection (mycelial growth)	
	Protective	**Curative**
Inoculated	30%	80%
Control	0%	0%
Strobilurin	5 %	70%
Phosphonate	0%	5%
Phosphate	5%	40%
ß-1, 3-glucan	20%	60%
Myco Sin® VIN	10%	50%
Algin Biovital	5%	60%
Frutogard®	10%	20%

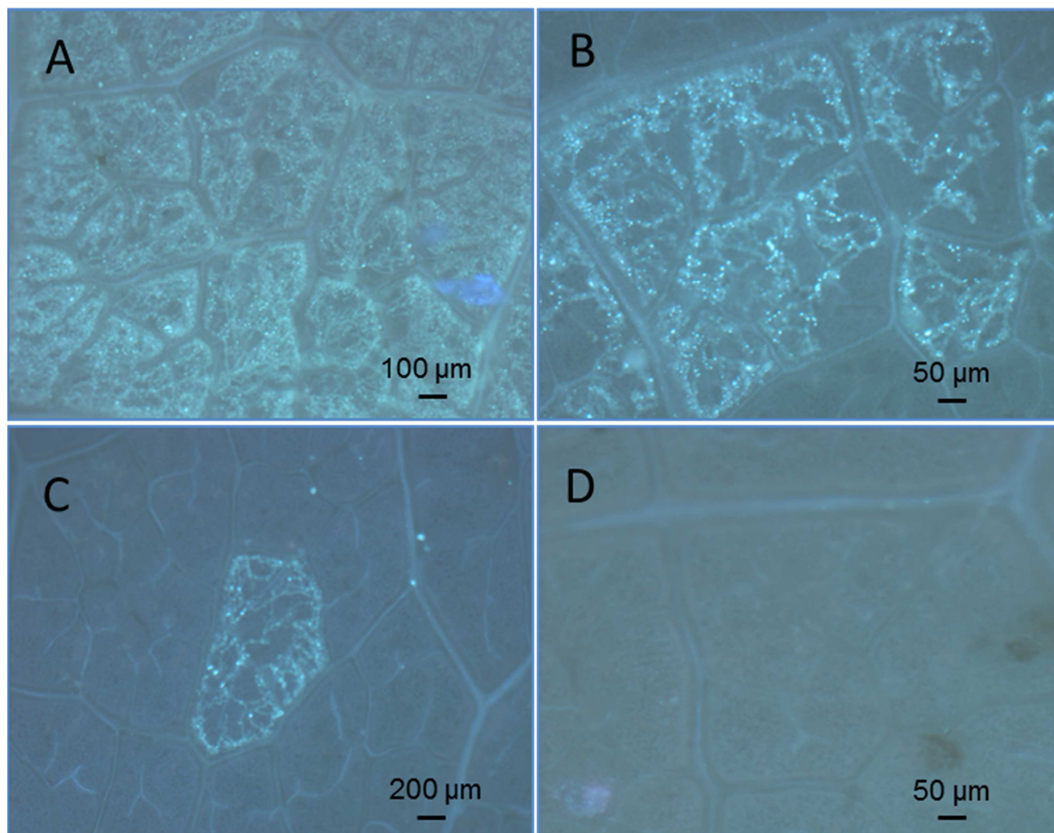

Figure 3.9: Observation with epifluorescence microscope after aniline blue staining of the lower leaf surface of the susceptible grapevine cultivar (Riesling) infected with *P. viticola* 5 dai
(A) Massive mycelial growth within the leaf tissue (inoculated plants)
(B) Less mycelial growth within the leaf tissue (treated and inoculated plants)
(C) Local mycelial growth within the leaf tissue (treated and inoculated plants)
(D) No mycelial growth within the leaf tissue (control plants)

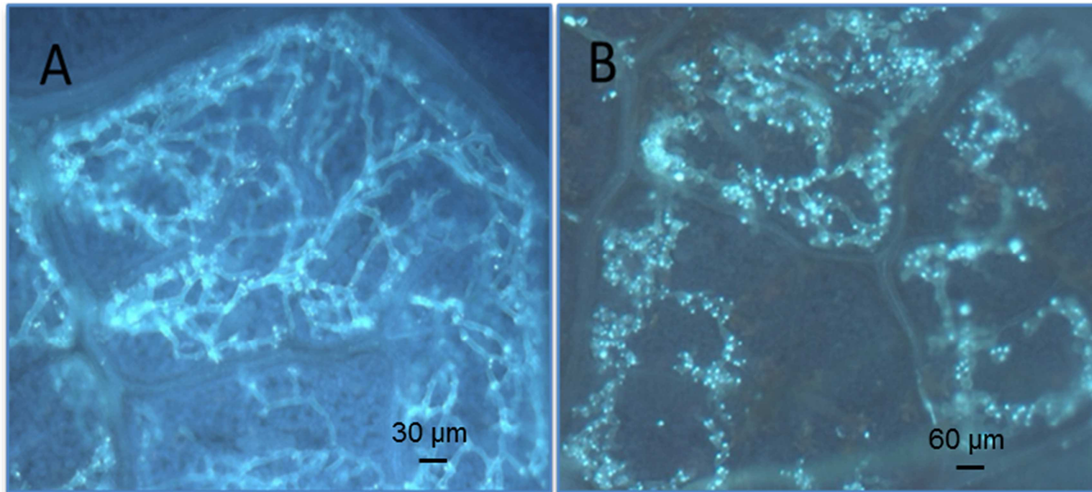

Figure 3.10: Observation with epifluorescence microscope after aniline blue staining of the lower leaf surface of the non-treated susceptible grapevine cultivar (Riesling) infected with *P. viticola* 5 dai
(A) Mycelia with haustoria within the leaf tissue
(B) Haustoria are represented as shiny spots along the mycelia (magnified)

Sporulation

Emergence of sporangiophores from the stomata was observed later in samples collected after the plastic wrap was removed, 8 dai in case of plants treated protectively and 9 dai in case of plants treated curatively. Non-treated plants showed more sporangiophores than treated plants. The quantity of sporangiophores was corresponding to the mycelial growth rate (Table 3.5), where tissues with more mycelia had more sporangiophores. Figure 3.11, A demonstrates the emergence of sporangiophores from the stomata, while Figure 3.11, B shows a fully developed sporangiophore. Figure 3.11, C and D demonstrates egg-shaped sporangia carried on sporangiophores, which signifies the end of the successful infection cycle of *P. viticola* on the susceptible grapevine (Riesling) that ends normally with sporulation.

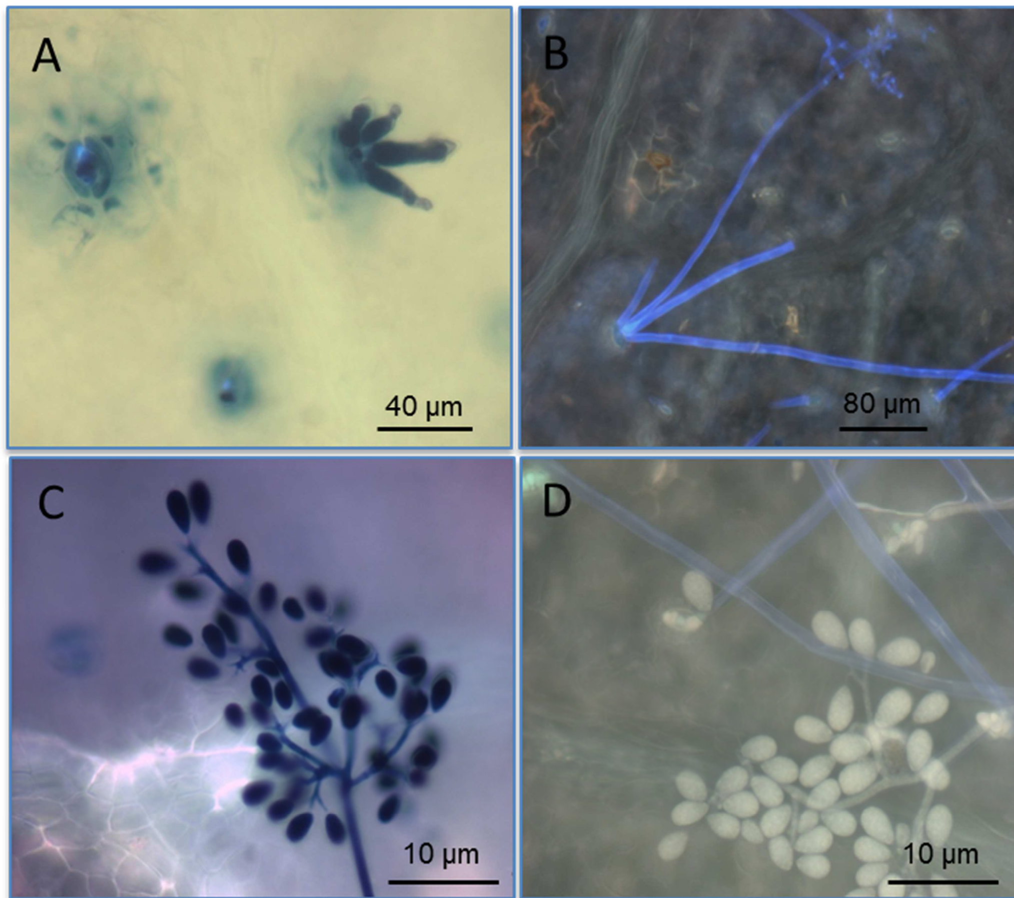

Figure 3.11: Observation with epifluorescence microscope of sporangiophores on the susceptible grapevine cultivar (Riesling) after trypan blue and aniline blue staining
(A) Emergence of sporangiophores from the stomata (trypan blue)
(B) Fully developed sporangiophores (aniline blue)
(C) Sporangiophores carrying egg-shaped sporangia (trypan blue)
(D) Sporangiophores carrying egg-shaped sporangia (aniline blue)

3.3.2 Plant responses

Callose deposition at infection site (stomata)

Callose, a polymer of glucose with ß-1,3 glycosidic linkages, is thought to function as a mechanical barrier to inhibit the entrance of infection structure through the stomata and limit pathogen growth by inhibiting the sporangiophores from emerging and releasing the zoospores carried in their egg-shaped sporangia. Callose was the only plant-originating molecule that was selectively stained with aniline blue, while trypan blue stained both callose and guard cells of the stomata. Little callose deposition was seen in case of only inoculated plants (Table 3.6). Elicited plants showed callose deposition at the stomatal cavity (Figure 3.12). The degree of deposition was different between treatments (Table 3.6). Callose deposition started at 2nd dai in the protective treatment, while in the curative treatment it started at 3rd dai. Callose was

deposited only in the infected area except for ß-1,3-glucan where it was deposited in both infected and non-infected area. Myco-Sin® VIN showed the highest rate of callose deposition in both protective and curative treatment. Plants treated with phosphate, phosphonate and strobilurin did not show any callose deposition. No callose deposition was visible in non-treated plants (neither inoculated nor elicited). Stomata guard cells were strongly fluorescent after staining with trypan blue eventually suggesting callose deposition inside these cells (Figure 3.12, C). Figure 3.12, D shows premature (incomplete) sporangiophores without sporangia that could not reach to the sporulation stage due to callose deposition.

Table 3.6: Average of callose deposition (in %) at the stomata in Riesling 2009

	Callose deposition	
	Protective	**Curative**
Inoculated	20%	50%
Control	0%	0%
Strobilurin	0%	0%
Phosphonate	0%	0%
Phosphate	0%	0%
ß-1,3-glucan	20%	10%
Myco Sin® VIN	40%	60%
Algin Biovital	10%	60%
Frutogard®	40 %	40%

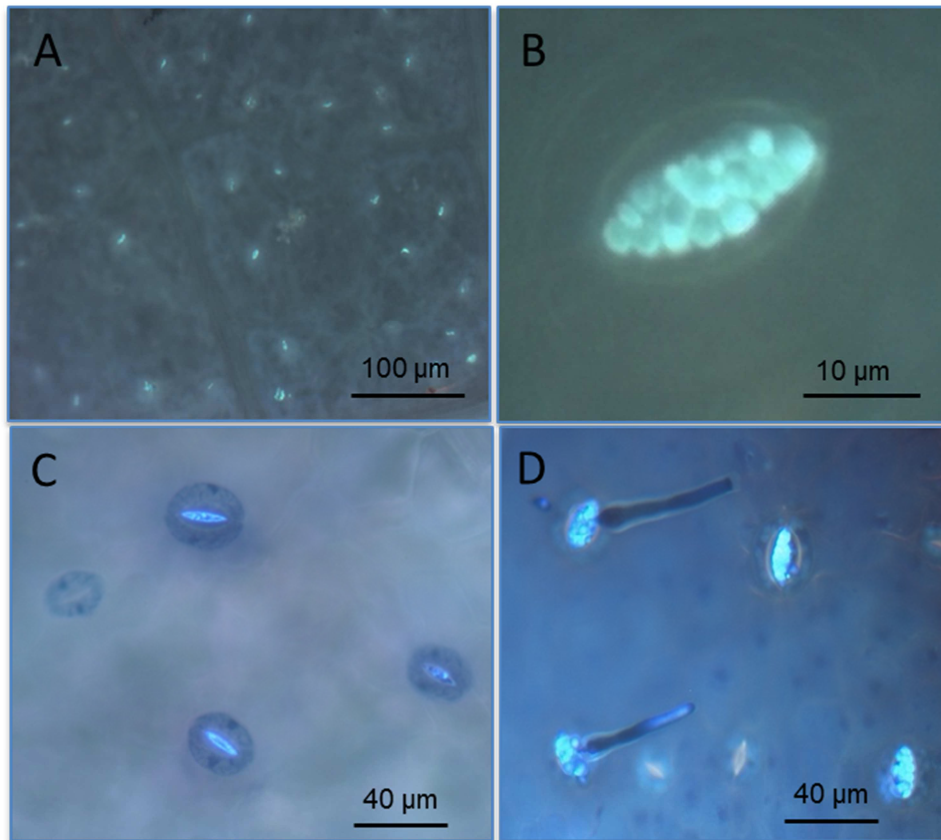

Figure 3.12: Observation with epifluorescence microscope of callose deposition at the stomata after aniline blue and trypan blue staining
(A) Stomatal cavity filled with callose (stained with aniline blue)
(B) Magnification of (A)
(C) Stomatal cavity filled with callose (stained with trypan blue)
(D) Premature sporangiophores

Hypersensitive-like response

The hypersensitive response (HR) is a form of programmed cell death. It is localized rapid cell death of one or a few host cells, represented by micro-necrotic zones, in response to pathogen infection during incompatible reaction; it limits the spread of the pathogen to adjacent cells as well as it stops the emergence of sporangiophores and hence the sporulation, thereby inhibiting further secondary infections. It is very effective against biotrophic pathogens, as they require living host cells for nutrition. It is triggered by the production of reactive oxygen species (ROS) e.g. O^{2-}, •OH, H_2O_2 and NO.

Substantial differences of timing and extent of the symptoms between treatments and control plants were observed (Table 3.7). Inoculated plants showed very few hypersensitive-like response symptoms around some stomata (Figure 3.13 A and B). The strategy of application (protective or curative) of elicitors did not have an effect on the rate of hypersensitive-like response symptoms. Plants elicited with Algin

Biovital® and Myco-Sin® VIN showed the highest rate of HR symptoms, while Frutogard® displayed also relatively high rate of the symptoms. Phosphonate, phosphate and Strobilurin treated plants showed very few hypersensitive-like response symptoms. ß-1,3-glucan treated plants did not show any symptoms. However, it is difficult to determine if these symptoms are because of the induction of programmed cell death or because of necrosis since no •OH, H_2O_2 or NO levels were measured.

Table 3.7: HR-like symptoms (in %) in Riesling 2009

	HR-like symptoms	
	Protective	**Curative**
Inoculated	5%	5%
Control	0%	0%
Strobilurin	5%	5%
Phosphonate	5%	5%
Phosphate	5%	5%
ß-1,3-glucan	0%	0%
Myco Sin® VIN	20%	20%
Algin Biovital	20%	20%
Frutogard®	10%	10%

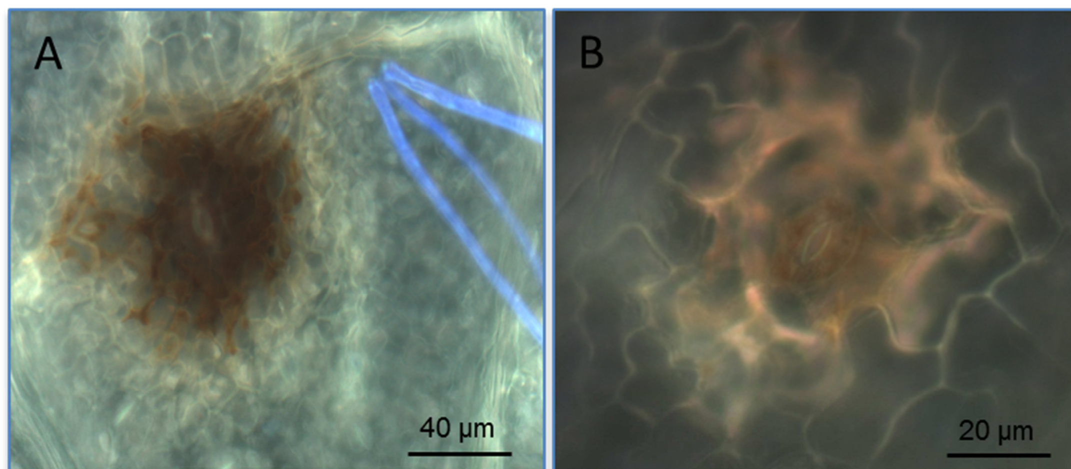

Figure 3.13: Observation with epifluorescence microscope of necrotic areas around the stomata after staining with aniline blue
(A) Necrotic zones surrounding the stoma, while sporangiophores are emerging from an adjacent stoma.
(B) Same as (A) but magnified.

3.4 Investigating the time course of the infection by targeted gene expression studies

In order to determine the time point where the defense responses are fully expressed, several defense-related genes known to be regulated during the *P. viticola*/grapevine interaction and/or the treatment with resistance inducers were investigated by qPCR. The expression of grapevine defense-related genes was analyzed on samples taken from protective experiments using all elicitors. Samples were taken from time points that range from 0 until 7 dat (day after treatment) or 6 dai (dai after inoculation).

Three defense-related genes were analyzed: stilbene synthase (STS), 9-lipoxygenase (LOX) and chitinase (CHIT_1b), while actin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and elongation factor 1 (EF1) were used as reference genes for normalization during gene expression analysis. PCRs displayed efficiencies between 92% and 105% and the specificity of the primer pairs was confirmed by the presence of a single peak during the final dissociation curve step.

Gene expression results were represented as heat-maps (Figure 3.14, 15, 16) through hierarchical clustering showing the level of expression during the time course of the experiment. They show the relative gene expression, in which high expression is depicted as light red squares and low expression as dark red squares. Black squares indicate that the treatment had no effect on the gene expression, while gray squares indicate no data. P0 (no *P. viticola*) represents plants that are only elicited and P1 (with *P. viticola*) represents plants that are elicited and inoculated.

3.4.1 Expression of stilbene synthase (STS) after elicitation and/or inoculation

Our results showed that the expression of STS was different between treatments (Figure 3.14); the expression started early in some cases then decreased with time, while in other cases the trend was reversed. Plants that were only elicited (P0), with Strobilurin, phosphate, phosphonate, Algin Biovital® and Frutogard® showed an early strong expression of STS at T2, which is 24 h after elicitation, except for Strobilurin that showed a strong expression at T3. Thereafter, the expression of STS was reduced to almost no expression. Plants treated with ß-1, 3-glucan did not show any significant expression of STS. Control plants (neither treated nor inoculated) and plants treated with water showed a slight expression of STS in the first two days. Inoculation had a strong impact on the expression of STS. Inoculated plants showed

a strong expression at T3 which is 24 h after inoculation, which decreased at T4 then strongly increased in a steady trend until T7. In plants that are elicited and inoculated (P1) the expression of STS was strong in the early days after infection then decreased, then again increased. This trend was observed in plants treated with Strobilurin, Algin Biovital®, Frutogard® and ß-1,3-glucan and Myco-Sin® VIN. Plants that are inoculated and treated with phosphonate and phosphate showed a slight expression in the early days after elicitation and inoculation that decreased with time (Figure 3.14).

3.4.2 Expression of 9-Lipoxygenase (9-LOX) after elicitation and/or inoculation

Plants treated with Strobilurin, phosphate, Algin Biovital®, Frutogard®, ß-1, 3-glucan and Myco-Sin® VIN (P0) showed a steady moderate expression of 9-LOX, except for Algin Biovital® that showed a relatively strong expression in the beginning followed by a decrease (Figure 3.15). Plants treated with phosphonate showed a high expression at the 5th day after elicitation followed by a decrease. Control plants and plants treated with water showed a steady low expression. Inoculated plants and plants inoculated and elicited (P1) with Algin Biovital®, Frutogard®, ß-1, 3-glucan and Myco-Sin® VIN showed an early expression of 9-LOX that increased with the time course to reach its maximum at T7. Plants inoculated and elicited with phosphonate, phosphate and Strobilurin showed a moderate expression throughout the time course (Figure 3.15).

3.4.3 Gene expression of chitinase (CHIT_1b) after elicitation and/or inoculation

Chitinases, antimicrobial proteins, are one of the PR-proteins’ classes. They were found to be induced under pathogen attack and elicitor treatment. CHIT_1b is a PR-3 basic class I chitinase.

In general, plants treated with elicitors (P0) and plants treated and inoculated (P1) exhibited an early strong expression of CHIT_1b that decreased afterwards except for inoculated plants and plants treated with ß-1, 3-glucan, showing a low level of expression that increased with time (Figure 3.16). Plants treated with phosphonate and phosphate had the same expression trend, where they had a high expression at T2, T3 and T5 followed by a reduced expression at T5 and T6 (Figure 3.16).

3.4.4 Selecting the suitable sampling point for microarray analysis

Investigating the time of the infection by targeted gene expression studies was done with the objective to determine the best sampling point for the microarray analysis. The third sampling point (T3), that is 24 h after inoculation and 48 h after elicitation, was chosen. At this time point the defense responses are fully expressed for the above mentioned genes. This was observed in the expression of the tested defense-related genes that are known to be regulated during the *P. viticola*/grapevine interaction and/or the treatment with resistance inducers such as stilbene synthase and chitinase.

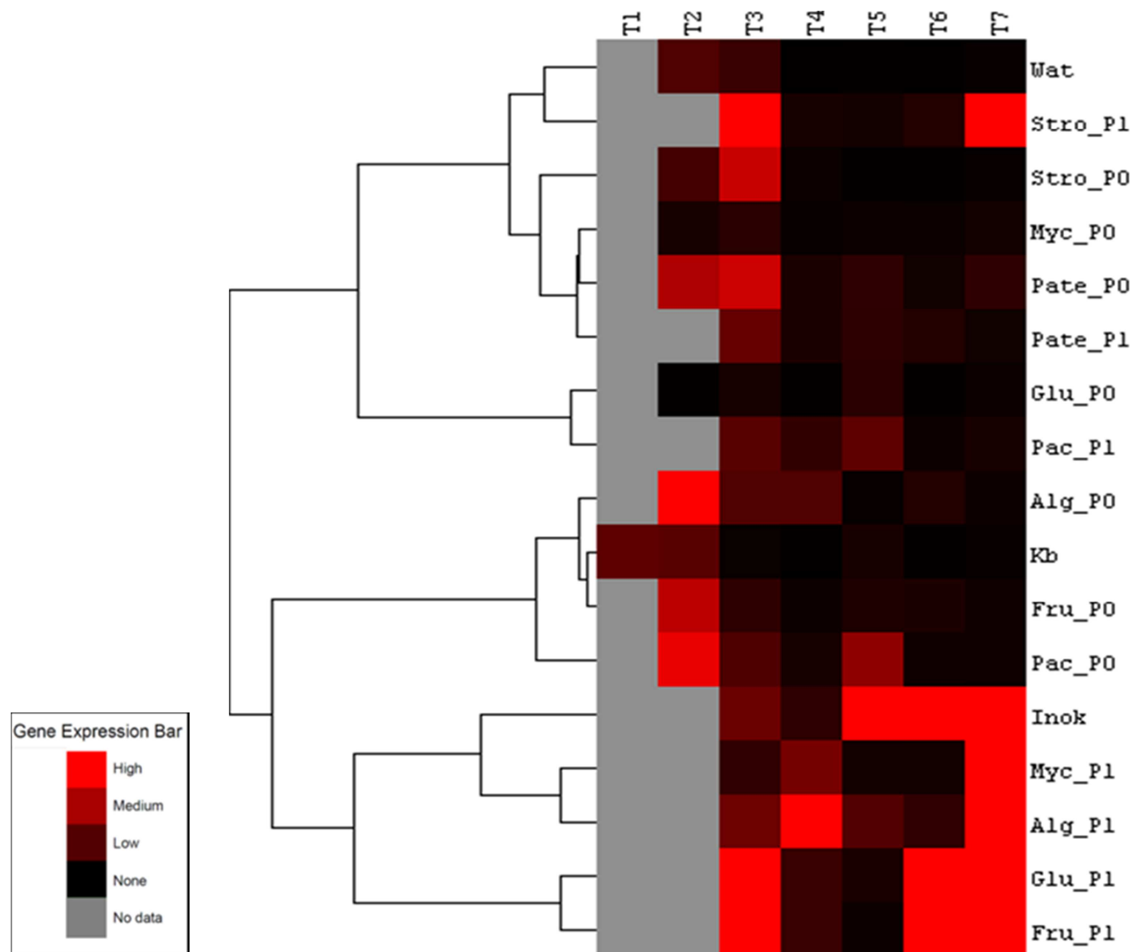

Figure 3.14: Heat-map and hierarchical cluster analysis of the differential expression of stilbene synthase during the protective treatment (Riesling, 2009)

Columns represent gene expression at different time points [T1 (0dai)-T7 (6dai)]. Elicitation was carried out at day 1 (T1) and inoculation at day 2 (T2). Rows represent different treatments, where (P0) indicates elicitation and (P1) elicitation and inoculation. Grey = no samples collected; black = no increase in gene expression; dark red = low expression; medium red = moderate expression; red = high expression. (Kb) control, (Wat) water, (Inok) inoculated, (Stro) Strobilurin, (Pac) phosphonate, (Pate) phosphate, (Alg) Algin Biovital®, (Fru) Frutogard®, (Myc) Myco-sin VIN® and (Glu) ß-1, 3-glucan.

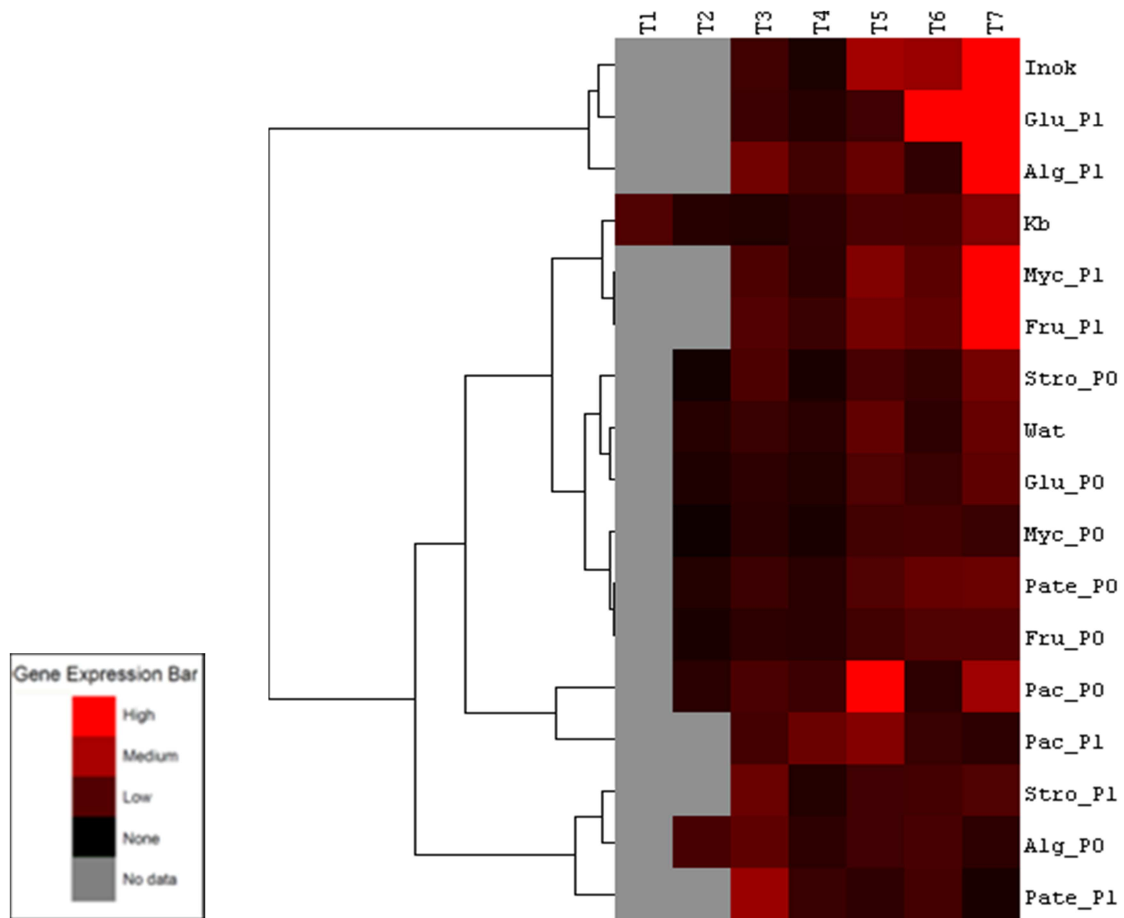

Figure 3.15: Heat-map and hierarchical cluster analysis of the differential expression of lipoxygenase during the protective treatment (Riesling, 2009)

Columns represent gene expression at different time points [T1 (0dai)-T7 (6dai)]. Elicitation was carried out at day 1 (T1) and inoculation at day 2 (T2). Rows represent different treatments, where (P0) indicates elicitation and (P1) elicitation and inoculation. Grey = no samples collected; black = no increase in gene expression; dark red = low expression; medium red = moderate expression; red = high expression. Kb) control, (Wat) water, (Inok) inoculated, (Stro) Strobilurin, (Pac) phosphonate, (Pate) phosphate, (Alg) Algin Biovital®, (Fru) Frutogard®, (Myc) Myco-sin VIN® and (Glu) ß-1, 3-glucan.

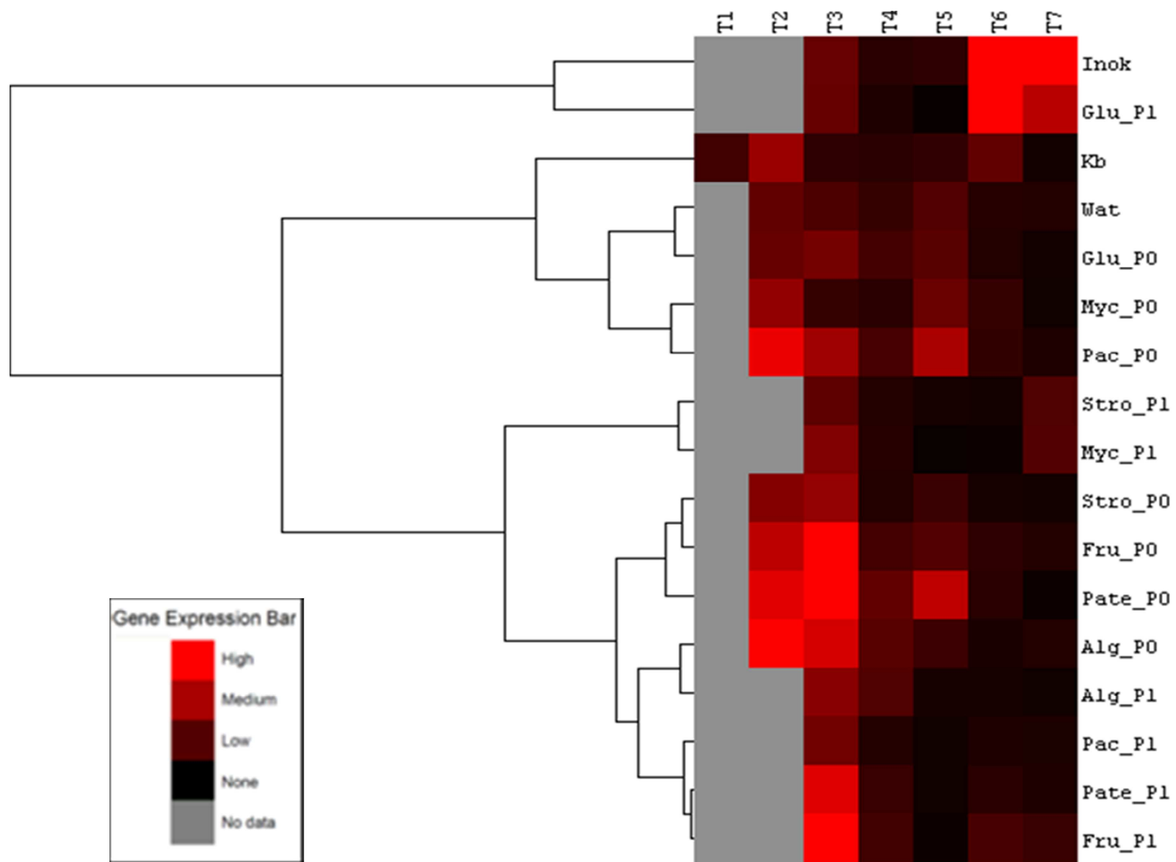

Figure 3.16: Heat-map and hierarchical cluster analysis of the differential expression of the enzyme chitinase_1b during the protective treatment (Riesling, 2009)

Columns represent gene expression at different time points [T1 (0dai)-T7 (6dai)]. Elicitation was carried out at day 1 (T1) and inoculation at day 2 (T2). Rows represent different treatments, where (P0) indicates elicitation and (P1) elicitation and inoculation. Grey = no samples collected; black = no increase in gene expression; dark red = low expression; medium red = moderate expression; red = high expression. Kb) control, (Wat) water, (Inok) inoculated, (Stro) Strobilurin, (Pac) phosphonate, (Pate) phosphate, (Alg) Algin Biovital®, (Fru) Frutogard®, (Myc) Myco-sin VIN® and (Glu) ß-1, 3-glucan.

3.5 Differential gene expression after elicitation and/or inoculation

The major aim of this work is to elucidate the molecular mechanisms underlying induced resistance in the susceptible grapevine (*Vitis vinifera* L. cv. Riesling) against *Plasmopara viticola* after elicitation with the different resistance inducers. Therefore, a whole transcriptome analysis of the grapevine leaves was performed to provide information on the genes involved in this pathosystem as well as genes involved in induced resistance mechanisms. To this end, microarray experiments were performed on the samples collected in 2009 and 2011. However, we will concentrate on the results from 2011 since disease severity in control, only inoculated plants in that year was sufficiently high (85% compared to 15% in 2009). Based on the investigation of the time course of the infection by qPCR (see chapter 3.4), samples were taken 24 h after inoculation and/or 48 h after treatment with the elicitors. Only samples from the protective treatment were considered for this experiment. Thus, eight conditions comprising 3 elicitors and their proper controls were used for microarrays (see material and methods).

Microarray results were lists of differentially expressed genes (DEGs), after statistical analysis of the data (p-value < 0.05, fold change threshold: $\log_2$ ratio >+1 or <-1), between different conditions. Differential expression analysis was performed with LIMMA (Linear Models Microarray Analysis) R package. P values were adjusted for multiplicity with Benjamini Hochberg method. Table 3.5 shows the number of differentially expressed genes (DEGs) in each comparison analyzed.

Table 3.5: Numbers of differentially expressed genes (DEGs) in 2011

Comparisons within each experiment	DEGs	Description of the DEGs
Inoculated – control	3466	DEGs due to inoculation only
Frutogard (P0) – control	462	DEGs due to elicitation with Frutogard
Frutogard (P1) – Inoculated	47	DEGs due to elicitation with Frutogard and inoculation
Phosphonate (P0) – control	1422	DEGs due to elicitation with Phosphonate
Phosphonate (P1) – Inoculated	2848	DEGs due to elicitation with Phosphonate and inoculation
Phosphate (P0) – control	1529	DEGs due to elicitation with Phosphate
Phosphate (P1) – Inoculated	3390	DEGs due to elicitation with Phosphate and inoculation

P (0) = Elicitation but no inoculation with *P. viticola*
P (1) = Elicitation and inoculation with *P. viticola*
Control = neither inoculated nor elicited

Classification and visualization of DEGs in Mapman

MapMan, a software that classifies genes and metabolites, visualizes the gene expression results as pictorial diagrams, where genes are assigned based on their annotation into non-redundant and hierarchically organized BINs. Each BIN consists of genes that share similar function (pathway) and can be further split into sub-BINs corresponding to sub nodes of the biological function. Each bin has a different number of clones, each of which corresponds to one gene. According to Mapman, there are 35 major bins and more than 1200 sub-bins. A special interest will be allocated to DEGs in the stress pathways such as genes involved in the biosynthesis of PR-proteins and secondary metabolites as well as R-genes. Table 3.6 shows the organization of MapMan bins.

Table 3.6: MapMan BINs. Taken from Rotter et al. (2009)

Bin	Bin name	No. of clones in the BIN
1	Photosynthesis	494
2	Major carbohydrate metabolism	165
3	Minor carbohydrate metabolism	162
4	Glycolysis	123
5	Fermentation	52
6	Gluconeogenese/glyoxylate cycle	22
7	Oxidative pentose phosphate pathway	42
8	TCA – organic transformation	123
9	Electron transport / ATP synthesis	156
10	Cell wall	595
11	Lipid metabolism	459
12	N-metabolism	59
13	Amino acid metabolism	459
14	S-assimilation	15
15	Metal handling	142
16	Secondary metabolism	543
17	Hormone metabolism	502
18	Co-factor and vitamine metabolism	45
19	Tetrapyrrole synthesis	56
20	Stress	948
21	Redox	282
22	Polyamine metabolism	18
23	Nucleotide metabolism	147
24	Biodegradation of xenobiotics	24
25	C1-metabolism	33
26	Miscellaneous enzyme groups	1219
27	RNA	2296
28	DNA	422
29	Protein	3628
30	Signalling	1157
31	Cell	655
33	Development	405
34	Transport	951
35	35.1. not assigned. no ontology	3276
	35.2. not assigned. unknown	15571

3.5.1 Changes in the gene expression pattern upon inoculation of grapevine with *P. viticola*

Inoculation with *P. viticola* led to differential expression of 3466 genes. The bins that had the highest relative number of DEGs were cell wall (16%), stress (12%), secondary metabolism (33%), hormone metabolism (23%), signalling (14%), cell (16%), transport (14%) and carbohydrate metabolism (major 12%, minor 9%). DEGs corresponding to bins photosynthesis, carbohydrate metabolism, cell wall, lipid metabolism, secondary metabolism, hormone metabolism, redox and RNA processing and regulation were majorly repressed (Table 3.7), whereas most of the DEGs involved in stress pathways and signalling were up-regulated (Table 3.7).

Interestingly, genes involved in stress pathways such as genes coding for dirigent proteins, that are involved in the formation of lignans, a class of proteins exhibiting plant defense activities, and germin like proteins and some PR-proteins such as beta-1,3-glucanase (PR-2), acidic endochitinases (PR-3), thaumatin (PR-5) and ribonuclease-like protein (PR-10) were down-regulated (Table 3.8). Additionally, many genes involved in secondary metabolism were down-regulated (69%), for example, genes coding for enzymes such as isoflavone reductase involved in isoflavonol biosynthesis as well as terpene synthase and terpenoid synthase, that are involved in terpenoid biosynthesis. In contrast, genes coding for enzymes implicated in stilbene biosynthesis such as stilbene synthase and resveratrol synthase were up-regulated (Table 3.9).

Hormone responsive genes were mainly down-regulated (70%). Genes coding for enzymes involved in the biosynthesis of abscisic acid such as ABA-responsive protein and carotenoid cleavage dioxygenase were down-regulated. Also genes implicated in auxin metabolism such as PIN1-like auxin transport protein, auxin-induced SAUR-like protein and Aux/IAA protein were down-regulated. Genes involved in the biosynthesis of brassinosteroids, a class of polyhydroxysteroids recognized as a sixth class of plant hormones, such as squalene monooxygenase, brassinosteroid biosynthetic protein and sterol-C-methyltransferase were down-regulated (Table 3.10). Genes coding for salicylic acid carboxyl methyltransferase, an enzyme involved in salicylic acid metabolism was strongly down-regulated. Most of the genes coding for lipoxygenases, which are involved in jasmonic acid metabolism, were down-regulated. However, a gene that codes for 13-

lipoxygenase was up-regulated. Genes coding for enzymes involved in gibberellin metabolism such as dioxygenase and GA 2-oxidase 2 were up-regulated, while gibberellin regulated protein was strongly down-regulated. Genes coding for enzymes involved in ethylene metabolism such as ethylene-responsive protein, aldo-keto reductase, leucoanthocyanidin dioxygenase and GA 20-oxidase were up-regulated (Table 3.10). Genes involved in signalling pathway had no clear trend (44% down and 56% up). Some of the genes coding for plant receptors such as protein kinases were up-regulated, while others were down-regulated. Among those up-regulated were genes coding for S-locus receptor protein kinases such as KI domain interacting kinases and receptor-like protein kinases. Moreover, genes coding for protein kinases (leucine rich repeat, class VIII) were up-regulated, while genes coding for protein kinases (leucine rich repeat, class III) were down-regulated. Genes coding for proteins involved in calcium signalling, calcium-modulated protein (calmodulin), calcium-binding proteins, were mostly up-regulated (Table 3.11).

As for cell wall related genes, 81% of DEGs were down-regulated. Some of these genes code for enzymes responsible for softening of plant tissues and break down of cell wall such as pectate lyase and pectin methylesterase respectively. Expansin that is thought to cause cell wall stress relaxation and irreversible cell wall extension and endo-1,4-beta-glucanase that is responsible for cell wall degradation were also down-regulated (Table 3.12). Genes implicated in RNA processing and regulations were mostly down-regulated such as genes coding for MYB transcription factor. However, genes coding for WRKY transcription factor were up-regulated (Table 3.13).

Most of genes involved in photosynthesis were down regulated. These were genes involved in photosystem II such as chloroplast chlorophyll a/b binding protein and light-harvesting complex protein (Table 3.14). Genes involved in carbohydrate metabolism such as fructokinase and sucrose synthase that regulate starch biosynthesis were down-regulated (Table 3.14). Genes involved in the biosynthesis of trehaloses, disaccharides involved in plant defense, such as trehalose-phosphatase were down-regulated. Genes coding for cyclins, proteins that control the progression of cell cycle, were down regulated such as genes coding for Glutathione S-transferase (Table 3.14).

Table 3.7: Differential regulation of genes grouped to 'bins' using the MapMan software during *P. viticola*/grapevine interaction. Up-, down-regulated genes, total no. of DEGs as well as no. and % of clones in each bin are shown. Gray shades represent the pathways discussed in text.

Bin	**Bin Name**	**Inoculated – Control**				
		Down	**Up**	**∑**	**No. of clones in the BIN**	**% of DEGs in the BIN**
1	Photosynthesis	23 (88%)	3 (12%)	26	494	5 %
2	Major carbohydrate metabolism	12 (63%)	7 (37%)	19	165	12 %
3	Minor carbohydrate metabolism	14 (93%)	1 (7%)	15	162	9 %
4	Glycolysis	4 (67%)	2 (33%)	6	123	5 %
5	Fermentation	1 (25%)	3 (75%)	4	52	8 %
6	Gluconeogenesis	-	-	0	22	0 %
7	Oxidative pentose phosphate pathway	4 (100%)	-	4	42	10 %
8	TCA – organic transformation	6 (67%)	3 (33%)	9	123	7 %
9	Electron transport / ATP synthesis	-	3 (100%)	3	156	2 %
10	Cell wall	75 (81%)	18 (19%)	93	595	16 %
11	Lipid metabolism	34 (77%)	10 (23%)	44	459	10 %
12	Nitrogen metabolism	3 (75%)	1 (25%)	4	59	7 %
13	Amino acid metabolism	20 (51%)	19 (49%)	39	459	8 %
14	Sulfur assimilation	1 (100%)	-	1	15	7 %
15	Metal handling	5 (63%)	3 (38%)	8	142	6 %
16	Secondary metabolism	124 (69%)	55 (31%)	179	543	33 %
17	Hormone metabolism	80 (70%)	34 (30%)	114	502	23 %
18	Co-factor – Vitamine metabolism	-	2 (100%)	2	45	4 %
19	Tetrapyrrole synthesis	5 (100%)	-	5	56	9 %
20	Stress	45 (40%)	67 (60%)	112	948	12 %
21	Redox	20 (91%)	2 (9%)	22	282	8 %
22	Polyamine metabolism	3 (100%)	-	3	18	17 %
23	Nucleotide metabolism	6 (86%)	1 (14%)	7	147	5 %

Table 3.7: Continued…

Bin	Bin Name	Down	Up	∑	No. of clones in the BIN	% of DEGs in the BIN
24	Biodegradation of Xenobiotics	-	1 (100%)	1	24	4 %
25	C1-metabolism	1 (100%)	-	1	33	3 %
26	Miscellaneous	162 (56%)	127 (44%)	289	1219	24 %
27	RNA processing and regulation	134 (60%)	89 (40%)	223	2296	10 %
28	DNA synthesis and repair	27 (82%)	6 (18%)	33	422	8 %
29	Protein metabolism	118 (52%)	109 (48%)	227	3628	6 %
30	Signalling	72 (44%)	93 (56%)	165	1157	14 %
31	Cell cycle and organization	82 (79%)	22 (21%)	104	655	16 %
33	Development	20 (77%)	6 (23%)	26	405	6 %
34	Transport	99 (73%)	37 (27%)	136	951	14 %
35	Not assigned – no ontology	649 (58%)	468 (42%)	1117	3276	34 %
∑	Total no. of genes in all bins	1849 (60%)	1192 (40%)	3041	19675	

Table 3.8: DEGs in selected sub-bins from stress pathways in inoculated plants

Pathway	Bin Name	Gene ID	Annotation	Fold change
Stress pathways	Biotic receptors	chr3_jgvv38_261_t01	weakly similar to UP|Q27JA5_PICGL (Q27JA5) Dirigent-like protein pDIR7, partial (77%)	-2,999
		chr2_jgvv25_69_t01	similar to UP|Q9SDR7_FORIN (Q9SDR7) Dirigent protein, partial (81%)	-2,106
		chr12_jgvv34_11_t01	GB|AF365881.1|AAQ15193.1 resistance protein [Vitis vinifera]	-1,751
		chr7_random_jgvv224_1_t01	weakly similar to UP|Q3A3E7_PELCD (Q3A3E7) Peptide ABC transporter, permease protein, partial (6%)	-1,722
		chr13_random_jgvv143_24_t01	weakly similar to UP|Q27J98_9CONI (Q27J98) Dirigent-like protein pDIR14, partial (74%)	-1,368
		chr18_jgvv75_1_t01	weakly similar to UP|Q9SCZ3_ARATH (Q9SCZ3) Disease resistance-like protein, partial (3%)	-1,191
		chr7_jgvv197_20_t01	weakly similar to PIR|T48468|T48468 disease resistance-like protein – Arabidopsis thaliana, partial (7%)	-1,095
		chr18_jgvv41_80_t01	similar to GB|BAD82812.1|56790017|AB182389 CLV1-like LRR receptor kinase {Oryza sativa (japonica cultivar-group)} partial (3%)	-1,057
		chr8_jgvv7_199_t01	similar to UP|Q45W75_ARAHY (Q45W75) Disease resistance-responsive family protein, partial (70%)	-1,036
		chr12_jgvv121_5_t01	weakly similar to GB|BAB01339.1|11994217|AB028617 disease resistance comples protein {Arabidopsis thaliana}, partial (11%)	1,018
		chrun_pdvv288_2_t01	weakly similar to GP|15787905|gb|AAL07544.1 resistance gene analog NBS9 {Helianthus annuus}, partial (49%)	1,033
		chr13_jgvv158_41_t01	weakly similar to GB|BAB01339.1|11994217|AB028617 disease resistance comples protein {Arabidopsis thaliana}, partial (11%)	1,051
		chrun_jgvv2390_1_t01	GB|AF369833.1|AAM21290.1 resistance gene analog [Vitis vinifera]	1,138
		chr19_jgvv27_54_t01	GB|AF365880.1|AAQ15192.1 resistance protein [Vitis vinifera]	1,141
		chr5_jgvv29_44_t01	weakly similar to UP|Q6JN46_LYCES (Q6JN46) EIX receptor 2, partial (11%)	1,356
		chr16_jgvv50_65_t01	weakly similar to UP|Q6JN46_LYCES (Q6JN46) EIX receptor 2, partial (11%)	1,395
		chr7_jgvv5_549_t01	similar to UP|Q2CML8_9EURY (Q2CML8) AAA ATPase, central region:ATPase associated with various cellular activities, AAA_3, partial (8%)	1,46
		chr1_jgvv11_541_t01	similar to UP|Q2CML8_9EURY (Q2CML8) AAA ATPase, central region:ATPase associated with various cellular activities, AAA_3, partial (8%)	1,475

Table 3.8: continued...

Pathway	Bin Name	Gene ID	Annotation	Fold change
Stress pathways	PR-proteins	chr4_jgvv23_37_t01	similar to UP|O22973_ARATH (O22973) Thaumatin isolog, partial (78%)	-1,309
		chr15_jgvv46_191_t01	similar to UP|CHIA_TOBAC (P29060) Acidic endochitinase precursor , partial (91%)	-1,24
		chr15_jgvv46_183_t01	UP|CHIT3_VITVI (P51614) Acidic endochitinase precursor , complete	-1,217
		chr15_jgvv46_181_t01	UP|CHIT3_VITVI (P51614) Acidic endochitinase precursor , complete	-1,011
		chr11_jgvv149_26_t01	weakly similar to UP|Q4W6L6_CYCRE (Q4W6L6) Chitinase A, partial (13%)	1,22
		chr5_jgvv94_19_t01	similar to UP|Q7XB39_VITVI (Q7XB39) Class IV chitinase, partial (94%)	1,242
		chr5_jgvv77_126_t01	homologue to UP|Q20BD2_9ROSI (Q20BD2) Pathogenesis-related protein 10.3, complete	1,284
		chr5_jgvv94_32_t01	UP|Q7XB39_VITVI (Q7XB39) Class IV chitinase, partial (98%)	1,337
		chr5_jgvv77_128_t01	UP|Q9FS42_VITVI (Q9FS42) Pathogenesis-related protein 10, complete	1,557
		chr18_jgvv1_1192_t01	weakly similar to UP|PRPX_HORVU (P16273) Pathogen-related protein, partial (70%)	1,662
		chr14_jgvv81_66_t01	UP|O81228_VITVI (O81228) PR-4 type protein, complete	1,702
		chr5_jgvv94_28_t01	similar to UP|Q7XB39_VITVI (Q7XB39) Class IV chitinase, partial (95%)	2,29
		chr7_jgvv5_248_t01	UP|Q9FS45_VITVI (Q9FS45) Chitinase precursor , complete	2,74
		chr5_jgvv77_124_t01	UP|Q9FS42_VITVI (Q9FS42) Pathogenesis-related protein 10, complete	3,159
	beta 1,3 glucan hydrolases	chr19_jgvv14_8_t01	weakly similar to RF|NP_913624.1|34904554|NM_188735 beta-1,3-glucanase-like protein {Oryza sativa (japonica cultivar-group)} (exp=-1; wgp=0; cg=0), partial (87%)	-2,073
		chr12_jgvv59_125_t01	similar to RF|NP_178637.2|30678225|NM_126593 hydrolase, hydrolyzing O-glycosyl compounds {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (95%)	-1,56
		chr18_jgvv122_40_t01	similar to RF|NP_178637.2|30678225|NM_126593 hydrolase, hydrolyzing O-glycosyl compounds {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (95%)	-1,423
		chr8_jgvv7_284_t01	weakly similar to UP|Q9M3U4_VITVI (Q9M3U4) Beta 1-3 glucanase, partial (91%)	-1,091
		chr5_jgvv62_116_t01	similar to RF|NP_178637.2|30678225|NM_126593 hydrolase, hydrolyzing O-glycosyl compounds {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (95%)	-1,034
		chr2_jgvv12_120_t01	UP|Q9M563_VITVI (Q9M563) Beta-1,3-glucanase, complete	2,208
	Germin-like protein	chr8_jgvv7_528_t01	similar to UP|GL32_ARATH (Q9SR72) Germin-like protein subfamily 3 member 2 precursor, partial (73%)	-2,451
		chr1_jgvv26_159_t01	similar to GB|AAB51752.1|1934730|ATU95036 germin-like protein {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (96%)	-1,886
		chr7_jgvv5_230_t01	similar to GB|AAB51752.1|1934730|ATU95036 germin-like protein {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (96%)	-1,71
		chr17_jgvv0_447_t01	weakly similar to UP|Q8H2A6_ANACO (Q8H2A6) Germin-like protein, partial (87%)	2,856
		chr14_jgvv60_240_t01	similar to GB|AAB51752.1|1934730|ATU95036 germin-like protein {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (96%)	-1,683
		chr16_jgvv98_4_t01	similar to UP|GL32_ARATH (Q9SR72) Germin-like protein subfamily 3 member 2 precursor, partial (73%)	-1,51

Table 3.9: DEGs in selected sub-bins from secondary metabolism pathways in inoculated plants

Pathway	Bin Name	Gene ID	Annotation	Fold change
Secondary metabolism	Flavonoids.chalcones stilbene synthase	chr16_jgvv100_29_t01	homologue to UP|Q9SPW2_VITRI (Q9SPW2) Stilbene synthase, complete	1,699
		chr16_jgvv100_33_t01	homologue to UP|Q9SPW2_VITRI (Q9SPW2) Stilbene synthase, complete	1,783
		chr16_jgvv100_31_t01	homologue to UP|Q9SPW2_VITRI (Q9SPW2) Stilbene synthase, complete	1,841
		chr10_jgvv42_68_t01	homologue to UP|Q9SPW2_VITRI (Q9SPW2) Stilbene synthase, complete	1,965
		chr16_jgvv100_46_t01	UP|THS1_VITVI (P28343) Stilbene synthase 1 (Resveratrol synthase 1) (Trihydroxystilbene synthase 1) (PSV25) , complete	2,08
		chr16_jgvv100_45_t01	homologue to UP|Q9SPW2_VITRI (Q9SPW2) Stilbene synthase, complete	2,086
		chr16_jgvv100_42_t01	homologue to UP|Q9SPW2_VITRI (Q9SPW2) Stilbene synthase, complete	2,106
		chr16_jgvv100_34_t01	UP|THS1_VITVI (P28343) Stilbene synthase 1 (Resveratrol synthase 1) (Trihydroxystilbene synthase 1) (PSV25) , complete	2,193
		chr10_jgvv42_63_t01	UP|Q6BAM2_VITVI (Q6BAM2) Stilbene synthase (Fragment), complete	2,282
		chr16_jgvv100_41_t01	UP|THS1_VITVI (P28343) Stilbene synthase 1 (Resveratrol synthase 1) (Trihydroxystilbene synthase 1) (PSV25) , complete	2,358
		chr10_jgvv42_66_t01	homologue to UP|Q9SPW2_VITRI (Q9SPW2) Stilbene synthase, complete	2,391
		chr16_jgvv100_36_t01	homologue to UP|Q9SPW2_VITRI (Q9SPW2) Stilbene synthase, complete	2,435
		chr16_jgvv100_43_t01	homologue to UP|Q9SPW2_VITRI (Q9SPW2) Stilbene synthase, complete	2,483
		chr16_jgvv100_37_t01	homologue to UP|Q9SPW2_VITRI (Q9SPW2) Stilbene synthase, complete	2,506
		chr10_jgvv42_64_t01	UP|THS1_VITVI (P28343) Stilbene synthase 1 (Resveratrol synthase 1) (Trihydroxystilbene synthase 1) (PSV25) , complete	2,546
		chr10_jgvv42_70_t01	homologue to UP|Q9SPW2_VITRI (Q9SPW2) Stilbene synthase, complete	2,547
		chr10_jgvv42_69_t01	homologue to UP|Q9SPW2_VITRI (Q9SPW2) Stilbene synthase, complete	2,59
		chr16_jgvv100_47_t01	homologue to UP|Q9SPW2_VITRI (Q9SPW2) Stilbene synthase, complete	2,655
		chr16_jgvv100_32_t01	UP|THS1_VITVI (P28343) Stilbene synthase 1 (Resveratrol synthase 1) (Trihydroxystilbene synthase 1) (PSV25) , complete	2,824
		chr16_jgvv100_38_t01	UP|THS1_VITVI (P28343) Stilbene synthase 1 (Resveratrol synthase 1) (Trihydroxystilbene synthase 1) (PSV25) , complete	2,866
		chr16_jgvv100_44_t01	UP|THS1_VITVI (P28343) Stilbene synthase 1 (Resveratrol synthase 1) (Trihydroxystilbene synthase 1) (PSV25) , complete	3,134

Table 3.9: continued...

Pathway	Bin Name	Gene ID	Annotation	Fold change
Secondary metabolism	flavonoids. isoflavonols	chr18_jgvv1_918_t01	UP|Q3KN69_VITVI (Q3KN69) Isoflavone reductase-like protein 4, complete	-2,786
		chr7_jgvv31_276_t01	UP|Q3KN70_VITVI (Q3KN70) Isoflavone reductase-like protein 3, complete	-2,763
		chr2_jgvv33_20_t01	UP|Q3KN72_VITVI (Q3KN72) Isoflavone reductase-like protein 1, complete	-2,386
		chr3_pdvv38_10_t01	UP|Q3KN67_VITVI (Q3KN67) Isoflavone reductase-like protein 6, partial (81%)	-1,76
		chr17_jgvv53_37_t01	UP|Q3KN72_VITVI (Q3KN72) Isoflavone reductase-like protein 1, complete	-1,112
	Isoprenoids. terpenoids	chr19_jgvv14_429_t01	homologue to UP|Q6Q3H3_VITVI (Q6Q3H3) Terpene synthase, complete	-2,976
		chr19_jgvv14_228_t01	homologue to UP|Q6Q3H3_VITVI (Q6Q3H3) Terpene synthase, complete	-2,888
		chr19_jgvv14_436_t01	homologue to UP|Q6Q3H3_VITVI (Q6Q3H3) Terpene synthase, complete	-2,798
		chr19_jgvv14_430_t01	homologue to UP|Q6Q3H3_VITVI (Q6Q3H3) Terpene synthase, complete	-2,746
		chr19_jgvv14_437_t01	homologue to UP|Q6Q3H3_VITVI (Q6Q3H3) Terpene synthase, complete	-2,704
		chr19_jgvv14_426_t01	homologue to UP|Q6Q3H3_VITVI (Q6Q3H3) Terpene synthase, complete	-2,48
		chr19_jgvv14_425_t01	homologue to UP|Q6Q3H3_VITVI (Q6Q3H3) Terpene synthase, complete	-2,433
		chr19_jgvv14_433_t01	homologue to UP|Q6Q3H3_VITVI (Q6Q3H3) Terpene synthase, complete	-2,28
		chr19_jgvv14_435_t01	homologue to UP|Q6Q3H3_VITVI (Q6Q3H3) Terpene synthase, complete	-2,158
		chrun_jgvv271_3_t01	UP|Q6PWU2_VITVI (Q6PWU2) Terpenoid synthase, complete	-2,134
		chrun_jgvv572_1_t01	homologue to UP|Q6Q3H3_VITVI (Q6Q3H3) Terpene synthase, complete	-2,081
		chrun_jgvv372_7_t01	UP|Q6PWU2_VITVI (Q6PWU2) Terpenoid synthase, complete	-1,955
		chrun_jgvv271_1_t01	UP|Q6PWU2_VITVI (Q6PWU2) Terpenoid synthase, complete	-1,946
		chrun_jgvv372_6_t01	UP|Q6PWU2_VITVI (Q6PWU2) Terpenoid synthase, complete	-1,895
		chrun_jgvv847_2_t01	UP|Q6PWU2_VITVI (Q6PWU2) Terpenoid synthase, complete	-1,895
		chrun_jgvv522_2_t01	UP|Q6PWU2_VITVI (Q6PWU2) Terpenoid synthase, complete	-1,808
		chrun_jgvv385_1_t01	homologue to UP|Q6Q3H3_VITVI (Q6Q3H3) Terpene synthase, complete	-1,801
		chrun_jgvv266_2_t01	UP|Q6PWU2_VITVI (Q6PWU2) Terpenoid synthase, complete	-1,785
		chrun_jgvv372_2_t01	UP|Q6PWU2_VITVI (Q6PWU2) Terpenoid synthase, complete	-1,699
		chrun_jgvv385_2_t01	homologue to UP|Q6Q3H3_VITVI (Q6Q3H3) Terpene synthase, complete	-1,67
		chr12_jgvv134_8_t01	UP|Q6PWU2_VITVI (Q6PWU2) Terpenoid synthase, complete	-1,552
		chrun_jgvv266_6_t01	UP|Q6PWU2_VITVI (Q6PWU2) Terpenoid synthase, complete	-1,417
		chr12_jgvv134_5_t01	UP|Q6PWU2_VITVI (Q6PWU2) Terpenoid synthase, complete	-1,389

Table 3.10: DEGs in selected sub-bins from hormone metabolism pathways in inoculated plants

Pathway	Bin Name	Gene ID	Annotation	Fold change
Hormone metabolism	abscisic acid metabolism	chr13_jgvv64_95_t01	UP|Q3T4H1_VITVI (Q3T4H1) 9,10[9\',10\']carotenoid cleavage dioxygenase, complete	-1,003
		chr10_jgvv71_71_t01	similar to UP|Q6H5X2_ORYSA (Q6H5X2) ABA-responsive protein-like, partial (58%)	-1,782
		chr4_jgvv23_328_t01	similar to UP|Q6H5X2_ORYSA (Q6H5X2) ABA-responsive protein-like, partial (58%)	-1,358
	Auxin metabolism	chr8_jgvv7_595_t01	weakly similar to UP|Q946K0_9BRAS (Q946K0) IAA amidohydrolase (IAA-amino acid hydrolase), partial (76%)	1,311
		chr18_jgvv1_1172_t01	similar to UP|Q8LKH1_9ROSI (Q8LKH1) PIN1-like auxin transport protein, partial (46%)	-2,548
		chr14_jgvv108_2_t01	similar to UP|Q8LKH1_9ROSI (Q8LKH1) PIN1-like auxin transport protein, partial (46%)	-1,889
		chr6_jgvv61_30_t01	similar to UP|Q9LW29_ARATH (Q9LW29) Transport inhibitor response-like protein (At3g26830), partial (74%)	-1,883
		chr11_jgvv52_128_t01	similar to UP|Q8LKH1_9ROSI (Q8LKH1) PIN1-like auxin transport protein, partial (46%)	-1,546
	Auxin.induced-regulated-responsive-activated	chr3_jgvv38_333_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	-2,556
		chr7_jgvv141_16_t01	similar to UP|Q8LSK7_9ROSI (Q8LSK7) Auxin-regulated protein, partial (78%)	-2,053
		chr3_jgvv38_322_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	-2,032
		chr3_jgvv38_335_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	-2,024
		chr3_jgvv38_334_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	-1,959
		chr3_jgvv38_337_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	-1,957
		chr3_jgvv38_336_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	-1,832
		chr3_jgvv38_326_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	-1,633
		chr11_jgvv16_507_t01	weakly similar to UP|Q8RVH8_9ROSI (Q8RVH8) Aux/IAA protein, partial (9%)	-1,526
		chr3_jgvv38_328_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	-1,491

Table 3.10: continued...

Pathway	Bin Name	Gene ID	Annotation	Fold change
Hormone metabolism	Auxin.induced-regulated-responsive-activated	chr10_jgvv3_134_t01	similar to UP|Q9FKP3_ARATH (Q9FKP3) Similarity to auxin-independent growth promoter, partial (61%)	-1,314
		chr3_jgvv38_332_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	-1,174
		chr7_jgvv104_95_t01	similar to UP|GH35_ORYSA (Q6I581) Probable indole-3-acetic acid-amido synthetase GH3.5 (Auxin-responsive GH3-like protein 5) (OsGH3-5) , partial (94%)	1,07
		chr9_jgvv2_51_t01	similar to UP|Q5JM48_ORYSA (Q5JM48) Auxin-induced-related / indole-3-acetic acid induced-related-like, partial (41%)	1,42
		chr5_jgvv62_80_t01	similar to UP|Q52QX9_MANES (Q52QX9) Aldo/keto reductase AKR, partial (98%)	1,628
	Brassinosteroid metabolism	chr15_jgvv48_148_t01	homologue to UP|Q2QDF7_GOSHI (Q2QDF7) 24-sterol C-methyltransferase, partial (34%)	1,243
		chr3_jgvv38_176_t01	similar to UP|SMT2_ARATH (Q39227) 24-methylenesterol C-methyltransferase 2 (24-sterol C-methyltransferase 2) (Sterol-C-methyltransferase 2) , complete	-1,033
		chr1_jgvv11_272_t01	similar to UP|Q4ACU1_ZINEL (Q4ACU1) Delta7 sterol C-5 desaturase, partial (94%)	-1,938
		chr1_jgvv11_273_t01	similar to UP|Q4ACU1_ZINEL (Q4ACU1) Delta7 sterol C-5 desaturase, partial (94%)	-1,196
		chr14_jgvv6_168_t01	similar to UP|Q4ACU1_ZINEL (Q4ACU1) Delta7 sterol C-5 desaturase, partial (94%)	-1,076
		chr1_jgvv10_84_t01	similar to UP|Q4ACU2_ZINEL (Q4ACU2) Sterol C-24 reductase, partial (51%)	-1,22
		chrun_jgvv1011_1_t01	similar to UP|Q9ATR0_PEA (Q9ATR0) Brassinosteroid biosynthetic protein LKB, partial (34%)	-1,028
		chrun_jgvv525_2_t01	similar to UP|Q506K3_DATIN (Q506K3) Squalene monooxygenase, partial (89%)	-2,429
		chrun_jgvv441_2_t01	similar to UP|Q506K3_DATIN (Q506K3) Squalene monooxygenase, partial (89%)	-2,068
		chr3_jgvv88_14_t01	similar to UP|Q506K3_DATIN (Q506K3) Squalene monooxygenase, partial (89%)	-1,053
	Ethylene.synthesis-degradation	chrun_jgvv299_5_t01	similar to RF|NP_174753.1|15219523|NM_103217 iron ion binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (56%)	1,065
		chrun_jgvv2197_1_t01	similar to RF|NP_174753.1|15219523|NM_103217 iron ion binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (78%)	1,164
		chr6_jgvv4_707_t01	weakly similar to UP|Q9LTH8_ARATH (Q9LTH8) Leucoanthocyanidin dioxygenase-like protein (At5g59530) (1-aminocyclopropane-1-carboxylate oxidase-like protein), partial (18%)	1,203
		chrun_jgvv299_4_t01	similar to RF|NP_174753.1|15219523|NM_103217 iron ion binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (78%)	1,57
		chr5_jgvv77_104_t01	weakly similar to UP|Q48IK0_PSE14 (Q48IK0) Aldo-keto reductase family protein, partial (27%)	2,143
		chrun_jgvv250_8_t01	weakly similar to UP|Q4JH25_SESRO (Q4JH25) GA 20-oxidase, partial (25%)	2,993

Table 3.10: continued...

Pathway	Bin Name	Gene ID	Annotation	Fold change
Hormone metabolism	Ethylene metabolism	chr5_jgvv77_104_t01	weakly similar to UP|Q48IK0_PSE14 (Q48IK0) Aldo-keto reductase family protein, partial (27%)	2,143
		chrun_jgvv250_8_t01	weakly similar to UP|Q4JH25_SESRO (Q4JH25) GA 20-oxidase, partial (25%)	2,993
	Ethylene.induced-regulated-responsive-activated	chr11_jgvv16_365_t01	similar to UP|Q9LV58_ARATH (Q9LV58) Ethylene-responsive transcriptional coactivator-like protein (At3g24500), partial (97%)	1,566
	Gibberellin metabolism.GA2 oxidase	chr19_jgvv140_8_t01	similar to UP|O04162_9ROSI (O04162) Dioxygenase, partial (39%)	2,174
		chr19_jgvv140_9_t01	similar to UP|Q53B81_NEROL (Q53B81) GA 2-oxidase 2, partial (52%)	2,409
	Gibberelin.induced-regulated-responsive-activated	chr8_jgvv7_298_t01	similar to UP|Q2HRH3_MEDTR (Q2HRH3) Gibberellin regulated protein, partial (64%)	-4,157
		chr8_jgvv7_297_t01	similar to UP|Q2HRH3_MEDTR (Q2HRH3) Gibberellin regulated protein, partial (64%)	-3,69
		chr9_jgvv2_100_t01	similar to GB|AAY28970.1|63054405|DQ006269 GIA/RGA-like gibberellin response modulator {Gossypium hirsutum} (exp=-1; wgp=0; cg=0), partial (55%)	1,567
	Jasmonate.metabolism lipoxygenase	chr6_jgvv4_645_t01	similar to UP|Q96573_LYCES (Q96573) Lipoxygenase , partial (54%)	-1,473
		chr6_jgvv4_642_t01	similar to UP|Q96573_LYCES (Q96573) Lipoxygenase , partial (54%)	-1,262
		chr6_jgvv4_646_t01	similar to UP|Q96573_LYCES (Q96573) Lipoxygenase , partial (54%)	-1,19
		chr6_jgvv4_647_t01	similar to UP|Q96573_LYCES (Q96573) Lipoxygenase , partial (54%)	-1,19
		chr5_jgvv20_298_t01	similar to UP|Q7X9G5_FRAAN (Q7X9G5) Lipoxygenase , partial (31%)	-1,013
		chr9_jgvv2_89_t01	similar to UP|O24371_SOLTU (O24371) 13-lipoxygenase , partial (29%)	1,071
		chr14_jgvv128_73_t01	UP|Q6YCG7_VITVI (Q6YCG7) Lipoxygenase (Fragment), complete	1,309
		chr14_jgvv128_74_t01	UP|Q6YCG7_VITVI (Q6YCG7) Lipoxygenase (Fragment), complete	1,319
	Salicylic acid metabolism	chr4_jgvv23_152_t01	similar to UP|Q9SPV4_CLABR (Q9SPV4) S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase, partial (76%)	-4,626
		chr4_jgvv23_157_t01	similar to UP|Q9SPV4_CLABR (Q9SPV4) S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase, partial (76%)	-4,105
		chr4_jgvv23_150_t01	similar to UP|Q9SPV4_CLABR (Q9SPV4) S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase, partial (76%)	-2,828
		chr4_jgvv23_151_t01	similar to UP|Q9SPV4_CLABR (Q9SPV4) S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase, partial (76%)	-2,323
		chr4_jgvv23_156_t01	similar to UP|Q9SPV4_CLABR (Q9SPV4) S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase, partial (76%)	-1,956
		chr4_jgvv23_159_t01	similar to UP|Q9SPV4_CLABR (Q9SPV4) S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase, partial (76%)	5,22

Table 3.11: DEGs in selected sub-bins from signalling pathways in inoculated plants

Pathway	Bin Name	Gene ID	Annotation	Fold change
Signalling pathways	Receptor kinases. S-locus glycoprotein like	chrun_jgvv286_11_t01	similar to UP|Q9ZT07_ARATH (Q9ZT07) Receptor-like protein kinase, partial (14%)	-1,138
		chr7_jgvv95_43_t01	weakly similar to UP|O49974_MAIZE (O49974) KI domain interacting kinase 1, partial (21%)	1,028
		chr15_jgvv24_17_t01	similar to UP|O81906_ARATH (O81906) Serine/threonine kinase-like protein, partial (20%)	1,294
		chrun_jgvv743_2_t01	similar to UP|O81906_ARATH (O81906) Serine/threonine kinase-like protein, partial (20%)	1,308
		chrun_jgvv286_5_t01	similar to UP|Q9ZT07_ARATH (Q9ZT07) Receptor-like protein kinase, partial (19%)	1,314
		chrun_jgvv2770_1_t01	weakly similar to UP|O49974_MAIZE (O49974) KI domain interacting kinase 1, partial (21%)	1,328
		chrun_jgvv1458_1_t01	similar to UP|Q9ZT07_ARATH (Q9ZT07) Receptor-like protein kinase, partial (19%)	1,422
		chrun_jgvv262_1_t01	similar to UP|O81906_ARATH (O81906) Serine/threonine kinase-like protein, partial (20%)	1,572
		chrun_jgvv2485_1_t01	weakly similar to UP|O49974_MAIZE (O49974) KI domain interacting kinase 1, partial (21%)	1,945
		chr19_jgvv14_373_t01	similar to UP|Q9ZT07_ARATH (Q9ZT07) Receptor-like protein kinase, partial (19%)	2,132
	Rreceptor kinases.leucine rich repeat III	chr6_jgvv9_185_t01	similar to UP|Q9LEA2_ARATH (Q9LEA2) Receptor protein kinase-like (Fragment), partial (66%)	-2,37
		chr1_jgvv11_274_t01	similar to RF|NP_177007.1|15221403|NM_105511 ATP binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (36%)	-1,65
		chr19_jgvv90_153_t01	weakly similar to RF|NP_180241.1|15225780|NM_128230 ATP binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (12%)	-1,442
		chr6_jgvv4_28_t01	similar to GB|BAA96921.1|8777331|AB019228 receptor-like protein kinase {Arabidopsis thaliana} (exp=0; wgp=1; cg=0), partial (35%)	-1,055
	Receptor kinases.leucine rich repeat VIII	chr10_jgvv3_177_t01	weakly similar to UP|Q6EQG8_ORYSA (Q6EQG8) Leucine-rich repeat family protein /protein kinase family protein-like, partial (16%)	1,194
		chr10_jgvv3_171_t01	weakly similar to UP|O22579_ARATH (O22579) Receptor-like serine/threonine kinase, partial (19%)	1,435
		chr10_jgvv3_174_t01	weakly similar to UP|Q6EQG8_ORYSA (Q6EQG8) Leucine-rich repeat family protein /protein kinase family protein-like, partial (16%)	1,767
		chr10_jgvv3_181_t01	weakly similar to UP|O22579_ARATH (O22579) Receptor-like serine/threonine kinase, partial (19%)	2,068

Table 3.11: continued...

Pathway	Bin Name	Gene ID	Annotation	Fold change
Signalling	Calcium Signalling	chr5_jgvv20_395_t01	similar to RF|NP_188931.1|15228891|NM_113191 calcium-transporting ATPase/ calmodulin binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0) , partial (24%)	-2,322
		chr16_jgvv13_110_t01	UP|Q6UQE4_DAUCA (Q6UQE4) Calmodulin 8 (Calmodulin 4) (Fragment), complete	-1,309
		chr14_jgvv30_23_t01	similar to RF|NP_188931.1|15228891|NM_113191 calcium-transporting ATPase/ calmodulin binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0) , partial (24%)	-1,291
		chr14_jgvv30_41_t01	similar to RF|NP_193211.2|30682982|NM_117560 calmodulin binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (17%)	-1,106
		chr5_jgvv77_8_t01	weakly similar to UP|Q9SWP6_PHAVU (Q9SWP6) Hypersensitive reaction associated Ca2+-binding protein, partial (16%)	1,034
		chr7_jgvv289_3_t01	similar to UP|Q9SCA1_LOTJA (Q9SCA1) Calcium-binding protein, partial (63%)	1,035
		chr14_jgvv30_16_t01	homologue to UP|Q39890_SOYBN (Q39890) Calmodulin, complete	1,237
		chr8_jgvv56_27_t01	similar to UP|ALLB3_BETVE (P43187) Calcium-binding allergen Bet v 3 (Bet v III), partial (80%)	1,251
		chr14_jgvv6_74_t01	weakly similar to UP|ALL8_OLEEU (Q9M7R0) Calcium-binding allergen Ole e 8 (PCA18/PCA23), partial (76%)	1,322
		chrun_jgvv179_23_t01	similar to RF|NP_188931.1|15228891|NM_113191 calcium-transporting ATPase/ calmodulin binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0) , partial (24%)	1,417
		chr17_jgvv0_626_t01	similar to UP|Q9FJI9_ARATH (Q9FJI9) Similarity to calmodulin-binding protein, partial (46%)	1,671
		chrun_jgvv179_25_t01	homologue to UP|Q39890_SOYBN (Q39890) Calmodulin, complete	2,21
		chr17_jgvv0_627_t01	similar to UP|Q9FJI9_ARATH (Q9FJI9) Similarity to calmodulin-binding protein, partial (46%)	2,973

Table 3.12: DEGs in selected sub-bins from cell wall pathways in inoculated plants

Pathway	Bin Name	Gene ID	Annotation	Fold change
Cell wall	Cel wall.degradation. cellulases and beta -1,4-glucanases	chr19_jgvv90_95_t01	similar to RF|NP_180858.1|15225764|NM_128859 hydrolase, hydrolyzing O-glycosyl compounds {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (33%)	-2,694
		chr13_jgvv73_18_t01	similar to UP|Q7XAS3_GOSHI (Q7XAS3) Beta-D-glucosidase, partial (23%)	-1,827
		chr19_jgvv85_53_t01	homologue to UP|Q6QLN2_POPTM (Q6QLN2) Endo-1,4-beta-glucanase , partial (76%)	-1,456
		chr6_jgvv9_248_t01	similar to UP|Q7XAS3_GOSHI (Q7XAS3) Beta-D-glucosidase, partial (38%)	-1,217
		chrun_jgvv2620_1_t01	homologue to UP|Q6QLN2_POPTM (Q6QLN2) Endo-1,4-beta-glucanase , partial (76%)	1,507
	Cell wall.degradation pectate lyases and polygalacturonases	chr1_jgvv127_46_t01	polygalacturonase [Vitis vinifera]	-6,602
		chr19_jgvv15_48_t01	similar to UP|Q4JLV6_GOSHI (Q4JLV6) Pectate lyase, partial (96%)	-4,238
		chr5_jgvv20_35_t01	similar to RF|NP_175244.1|15221078|NM_103706 polygalacturonase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (35%)	-3,334
		chr1_jgvv137_59_t01	similar to UP|Q4JLV6_GOSHI (Q4JLV6) Pectate lyase, partial (96%)	-2,958
		chr1_jgvv10_247_t01	homologue to UP|Q94FT5_FRAAN (Q94FT5) Pectate lyase (Fragment), complete	-2,571
		chr1_jgvv127_4_t01	similar to UP|GP1_LYCES (Q40161) Polygalacturonase-1 non-catalytic beta subunit precursor (AroGP1) (Polygalacturonase converter), partial (36%)	-2,457
		chr17_pdvv0_63_t01	similar to UP|Q4JLV6_GOSHI (Q4JLV6) Pectate lyase, partial (96%)	-2,297
		chr2_jgvv25_24_t01	similar to UP|Q84LI7_FRAAN (Q84LI7) Polygalacturonase-like protein, partial (91%)	-2,144
		chr1_jgvv127_47_t01	UP|Q94B15_VITVI (Q94B15) Polygalacturonase PG1, complete	-2,092
		chr15_jgvv46_153_t01	similar to UP|Q84LI7_FRAAN (Q84LI7) Polygalacturonase-like protein, partial (92%)	-2,059
		chr17_jgvv0_58_t01	similar to UP|Q4JLV6_GOSHI (Q4JLV6) Pectate lyase, partial (96%)	-1,452
		chr1_jgvv127_6_t01	weakly similar to GP|15912221|gb|AAL08244.1 At1g70370/F17O7_9 {Arabidopsis thaliana}, partial (30%)	-1,357
		chr2_jgvv25_123_t01	similar to RF|NP_194113.1|15236625|NM_118513 polygalacturonase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (40%)	-1,198
		chr5_jgvv51_45_t01	homologue to UP|Q94FT5_FRAAN (Q94FT5) Pectate lyase (Fragment), complete	-1,186

Table 3.12: continue…

Pathway	Bin Name	Gene ID	Annotation	Fold change
Cell wall	Degradation.pectate lyases and polygalacturonases	chr4_jgvv8_385_t01	weakly similar to UP|Q4VT47_VITVI (Q4VT47) RD22-like protein, partial (45%)	-1,142
		chr5_jgvv77_160_t01	similar to UP|O49721_ARATH (O49721) Polygalacturonase-like protein (Fragment), partial (19%)	1,955
		chr13_jgvv64_44_t01	UP|Q8LKV2_VITVI (Q8LKV2) Polygalacturonase-inhibiting protein, complete	2,365
		chr8_jgvv7_127_t01	UP|Q8LKV2_VITVI (Q8LKV2) Polygalacturonase-inhibiting protein, complete	2,819
	Cell wall.modification	chr14_jgvv108_94_t01	similar to UP|Q8L5J6_MALDO (Q8L5J6) Expansin 3, complete	-2,492
		chr8_jgvv7_384_t01	homologue to UP|Q8W3L8_9ROSI (Q8W3L8) Xyloglucan endo-transglycosylase, complete	-2,467
		chr5_jgvv77_13_t01	UP|Q84UT0_9ROSI (Q84UT0) Expansin, complete	-1,893
		chr17_jgvv53_69_t01	UP|Q84UT0_9ROSI (Q84UT0) Expansin, complete	-1,7
		chr8_jgvv7_809_t01	similar to UP|Q49QW6_9ROSI (Q49QW6) Expansin, partial (96%)	-1,613
		chr4_jgvv79_1_t01	similar to UP|Q49QW6_9ROSI (Q49QW6) Expansin, partial (96%)	-1,429
		chr6_jgvv4_48_t01	UP|Q84US7_9ROSI (Q84US7) Expansin, complete	-1,2
		chr11_jgvv52_57_t01	similar to UP|Q8S902_SOYBN (Q8S902) Syringolide-induced protein 19-1-5, partial (86%)	1,646
	Pectin*esterases.PME	chr11_jgvv16_14_t01	UP|Q94B16_VITVI (Q94B16) Pectin methylesterase PME1, complete	-3,777
		chr16_jgvv13_17_t01	similar to RF|NP_197474.1|15241163|NM_121978 pectinesterase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (83%)	-2,89
		chr10_jgvv116_28_t01	weakly similar to GP|20269071|emb|CAD29733. pectin methylesterase {Sesbania rostrata}, partial (25%)	-1,975
		chr3_jgvv17_118_t01	similar to RF|NP_197474.1|15241163|NM_121978 pectinesterase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (83%)	-1,776
		chr7_jgvv5_71_t01	weakly similar to UP|O81301_ARATH (O81301) T14P8.1 (Pectinesterase-like protein) (Pectinesterase family protein), partial (19%)	-1,603
		chr16_jgvv13_15_t01	weakly similar to RF|NP_177152.2|30697951|NM_105663 pectinesterase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (26%)	-1,156
		chr11_jgvv16_12_t01	UP|Q94B16_VITVI (Q94B16) Pectin methylesterase PME1, complete	1,502
		chr13_jgvv47_70_t01	UP|Q94B16_VITVI (Q94B16) Pectin methylesterase PME1, complete	1,668
	Pectin*esterases acetyl esterase	chr14_jgvv60_232_t01	similar to UP|Q2HRU2_MEDTR (Q2HRU2) Pectinacetylesterase, partial (64%)	-1,196
		chr16_jgvv50_201_t01	similar to UP|Q9FF93_ARATH (Q9FF93) Pectinacetylesterase, partial (79%)	1,537

Table 3.13: DEGs in selected sub-bins from RNA processing pathways in inoculated plants

Pathway	Bin Name	Gene ID	Annotation	Fold change
RNA processing	Transcription factor Family WRKY	chr14_jgvv68_169_t01	homologue to UP|Q5DJU0_CAPAN (Q5DJU0) WRKY transcription factor-b, partial (62%)	1,054
		chr15_jgvv46_234_t01	similar to RF|NP_849358.1|30681651|NM_179027 WRKY41; transcription factor {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (31%)	1,294
		chr1_jgvv11_568_t01	similar to UP|WRK57_ARATH (Q9C983) Probable WRKY transcription factor 57 (WRKY DNA-binding protein 57), partial (34%)	1,332
		chr4_jgvv69_66_t01	similar to UP|Q9SXP4_TOBAC (Q9SXP4) DNA-binding protein NtWRKY3, partial (38%)	1,401
		chr10_jgvv3_147_t01	similar to UP|Q3SAJ9_CAPAN (Q3SAJ9) WRKY-A1244, partial (57%)	1,433
		chr2_jgvv25_40_t01	similar to UP|Q6IEP2_ORYSA (Q6IEP2) WRKY transcription factor 39, partial (26%)	1,435
		chr11_jgvv52_127_t01	similar to GP|4894963|gb|AAD32676.1| DNA-binding protein WRKY3 {Avena sativa}, partial (34%)	1,622
		chr4_jgvv69_71_t01	homologue to UP|WRK71_ARATH (Q93WV4) Probable WRKY transcription factor 71 (WRKY DNA-binding protein 71), partial (32%)	1,905
		chr8_jgvv58_74_t01	weakly similar to UP|Q5QJ44_9SOLA (Q5QJ44) WRKY6, partial (12%)	2,268
	Transcription factor Family MYB	chr17_jgvv0_689_t01	similar to UP|O49021_GOSHI (O49021) MYB-like DNA-binding domain protein (Myb-like transcription factor 5), partial (56%)	-3,18
		chr4_jgvv8_168_t01	MYB-like protein [Vitis vinifera]	-2,987
		chr15_jgvv46_313_t01	MYB-like protein [Vitis vinifera]	-2,959
		chr14_jgvv6_69_t01	UP|Q6L973_VITVI (Q6L973) Myb-related transcription factor VvMYBA1 (Transcription factor MybA), complete	-2,636
		chr5_jgvv20_99_t01	similar to GP|28628949|gb|AAO49411.1 MYB2 {Dendrobium sp. XMW-2002-2}, partial (36%)	-2,311
		chr14_jgvv6_71_t01	homologue to UP|Q2VA85_VITVI (Q2VA85) Transcription factor MybA, partial (72%)	-2,273
		chr11_jgvv16_111_t01	MYB-like protein [Vitis vinifera]	-2,034
		chr17_jgvv0_171_t01	similar to UP|Q9SEI0_ARATH (Q9SEI0) Werewolf (Myb transcription factor werewolf (WER)/ MYB66), partial (25%)	-1,935
		chr6_jgvv4_586_t01	similar to GP|28628949|gb|AAO49411.1 MYB2 {Dendrobium sp. XMW-2002-2}, partial (36%)	-1,651
		chr14_jgvv66_111_t01	homologue to UP|Q8LRU4_CUCSA (Q8LRU4) Werewolf (Fragment), partial (16%)	-1,465
		chr11_jgvv16_109_t01	MYB-like protein [Vitis vinifera]	-1,385
		chr17_pdvv0_648_t01	similar to GP|9294065|dbj|BAB02022.1 contains similarity to myb proteins~gene_id:MRC8.8 {Arabidopsis thaliana}, partial (8%)	-1,208
		chr13_jgvv67_167_t01	similar to UP|Q8H0H0_TOBAC (Q8H0H0) Myb-like protein, partial (65%)	-1,154
		chr7_jgvv5_317_t01	MYB-like protein [Vitis vinifera]	1,714
		chr14_jgvv6_70_t01	UP|Q6L9M8_VITVI (Q6L9M8) Myb-relared transcription factor VvMYBA2, complete	-3,211

Table 3.14: DEGs in selected sub-bins from different pathways in inoculated plants

Pathway	Bin Name	Gene ID	Annotation	Fold change
Miscelleanous	Trehalose	chr16_jgvv50_19_t01	similar to RF|NP_179809.2|79560035|NM_127787 catalytic/ trehalose-phosphatase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (64%)	-2,258
		chr18_jgvv1_293_t01	similar to RF|NP_179809.2|79560035|NM_127787 catalytic/ trehalose-phosphatase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (64%)	-1,769
		chr11_jgvv37_65_t01	similar to UP|Q3ZTF5_TOBAC (Q3ZTF5) Trehalose-phosphate phosphatase, partial (54%)	-1,012
	Glutathione S transferases	chr4_jgvv79_54_t01	UP|Q56AY1_VITVI (Q56AY1) Glutathione S-transferase , complete	-6,246
		chr19_jgvv93_11_t01	glutathione-S-transferase [Vitis vinifera]	-1,391
		chr19_jgvv15_210_t01	similar to UP|O49821_CARPA (O49821) Glutathione transferase , partial (67%)	-1,379
		chr19_jgvv27_43_t01	similar to RF|NP_176758.1|15218828|NM_105255 glutathione transferase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (90%)	-1,25
		chr12_jgvv35_25_t01	similar to UP|Q84VH0_MALPU (Q84VH0) Glutathione S-transferase Z1, partial (54%)	-1,122
		chr18_jgvv1_46_t01	weakly similar to UP|Q9FQC9_MAIZE (Q9FQC9) Glutathione S-transferase GST 10 , partial (80%)	-1,095
		chr19_jgvv15_211_t01	similar to UP|Q84VH2_MALPU (Q84VH2) Glutathione S-transferase U1, partial (80%)	-1,007
		chr12_jgvv34_77_t01	similar to UP|Q84VH0_MALPU (Q84VH0) Glutathione S-transferase Z1, partial (97%)	-1,002
		chr5_jgvv49_97_t01	weakly similar to UP|Q9FQE4_SOYBN (Q9FQE4) Glutathione S-transferase GST 14 (Fragment) , partial (91%)	1,45
		chr5_jgvv49_99_t01	weakly similar to UP|Q9FQE4_SOYBN (Q9FQE4) Glutathione S-transferase GST 14 (Fragment) , partial (91%)	1,611
		chr8_jgvv40_229_t01	weakly similar to UP|GSTX6_SOYBN (P32110) Probable glutathione S-transferase (Heat shock protein 26A) (G2-4) , partial (69%)	2,508
	Cyclin	chr3_jgvv180_25_t01	similar to UP|Q1XGF1_TOBAC (Q1XGF1) Cyclin, partial (79%)	-2,68
		chr4_jgvv8_398_t01	similar to UP|Q40491_TOBAC (Q40491) Cyclin A-like protein, partial (57%)	-2,366
		chr8_jgvv40_228_t01	weakly similar to UP|O04398_TOBAC (O04398) B-type cyclin, partial (12%)	-2,316
		chrun_jgvv194_26_t01	similar to UP|Q1XGF1_TOBAC (Q1XGF1) Cyclin, partial (79%)	-2,271
		chr8_pdvv105_52_t01	similar to UP|Q1XGF1_TOBAC (Q1XGF1) Cyclin, partial (79%)	-1,607
		chr5_jgvv94_10_t01	similar to UP|Q1XGF1_TOBAC (Q1XGF1) Cyclin, partial (79%)	-1,121

Table 3.14: continued…

Pathway	Bin Name	Gene ID	Annotation	Fold change
Miscelleanous	PS.lightreaction. photosystem II	chr19_jgvv14_326_t01	homologue to UP|Q5DNZ6_TOBAC (Q5DNZ6) Chloroplast chlorophyll a-b binding protein (Fragment), partial (68%)	-2,112
		chr18_jgvv1_720_t01	similar to UP|Q9C639_ARATH (Q9C639) Light-harvesting complex protein (At1g45474/F2G19.4), partial (80%)	-1,425
		chrun_jgvv181_18_t01	homologue to UP|Q9SDT2_DAUCA (Q9SDT2) Chlorophyll a/b-binding protein, complete	-1,335
		chrun_jgvv181_20_t01	homologue to UP|Q9SDT2_DAUCA (Q9SDT2) Chlorophyll a/b-binding protein, complete	-1,262
	major carbohydrate metabolism synthesis.sucrose	chr5_jgvv29_35_t01	similar to UP|SPS1_CITUN (O22060) Sucrose-phosphate synthase 1 (UDP-glucose-fructose-phosphate glucosyltransferase 1) , partial (65%)	-2,797
		chr4_jgvv8_535_t01	similar to UP|SPS1_CITUN (O22060) Sucrose-phosphate synthase 1 (UDP-glucose-fructose-phosphate glucosyltransferase 1) , partial (65%)	-1,45
		chr5_jgvv20_269_t01	homologue to UP|GLGS_BRANA (Q9M462) Glucose-1-phosphate adenylyltransferase, partial (79%)	-1,119
		chr3_jgvv38_19_t01	similar to UP|O22658_CITLA (O22658) ADP-glucose pyrophosphorylase large subunit 1 , partial (29%)	1,103
		chr15_jgvv48_38_t01	similar to UP|Q84LK2_PHAVU (Q84LK2) Granule-bound starch synthase Ib precursor , partial (43%)	-4,38
		chr16_jgvv300_3_t01	similar to UP|O49447_ARATH (O49447) ADP, ATP carrier-like protein, partial (30%)	-1,244
		chr14_jgvv6_75_t01	similar to UP|Q6VWJ5_LYCES (Q6VWJ5) Fructokinase 3, partial (61%)	-1,315
		chr8_jgvv7_565_t01	homologue to UP|Q3L7K5_VITVI (Q3L7K5) Cell wall apoplastic invertase, partial (83%)	-1,27
		chrun_jgvv233_1_t01	homologue to UP|Q9S943_VITVI (Q9S943) Vacuolar invertase 2, GIN2, partial (46%)	1,175
		chrun_jgvv1562_1_t01	similar to PIR|S19125|YUMU sucrose synthase (EC 2.4.1.13) - Arabidopsis thaliana, partial (18%)	-1,909
		chr12_jgvv57_12_t01	similar to UP|Q9SLS2_CITUN (Q9SLS2) Sucrose synthase, partial (85%)	-1,234
		chr18_jgvv1_723_t01	similar to UP|Q9XGN4_AJURE (Q9XGN4) Galactinol synthase, isoform GolS-1 , partial (93%)	-1,467
		chr3_jgvv38_388_t01	similar to UP|Q8L794_ARATH (Q8L794) Xylulose kinase like protein, partial (50%)	-1,523

3.5.2 Changes in the gene expression pattern upon elicitation with phosphonate and elicitation with phosphate

Treatment with phosphate and phosphonate led to down-regulation of most of the DEGs (74% and 68% respectively). DEGs involved in photosynthesis, carbohydrate metabolism, stress, signalling, RNA processing and regulation were majorly repressed, while secondary metabolism is up-regulated (Table 3.15). DEGs involved in hormone metabolism were mostly down-regulated after phosphate treatment, while they were up-regulated after phosphonate treatment (Table 3.15).

Common and specific DEGs after elicitation

Inoculation induced differential expression of 3466 genes, while in plants treated with phosphonate and phosphate but not inoculated fewer DEGs (1422 and 1529, respectively) were observed and plants treated with Frutogard® showed only 462 DEGs (Table 3.5). It is interesting to know how many DEGs are common among different treatments. This will provide more information on the genes that are differentially regulated due to elicitation in general and those that are regulated after the treatment with specific elicitors. Figure 3.15 shows the common as well as the specific genes in each treatment. 507 DEGs are unique to phosphate treatment, 355 are unique to phosphonate treatment and 276 are unique to Frutogard® treatment. 104 DEG are found as a response to all 3 elicitors, while between phosphonate and Frutogard® treatments there are 168 common DEGs. Between phosphate treatment and Frutogard® treatment there are 123 common DEGs and between phosphate and phosphonate there are 1003 DGEs. These results indicate that the responses of phosphonate and phosphate are more similar to each other than any of them to Frutogard®, since the majority of the genes (1003 (“899+104” of 1422 and 1529 DEGs respectively) that are differentially regulated are common.

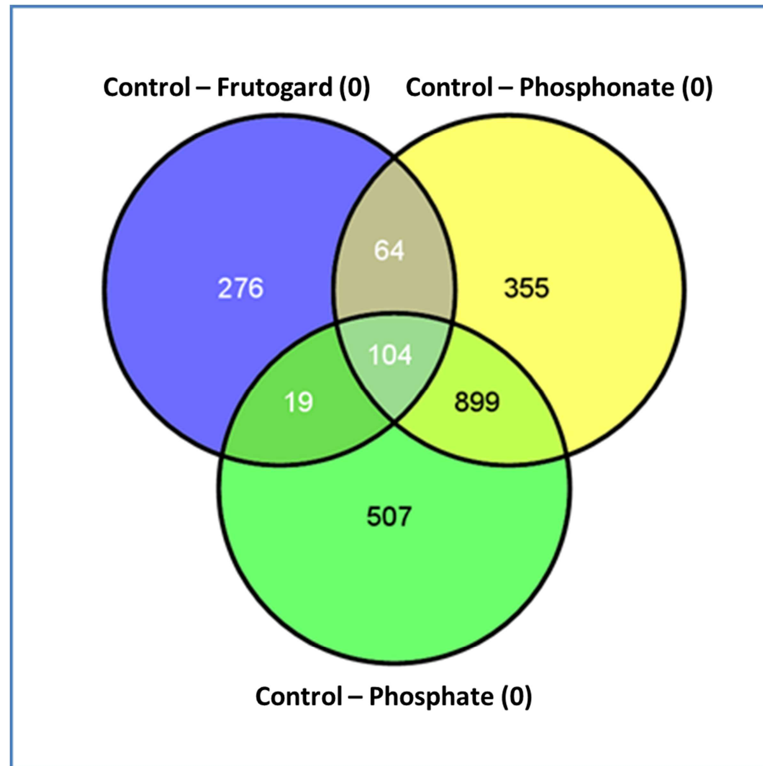

Figure 3.15: Venn-diagrams showing common and specific differentially expressed genes after elicitation with different elicitors. **Control:** DEGs in control condition (no elicitation, no inoculation); **Frutogard (0):** DEGs after elicitation with Frutogard; **Phosphonate (0):** DEGs after elicitation with phosphonate; **Phosphate (0):** DEGs after elicitation with phosphate.

Table 3.15: Differential regulation of genes grouped to 'bins' using the MapMan software after elicitation. Up-, down-regulated genes and total no. of genes are shown. Gray shades represent the pathways discussed in text. **Control:** no elicitation and no inoculation; **(P0):** elicitation only

Bin	Bin Name	Phosphate (P0) – control					Phosphonate (P0) – control			
		Down	Up	∑	No. of clones in the BIN	% of DEGs in the BIN	Down	Up	∑	% of DEGs in the BIN
1	Photosynthesis	40 (95%)	2 (5%)	42	494	9%	37 (97%)	1 (3%)	38	8%
2	Major carbohydrate metabolism	5 (83%)	1 (17%)	6	165	4%	7 (78%)	2 (22%)	9	5%
3	Minor carbohydrate metabolism	5 (63%)	3 (37%)	8	162	5%	5 (71%)	2 (29%)	7	4%
4	Glycolysis	-	-	0	123	0%	-	-	0	0%
5	Fermentation	1 (100%)	-	1	52	2%	-	-	0	0%
6	Gluconeogenesis	2 (100%)	-	2	22	9%	1 (100%)	-	1	5%
7	Oxidative pentose phosphate pathway	1 (100%)	-	1	42	2%	1 (100%)	-	1	2%
8	TCA – organic transformation	2 (40%)	3 (60%)	5	123	4%	-	1 (100%)	1	1%
9	Electron transport / ATP synthesis	11 (92%)	1 (8%)	12	156	8%	10 (91%)	1 (9%)	11	7%
10	Cell wall	10 (53%)	9 (47%)	19	595	3%	8 (32%)	17 (68%)	25	4%
11	Lipid metabolism	2 (18%)	9 (82%)	11	459	2%	2 (15%)	11 (85%)	13	3%
12	N-metabolism	3 (75%)	1 (25%)	4	59	7%	3 (75%)	1 (25%)	4	7%
13	Amino acid metabolism	4 (50%)	4 (50%)	8	459	2%	3 (25%)	9 (75%)	12	3%
14	S-assimilation	-	-	0	15	0%	-	-	0	0%
15	Metal handling	2 (50%)	2 (50%)	4	142	3%	3 (75%)	1 (25%)	4	3%
16	Secondary metabolism	7 (30%)	16 (70%)	23	543	4%	12 (27%)	33 (73%)	45	8%
17	Hormone metabolism	16 (67%)	8 (33%)	24	502	5%	10 (36%)	18 (64%)	28	6%
18	Vitamine metabolism	1 (100%)	-	1	45	2%	-	-	0	0%
19	Tetrapyrrole synthesis	-	1 (100%)	1	56	2%	-	1 (100%)	1	2%
20	Stress	49 (75%)	16 (25%)	65	948	7%	48 (75%)	16 (25%)	64	7%
21	Redox	1 (13%)	7 (87%)	8	282	3%	3 (38%)	5 (62%)	8	3%
22	Polyamine metabolism	-	-	0	18	0%	-	-	0	0%

Table 3.15: Continued…

Bin	Bin Name	Phosphate (P0) – control					Phosphonate (P0) – control			
		Down	Up	∑	No. of clones in the BIN	% of DEGs in the BIN	Down	Up	∑	% of DEGs in the BIN
23	Nucleotide metabolism	3 (100%)	-	3	147	2%	-	1 (100%)	1	1%
24	Biodegradation of Xenobiotics	-	-	0	24	0%	-	-	0	0%
25	C1-metabolism	-	1 (100%)	1	33	3%	-	1 (100%)	1	3%
26	Miscellaneous	33 (51%)	32 (49%)	65	1219	5%	37 (55%)	30 (45%)	67	5%
27	RNA processing and regulation	85 (79%)	23 (21%)	108	2296	5%	67 (74%)	23 (33%)	90	4%
28	DNA synthesis and repair	6 (40%)	9 (60%)	15	422	4%	6 (67%)	3 (33%)	9	2%
29	Protein metabolism	81 (78%)	23 (22%)	104	3628	3%	50 (66%)	26 (34%)	76	2%
30	Signalling	28 (74%)	10 (26%)	38	1157	3%	24 (67%)	12 (33%)	36	3%
31	Cell cycle and organization	19 (49%)	20 (51%)	39	655	6%	20 (51%)	19 (45%)	39	6%
33	Development	4 (57%)	3 (43%)	7	405	2%	5 (45%)	6 (55%)	11	3%
34	Transport	29 (64%)	16 (36%)	45	951	5%	20 (43%)	27 (57%)	47	5%
35	Not assigned.no ontology	493 (81%)	118 (19%)	611	3276	19%	430 (79%)	111 (21%)	541	17%
∑	Total no. of genes in all bins	943 (74%)	338 (26%)	1281	19675	7%	812 (68%)	378 (32%)	1190	6%

3.5.2.1 Similar changes in the gene expression pattern upon elicitation with phosphonate and phosphate

As mentioned before, treatments with phosphate and phosphonate had a similar effect on the gene expression (Figure 3.15). 1003 DEGs, which represent 70% of the DEGs after phosphonate treatment and 65% of the DEGs after phosphate treatment were common between both conditions (Figure 3.16 and Table 3.16). Therefore, in the following parts, the DEGs will be divided into common DEGs (between phosphonate treatment and phosphate treatment) and specific DEGs (for each condition separately) to eliminate the redundancy in the results and to make the discussion easier.

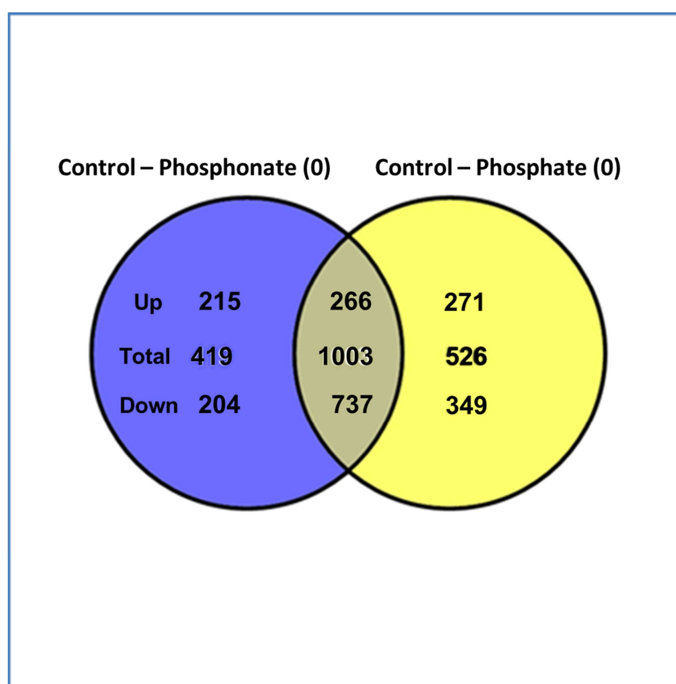

Figure 3.16: Common and specific DEGs after elicitation with phosphonate and phosphate. **Cont:** DEGs in control condition (no elicitation, no inoculation); **Pac0:** DEGs after elicitation with phosphonate; **Pate0:** DEGs after elicitation with phosphate

Table 3.16: Common and specific DEGs after elicitation with phosphonate and phosphate

	Total DEGs	**Common DEGs**		**Specific DEGs**
Phosphonate (Pac0)	1422	1003	70%	419 (30%)
Phosphate (Pate0)	1529		65%	526 (35%)

Common transcriptional responses between plants treated with phosphonate and plants treated with phosphate

The gene expression in plants treated with phosphonate and with phosphate followed similar trends though with slightly different fold changes of the differentially expressed genes. Genes involved in stress pathways, composed of different bins, were mostly down-regulated. Among these down-regulated DEGs were genes coding for biotic receptors such as resistance proteins. Some of the genes in the heat shock proteins bin were up-regulated, while others were down-regulated. Genes coding for germin proteins, that were down-regulated after inoculation, were up-regulated. (Table 3.17). A few genes involved in secondary metabolite pathways were up-regulated such as genes involved in phenylpropanoids, anthocyanins, flavonoids and isoflavonols biosynthesis, while genes involved in flavonols and isoprenoids biosynthesis were down-regulated (Table 3.18).

Common DEGs between plants treated with phosphonate and with phosphate involved in signalling pathways and hormone metabolism were also very few. Most of the genes involved in signalling pathways were mainly down-regulated, mostly coding for receptor-like serine/threonine kinases, leucine-rich repeat recptors and calcium signalling (Table 3.18). Many genes involved in hormone metabolism such as abscisic acid and jasmonate were down-regulated, while genes coding for enzymes involved in the metabolism of gibberellin and brassinosteroid were up-regulated (Table 3.19). Genes involved in cell wall synthesis such as cellulose synthase and cell wall modification such as xyloglucan endotransglycosylase were up-regulated, while genes leading to cell wall degradation such as beta-D-glucosidase, pectate lyase and endo-polygalacturonase were down-regulated (Table 3.19).

Most of DEGs involved in photosynthesis were down-regulated , such as genes coding for enzymes involved in light reaction in the photosystem II such as NADH-plastoquinone oxidoreductase and enzymes involved in calvin cycle such as RuBisCO, an enzyme involved in carbon fixation in the calvin cycle (Table 3.20). Genes involved in carbohydrate metabolism such as vacuolar invertase, sucrose synthase, alpha-glucosidase and raffinose synthase were down-regulated. However, beta-amylase was up-regulated. A gene coding for trehalose-phosphate phosphatase was up-regulated (Table 3.20). Most of DEGs involved in RNA

metabolism, such as genes coding for MYB and WRKY transcription factors, were down-regulated. (Table 3.21).
DEGs involved in lipid metabolism were up-regulated, such as genes involved in fatty acid synthesis, elongation and desaturation, e.g. stearoyl-ACP desaturase, pyruvate kinase and fatty acid hydroxylase (Table 3.21).

Table 3.17: Examples of common DEGs (in selected sub-bins from stress pathways) between plants treated with phosphonate and plants treated with phosphate

Pathway	Bin Name	Gene ID	Annotation	Fold change	
				Phosphonate	Phosphate
Stess pathways	Stress.biotic receptors	chr18_jgvv89_119_t01	GB|AF365879.1|AAQ15191.1 resistance protein [Vitis vinifera]	-1,629	-1,589
		chr19_jgvv93_3_t01	GB|AF365880.1|AAQ15192.1 resistance protein [Vitis vinifera]	-2,753	-3,193
		chr19_jgvv27_46_t01	GB|AF365880.1|AAQ15192.1 resistance protein [Vitis vinifera]	-2,711	-3,221
		chr12_jgvv35_155_t01	GB|AF365881.1|AAQ15193.1 resistance protein [Vitis vinifera]	-1,307	-1,41
		chrun_jgvv2381_1_t01	GB|AF369833.1|AAM21290.1 resistance gene analog [Vitis vinifera]	-2,373	-2,032
		chr13_jgvv139_3_t01	GB|AF369833.1|AAM21290.1 resistance gene analog [Vitis vinifera]	-2,732	-3,041
		chr5_jgvv51_23_t01	weakly similar to GP|15787897|gb|AAL07540.1 resistance gene analog NBS5 {Helianthus annuus}, partial (42%)	-1,118	-1,107
		chrun_jgvv184_11_t01	weakly similar to GP|15787905|gb|AAL07544.1 resistance gene analog NBS9 {Helianthus annuus}, partial (49%)	-2,192	-2,306
		chrun_pdvv288_2_t01	weakly similar to GP|15787905|gb|AAL07544.1 resistance gene analog NBS9 {Helianthus annuus}, partial (49%)	-2,047	-2,112
		chrun_jgvv160_27_t01	weakly similar to GP|15787905|gb|AAL07544.1 resistance gene analog NBS9 {Helianthus annuus}, partial (49%)	-1,387	-1,495
		chr9_random_jgvv162_7_t01	weakly similar to UP|Q8H6R0_PONTR (Q8H6R0) NBS-LRR type disease resistance protein, partial (19%)	-1,147	-1,432
		chr7_random_jgvv209_16_t01	weakly similar to UP|Q93VS9_PHAVU (Q93VS9) NBS-LRR resistance-like protein B8, partial (7%)	-1,627	-1,505
	Stress.abiotic.heat	chr14_pdvv83_107_t01	similar to UP|HSP81_ORYSA (P33126) Heat shock protein 81-1 (HSP81-1) (Heat shock protein 82), partial (12%)	1,232	1,184
		chrun_jgvv131_20_t01	homologue to UP|Q6UJX6_NICBE (Q6UJX6) Molecular chaperone Hsp90-1, partial (59%)	1,531	1,717
		chr13_jgvv19_375_t01	homologue to UP|Q9M4E6_CUCSA (Q9M4E6) Heat shock protein 70, complete	1,191	1,192
		chr2_pdvv12_8_t01	similar to UP|HSP81_ORYSA (P33126) Heat shock protein 81-1 (HSP81-1) (Heat shock protein 82), partial (12%)	-1,973	-2,158
		chr7_jgvv95_13_t01	similar to UP|HSP81_ORYSA (P33126) Heat shock protein 81-1 (HSP81-1) (Heat shock protein 82), partial (12%)	-2,163	-1,962
		chrun_jgvv279_1_t01	similar to UP|HSP81_ORYSA (P33126) Heat shock protein 81-1 (HSP81-1) (Heat shock protein 82), partial (12%)	-2,324	-2,168
	Stress.abiotic	chr10_pdvv3_429_t01	GB|AY298727.1|AAQ63185.1 germin-like protein 3 [Vitis vinifera]	2,387	2,696
		chr10_pdvv3_427_t01	GB|AY298727.1|AAQ63185.1 germin-like protein 3 [Vitis vinifera]	2,1	1,971

Table 3.18: Examples of common DEGs (in selected sub-bins from secondary metabolism and signalling pathways) between plants treated with phosphonate and plants treated with phosphate

Pathway	Bin Name	Gene ID	Annotation	Fold change	
				Phosphonate	Phosphate
Secondary metabolites	Isoprenoids	chr18_jgvv89_78_t01	similar to UP|Q2L8A7_9LAMI (Q2L8A7) Acetoacetyl-CoA thiolase, partial (92%)	-1,676	-1,508
	Phenylpropanoids.lignin biosynthesis	chr1_jgvv10_258_t01	similar to UP|Q2YHM9_PLAMJ (Q2YHM9) Caffeoyl-CoA O-methyltransferase (Fragment), partial (86%)	2,528	2,021
	Anthocyanins	chrun_jgvv361_4_t01	UP|Q7PCC4_VITVI (Q7PCC4) Anthocyanidin reductase, partial (87%)	1,026	1,064
	Dihydroflavonols.Flavonoids	chr8_jgvv7_364_t01	UP|Q3C210_VITVI (Q3C210) Flavonoid 3\',5\'-hydroxylase, complete	1,621	1,713
		chr6_jgvv9_76_t01	UP|Q3C210_VITVI (Q3C210) Flavonoid 3\',5\'-hydroxylase, complete	1,177	1,079
	Flavonols	chr10_jgvv3_227_t01	similar to UP|Q9SB32_ARATH (Q9SB32) SRG1-like protein (At4g25310), partial (32%)	-1,436	-1,296
	Isoflavonols	chr3_pdvv38_10_t01	UP|Q3KN67_VITVI (Q3KN67) Isoflavone reductase-like protein 6, partial (81%)	1,354	1,326
Signalling pathways	Receptor kinases.leucine rich repeat VIII.2	chr19_jgvv14_56_t01	weakly similar to UP|Q6EQG8_ORYSA (Q6EQG8) Leucine-rich repeat family protein /protein kinase family protein-like, partial (16%)	-1,623	-1,777
	Receptor kinases.leucine rich repeat VIII.2	chr10_jgvv3_171_t01	weakly similar to UP|O22579_ARATH (O22579) Receptor-like serine/threonine kinase, partial (19%)	-1,348	-1,218
	Receptor kinases.leucine rich repeat VIII.3	chrun_jgvv125_18_t01	weakly similar to UP|O22579_ARATH (O22579) Receptor-like serine/threonine kinase, partial (19%)	-1,587	-1,102
	Receptor kinases	chrun_jgvv398_2_t01	similar to GP|13506747|gb|AAK28316.1 receptor-like protein kinase 5 {Arabidopsis thaliana}, partial (20%)	1,734	2,111
		chr16_jgvv22_25_t01	similar to RF|NP_182083.1|15225518|NM_130121 ATP binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (24%)	-1,263	-1,55
	Receptor kinases.S-locus glycoprotein	chr7_jgvv185_7_t01	similar to UP|Q9ZT07_ARATH (Q9ZT07) Receptor-like protein kinase, partial (19%)	1,309	1,293
	Receptor kinases.misc	chr3_jgvv88_10_t01	similar to UP|Q56X19_ARATH (Q56X19) Leucine-rich repeat receptor-like kinase At1g09970, partial (55%)	-1,613	-1,799
		chr5_jgvv20_433_t01	weakly similar to RF|NP_186833.1|15232204|NM_111050 ATP binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (15%)	-2,111	-1,829
	Signalling.calcium	chrun_jgvv2438_2_t01	similar to SP|Q9LU41|ACA9_ARATH Potential calcium-transporting ATPase 9 plasma membrane-type (EC 3.6.3.8) (Ca(2+)-ATPase isoform 9), partial (6%)	-2,182	-2,375
		chr16_jgvv13_110_t01	UP|Q6UQE4_DAUCA (Q6UQE4) Calmodulin 8 (Calmodulin 4) (Fragment), complete	1,137	1,185
		chrun_jgvv179_23_t01	similar to RF|NP_188931.1|15228891|NM_113191 calcium-transporting ATPase/ calmodulin binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0) , partial (24%)	-2,425	-2,345

Table 3.19: Examples of common DEGs (in selected sub-bins hormone metabolism and cell wall pathways) between plants treated with phosphonate and plants treated with phosphate

Pathway	Bin Name	Gene ID	Annotation	Fold change	
				Phosphonate	Phosphate
Hormone metabolism	Abscisic acid metabolism	chr13_jgvv64_96_t01	UP|Q3T4H1_VITVI (Q3T4H1) 9,10[9\',10\']carotenoid cleavage dioxygenase, complete	-2,083	-1,861
	Abscisic acid.signal transduction	chr12_jgvv55_32_t01	similar to UP|Q6QPK1_LYCES (Q6QPK1) AREB-like protein, partial (70%)	-1,551	-1,355
	Abscisic acid.responsive-activated	chr3_jgvv132_8_t01	weakly similar to UP|Q2R1K3_ORYSA (Q2R1K3) AtHVA22a, partial (24%)	-2,086	-1,66
	Auxin.signal transduction	chr18_jgvv1_1172_t01	similar to UP|Q8LKH1_9ROSI (Q8LKH1) PIN1-like auxin transport protein, partial (46%)	-1,164	-2,1
		chr14_jgvv83_78_t01	similar to RF|NP_563915.1|18391439|NM_101152 IPS1; ubiquitin-protein ligase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (12%)	1,026	1,036
	Brassinosteroid.synthesis-degradation	chr1_jgvv11_272_t01	similar to UP|Q4ACU1_ZINEL (Q4ACU1) Delta7 sterol C-5 desaturase, partial (94%)	1,56	1,535
	Gibberelin metabolism	chr18_jgvv1_93_t01	similar to UP|O80417_TOBAC (O80417) Ntc12 protein, partial (72%)	1,14	1,357
	Jasmonate.synthesis-degradation	chr9_jgvv2_89_t01	similar to UP|O24371_SOLTU (O24371) 13-lipoxygenase , partial (29%)	-1,126	-1,576
		chr18_jgvv1_819_t01	similar to UP|Q84V85_CITSI (Q84V85) Allene oxide synthase, partial (26%)	-1,031	-1,239
Cell wall	Cellulose synthesis	chr2_jgvv25_180_t01	homologue to UP|P93156_GOSHI (P93156) Cellulose synthase (Fragment), partial (82%)	1,635	1,682
		chr14_jgvv6_202_t01	homologue to UP|Q6XP46_SOLTU (Q6XP46) Cellulose synthase, partial (39%)	1,214	1,07
	Cell wall proteins.LRR	chr5_jgvv20_175_t01	similar to UP|O18465_HIRME (O18465) Tractin, partial (6%)	1,556	1,081
		chr18_jgvv1_796_t01	similar to GP|9279698|dbj|BAB01255.1 extensin protein-like {Arabidopsis thaliana}, partial (49%)	-1,268	-1,742
	Cell wall.degradation	chr13_jgvv73_18_t01	similar to UP|Q7XAS3_GOSHI (Q7XAS3) Beta-D-glucosidase, partial (23%)	1,108	1,152
		chr5_jgvv77_101_t01	similar to GB|AAS17751.1|42495032|AY486104 beta xylosidase {Fragaria x ananassa} (exp=-1; wgp=0; cg=0), partial (54%)	-2,132	-2,341
		chr1_jgvv10_247_t01	homologue to UP|Q94FT5_FRAAN (Q94FT5) Pectate lyase (Fragment), complete	1,416	1,275
		chrun_pdvv2630_1_t01	similar to UP|Q9SMT3_ARATH (Q9SMT3) Endo-polygalacturonase-like protein (Glycoside hydrolase family 28 protein), partial (50%)	-1,391	-1,679
		chr13_jgvv64_44_t01	UP|Q8LKV2_VITVI (Q8LKV2) Polygalacturonase-inhibiting protein, complete	-2,261	-1,973
		chr8_jgvv7_127_t01	UP|Q8LKV2_VITVI (Q8LKV2) Polygalacturonase-inhibiting protein, complete	-2,012	-2,235
		chr11_jgvv52_52_t01	similar to UP|Q8S902_SOYBN (Q8S902) Syringolide-induced protein 19-1-5, partial (94%)	1,718	1,609
		chr18_pdvv41_129_t01	xyloglucan endotransglycosylase XET2 [Vitis vinifera]	1,483	1,526

Table 3.20: Examples of common DEGs (in selected sub-bins from photosynthesis and carbohydrate pathways) between plants treated with phosphonate and plants treated with phosphate

Pathway	Bin Name	Gene ID	Annotation	Fold change	
				Phosphonate	Phosphate
Photosynthesis	Lightreaction.photosystem II	chr3_pdvv38_356_t01	homologue to PRF|1011228A|224159|1011228A cytochrome b559. {Spinacia oleracea} (exp=-1; wgp=-1; cg=-1), complete	-2,588	-2,509
		chr13_pdvv101_15_t01	homologue to PRF|1011228A|224159|1011228A cytochrome b559. {Spinacia oleracea} (exp=-1; wgp=-1; cg=-1), complete	-3,538	-3,649
		chrun_jgvv396_1_t01	homologue to UP|Q3V538_ACOCL (Q3V538) PSII 43 kDa protein, complete	-3,067	-3,191
		chr6_jgvv9_194_t01	homologue to UP|Q70JE5_OLEEU (Q70JE5) PSII K protein, complete	-1,123	-1,129
		chr11_jgvv103_59_t01	homologue to UP|Q3V538_ACOCL (Q3V538) PSII 43 kDa protein, complete	-1,611	-2,192
		chrun_jgvv505_6_t01	UP|Q2L933_GOSHI (Q2L933) Cytochrome b6, partial (50%)	-2,007	-2,49
		chrun_jgvv504_1_t01	homologue to UP|Q3V538_ACOCL (Q3V538) PSII 43 kDa protein, complete	-1,54	-2,243
		chr11_jgvv37_102_t01	homologue to UP|Q49KZ4_EUCGG (Q49KZ4) NADH-plastoquinone oxidoreductase subunit K, complete	-1,025	-1,096
		chr7_jgvv151_46_t01	UP|Q2MII8_SOLBU (Q2MII8) Photosystem I P700 apoprotein A2, complete	-1,985	-2,485
	Calvin cycle.rubisco	chr14_pdvv68_174_t01	UP|Q9MVF6_9ROSI (Q9MVF6) Ribulose-1,5-bisphosphate carboxylase/oxygenase, large subunit, complete	-1,998	-2,475
		chr7_jgvv129_76_t01	UP|Q9MVF6_9ROSI (Q9MVF6) Ribulose-1,5-bisphosphate carboxylase/oxygenase, large subunit, complete	-1,524	-1,931
		chr16_jgvv13_30_t01	UP|Q9MVF6_9ROSI (Q9MVF6) Ribulose-1,5-bisphosphate carboxylase/oxygenase, large subunit, complete	-1,508	-1,758
Carbohydrate metabolism	Degradation.sucrose.invertases	chrun_jgvv233_1_t01	homologue to UP|Q9S943_VITVI (Q9S943) Vacuolar invertase 2, GIN2, partial (46%)	-1,471	-2,116
	Degradation.sucrose.Susy	chr10_pdvv71_111_t01	similar to UP|Q9SLS2_CITUN (Q9SLS2) Sucrose synthase, partial (85%)	-1,133	-1,145
	Degradation.starch.starch cleavage	chr10_jgvv92_23_t01	similar to UP|Q9LEC9_SOLTU (Q9LEC9) Alpha-glucosidase , partial (37%)	-2,351	-2,739
		chr12_jgvv59_253_t01	similar to UP|Q5F305_SOYBN (Q5F305) Beta-amylase , partial (85%)	1,432	1,036
	Raffinose family.raffinose synthases	chr5_jgvv77_59_t01	similar to GP|4106395|gb|AAD02832.1| raffinose synthase {Cucumis sativus}, partial (35%)	-1,924	-1,616
		chr7_jgvv5_164_t01	similar to GP|4106395|gb|AAD02832.1| raffinose synthase {Cucumis sativus}, partial (35%)	-2,004	-1,806
	Trehalose	chr11_jgvv37_65_t01	similar to UP|Q3ZTF5_TOBAC (Q3ZTF5) Trehalose-phosphate phosphatase, partial (54%)	1,665	1,579

Table 3.21: Examples of common DEGs (in selected sub-bins from RNA processing and lipid metabolism) between plants treated with phosphonate and plants treated with phosphate

Pathway	Bin Name	Gene ID	Annotation	Fold change	
				Phosphonate	Phosphate
RNA transcription	Transcription factor family.MYB	chr14_jgvv36_82_t01	homologue to UP|Q8W149_MAIZE (Q8W149) CDC5 protein, partial (21%)	-3,139	-3,056
		chr9_jgvv2_146_t01	similar to GP|28628949|gb|AAO49411.1 MYB2 {Dendrobium sp. XMW-2002-2}, partial (36%)	1,387	1,405
		chr9_jgvv2_329_t01	similar to UP|Q2LMD7_MALDO (Q2LMD7) MYBR2, partial (64%)	-1,24	-1,065
	Transcription factor family.WRKY	chr10_jgvv3_147_t01	similar to UP|Q3SAJ9_CAPAN (Q3SAJ9) WRKY-A1244, partial (57%)	-1,213	-1,021
		chr12_jgvv59_82_t01	homologue to UP|Q6B6R1_ORYSA (Q6B6R1) Transcription factor WRKY10, partial (24%)	-1,388	-1,785
Lipid metabolism	Fatty acid synthesis and elongation	chr12_jgvv34_189_t01	homologue to UP|Q4JIJ4_9ROSI (Q4JIJ4) Stearoyl-ACP desaturase , complete	1,296	1,176
		chr12_pdvv34_186_t01	homologue to UP|Q4JIJ4_9ROSI (Q4JIJ4) Stearoyl-ACP desaturase , complete	1,463	1,429
		chr14_jgvv108_155_t01	similar to UP|Q39350_BRANA (Q39350) Biotin carboxyl carrier protein, partial (32%)	1,479	1,193
		chr7_jgvv5_42_t01	similar to UP|KPYG_TOBAC (Q40546) Pyruvate kinase isozyme G, chloroplast precursor , partial (84%)	1,525	1,389
	Fatty acid desaturation	chr10_jgvv3_456_t01	similar to UP|O48916_ARATH (O48916) Fatty acid hydroxylase Fah1p (FAH1) (At2g34770/T29F13.2), partial (96%)	1,539	1,321

3.5.2.2 Specific transcriptional responses after elicitation with phosphonate

Among the DEGs that were specifically down-regulated by phosphonate were genes that code for PR-proteins such as chitinase, PR-4 and PR-5 (thaumatin) and other genes that code for biotic stress receptors and R-proteins (Table 3.22).
Most of DEGs involved in secondary metabolism were up-regulated (Table 3.22). These included genes involved in isoprenoids metabolism such as laccase and in chalcone biosynthesis such as chalcone synthase. However, stilbene synthase was down-regulated. Genes coding for enzymes in the phenylpropanoids pathway were mainly down-regulated, except for genes involved in lignin biosynthesis such as phenylalanine ammonia-lyase. Genes coding for enzymes involved in the biosynthesis of terpenoids such as terpene synthase were up-regulated. Genes coding for enzymes in the dihydroflavonols and isoflavonols pathways such as 2-hydroxy isoflavone-dihydroflavonol reductase and isoflavone reductase were up-regulated (Table 3.22).
Phosphonate treatment-specific DEGs that are involved in signalling pathways were very few (Table 3.23). However, genes involved in calcium signalling were mostly up-regulated such as hypersensitive reaction associated Ca2+-binding and calcium-transporting ATPase. Most of the DEGs involved in auxin metabolism were up-regulated, while all DEGs involved in ethylene, gibberellin, jasmonate and salicylic acid metabolism were up-regulated (Table 3.23). Genes coding for enzymes involved in cell wall such as xyloglucan endotransglycosylase, syringolide-induced and alpha-expansin were found up-regulated (Table 3.24). Nitrilases, enzymes that have a significant impact on the outcome of plant–microbe interactions by catalysing the hydrolysis of toxic nitrile compounds were down-regulated (Table 3.24). The rest of the pathways such as photosynthesis, carbohydrate metabolism and RNA metabolism were mainly down-regulated (data not shown).

Table 3.22: Examples of specific DEGs (in selected sub-bins from stress pathways and secondary metabolism) in plants treated with phosphonate (compared to control)

Pathway	Bin name	Gene ID	Annotation	Fold change
Stress pathways	Biotic stress receptors	chr18_jgvv117_40_t01	weakly similar to GP|15787897|gb|AAL07540.1 resistance gene analog NBS5 , partial (42%)	-1,067
		chrun_jgvv222_5_t01	weakly similar to UP|Q93VS9_PHAVU (Q93VS9) NBS-LRR resistance-like protein B8, partial (7%)	-1,393
		chr13_jgvv147_8_t01	weakly similar to UP|Q947E3_HELAN (Q947E3) Resistance gene analog NBS5 (Fragment), partial (46%)	-1,078
		chr18_jgvv41_86_t01	weakly similar to GP|15787905|gb|AAL07544.1 resistance gene analog NBS9 , partial (49%)	-1,198
		chr18_jgvv89_86_t01	weakly similar to UP|Q84ZU6_SOYBN (Q84ZU6) R 1 protein, partial (4%)	-1,618
		chr8_jgvv7_198_t01	similar to UP|Q45W75_ARAHY (Q45W75) Disease resistance-responsive family protein, partial (71%)	1,029
		chr19_jgvv27_54_t01	GB|AF365880.1|AAQ15192.1 resistance protein [Vitis vinifera]	-1,321
		chr15_jgvv24_13_t01	GB|AF365879.1|AAQ15191.1 resistance protein [Vitis vinifera]	-1,257
	PR-proteins	chr11_jgvv149_26_t01	weakly similar to UP|Q4W6L6_CYCRE (Q4W6L6) Chitinase A, partial (13%)	-1,132
		chr5_jgvv94_30_t01	homologue to UP|Q7XAU6_VITVI (Q7XAU6) Class IV chitinase, complete	-3,395
		chr5_jgvv94_29_t01	UP|O24530_VITVI (O24530) Class IV endochitinase , complete	-2,567
		chr14_jgvv81_66_t01	UP|O81228_VITVI (O81228) PR-4 type protein, complete	-1,61
		chr15_jgvv46_191_t01	similar to UP|CHIA_TOBAC (P29060) Acidic endochitinase precursor , partial (91%)	1,039
		chr2_jgvv25_393_t01	homologue to UP|Q9SPE0_VITRI (Q9SPE0) Thaumatin, complete	-2,45
		chr2_jgvv25_390_t01	UP|Q7XAU7_VITVI (Q7XAU7) Thaumatin-like protein, complete	-4.277
Secondary metabolism	Isoprenoids	chr18_jgvv75_78_t01	weakly similar to UP|Q94ID0_RHUVE (Q94ID0) Laccase (Fragment), partial (19%)	-2,242
		chr8_jgvv7_794_t01	similar to GP|1621467|gb|AAB17194.1| laccase {Liriodendron tulipifera}, partial (47%)	1,464
		chr4_jgvv69_69_t01	similar to UP|Q38757_ACEPS (Q38757) Laccase , partial (34%)	1,365
		chr8_jgvv40_147_t01	similar to GP|1621467|gb|AAB17194.1| laccase {Liriodendron tulipifera}, partial (47%)	1,406
	Isoprenoids.terpenoids	chr19_jgvv14_89_t01	homologue to UP|Q6Q3H3_VITVI (Q6Q3H3) Terpene synthase, complete	1,368
	Phenylpropanoids	chr10_jgvv3_44_t01	weakly similar to GP|28804594|dbj|BAC58012. daidzein 7-0-methyltransferase, partial (49%)	-2,561
		chr10_jgvv3_45_t01	weakly similar to UP|Q8GU24_ROSCH (Q8GU24) Orcinol O-methyltransferase 1 , partial (62%)	-2,984
	Phenylpropanoids. lignin biosynthesis	chr6_jgvv4_543_t01	similar to UP|PALY_CAMSI (P45726) Phenylalanine ammonia-lyase , partial (97%)	1,224
		chrun_jgvv174_26_t01	similar to UP|Q2R114_ORYSA (Q2R114) Oxidoreductase, zinc-binding dehydrogenase, partial (65%)	1,037
	Flavonoids. chalcones	chr5_jgvv136_15_t01	UP|Q8W3P6_VITVI (Q8W3P6) Chalcone synthase , complete	1,709
		chr16_jgvv100_35_t01	homologue to UP|Q9SPW2_VITRI (Q9SPW2) Stilbene synthase, complete	-2,303
		chr16_jgvv100_49_t01	UP|THS1_VITVI (P28343) Stilbene synthase 1 (Resveratrol synthase 1) (PSV25) , complete	-2,644
	Flavonoids.isoflavonols	chr15_jgvv48_189_t01	UP|Q3KN69_VITVI (Q3KN69) Isoflavone reductase-like protein 4, complete	1,544
		chr2_jgvv33_22_t01	UP|Q3KN72_VITVI (Q3KN72) Isoflavone reductase-like protein 1, complete	1,227

Table 3.23: Examples of specific DEGs (in selected sub-bins from signalling pathways and hormone metabolism) in plants treated with phosphonate (compared to control)

Pathway	Bin name	Gene ID	Annotation	Fold change
Signalling signalling	signalling.in sugar and nutrient physiology	chr19_jgvv14_159_t01	similar to GP|30013669|gb|AAP03877.1 Avr9/Cf-9 rapidly elicited protein 141 {Nicotiana tabacum}, partial (2%)	1,241
	Receptor kinases. leucine rich repeat VIII.2	chrun_jgvv173_11_t01	weakly similar to UP|Q6EQG8_ORYSA (Q6EQG8) Leucine-rich repeat family protein /protein kinase family protein-like, partial (16%)	-1,058
	Calcium	chr14_jgvv30_23_t01	similar to RF|NP_188931.1|15228891|NM_113191 calcium-transporting ATPase/ calmodulin binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0) , partial (24%)	1,036
		chr5_jgvv77_8_t01	weakly similar to UP|Q9SWP6_PHAVU (Q9SWP6) Hypersensitive reaction associated Ca2+-binding protein, partial (16%)	-1,123
		chrun_jgvv1658_1_t01	similar to SP|Q9LU41|ACA9_ARATH Potential calcium-transporting ATPase 9 plasma membrane-type (EC 3.6.3.8) (Ca(2+)-ATPase isoform 9), partial (6%)	-1,814
Hormone metabolism	Auxin.induced-regulated-responsive-activated	chr14_jgvv108_2_t01	similar to UP|Q8LKH1_9ROSI (Q8LKH1) PIN1-like auxin transport protein, partial (46%)	2,053
		chr19_jgvv14_276_t01	similar to UP|Q9LE80_ARATH (Q9LE80) Arabidopsis thaliana genomic DNA, chromosome 3, P1 clone: MJK13 (AT3g15450/MJK13_11) (MJK13.11 protein), partial (94%)	-3,063
		chr10_jgvv405_4_t01	weakly similar to RF|NP_199564.1|15238124|NM_124126 dopamine beta-monooxygenase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (32%)	1,412
		chr9_jgvv2_53_t01	similar to UP|Q8H0W7_ARATH (Q8H0W7) Expressed protein (At1g56220), partial (24%)	-1,293
		chr3_jgvv38_348_t01	similar to UP|Q8H6T6_PHAVU (Q8H6T6) Auxin-regulated protein, partial (72%)	1,109
	Ethylene metabolism	chr3_jgvv63_115_t01	weakly similar to UP|Q86B83_DROME (Q86B83) CG33099-PA, partial (7%)	1,404
		chr10_jgvv116_16_t01	similar to UP|Q84RC3_NICSY (Q84RC3) Gibberellin 2-oxidase 1, partial (33%)	1,278
		chrun_jgvv687_1_t01	weakly similar to UP|Q9LTH8_ARATH (Q9LTH8) Leucoanthocyanidin dioxygenase-like protein (At5g59530) (1-aminocyclopropane-1-carboxylate oxidase-like protein), partial (18%)	1,075
		chr3_jgvv63_122_t01	weakly similar to UP|Q86B83_DROME (Q86B83) CG33099-PA, partial (7%)	1,276
	Ethylene.induced-regulated-responsive-activated	chr4_jgvv23_141_t01	similar to RF|NP_174087.1|15217667|NM_102531 transcription factor {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (23%)	1,036
		chr8_jgvv32_46_t01	similar to UP|Q2V3W9_ARATH (Q2V3W9) Protein At3g11930, partial (41%)	1,041
	Gibberelin metabolism	chr16_jgvv22_18_t01	similar to UP|O80417_TOBAC (O80417) Ntc12 protein, partial (72%)	1,581
	Jasmonate metabolism	chr18_jgvv41_29_t01	similar to UP|Q76DL0_LITER (Q76DL0) LEDI-5c protein, partial (96%)	1,074
	Salicylic acid metabolism	chr1_jgvv11_75_t01	weakly similar to UP|Q9XI57_ARATH (Q9XI57) F9L1.6, partial (29%)	1,042

Table 3.24: Examples of specific DEGs (in selected sub-bins from cell wall and nitrilase pathways) in plants treated with phosphonate (compared to control)

Pathway	Bin name	Gene ID	Annotation	Fold change
Cell wall	Cell wall. modification	chr17_jgvv53_43_t01	similar to UP|Q4F986_LYCES (Q4F986) Xyloglucan endotransglycosylase/hydrolase 16 protein (Fragment) , partial (98%)	1,097
		chr8_jgvv7_384_t01	homologue to UP|Q8W3L8_9ROSI (Q8W3L8) Xyloglucan endo-transglycosylase, complete	1,339
		chr11_jgvv52_51_t01	similar to UP|Q8S902_SOYBN (Q8S902) Syringolide-induced protein 19-1-5, partial (93%)	1,719
		chr18_random_jgvv126_49_t01	similar to UP|Q8LKK0_GOSHI (Q8LKK0) Alpha-expansin precursor, partial (64%)	1,068
		chr11_jgvv52_49_t01	similar to UP|Q8S902_SOYBN (Q8S902) Syringolide-induced protein 19-1-5, partial (93%)	1,3
		chr11_jgvv52_64_t01	similar to UP|Q8S902_SOYBN (Q8S902) Syringolide-induced protein 19-1-5, partial (93%)	1,991
		chr11_jgvv52_55_t01	similar to UP|Q8S902_SOYBN (Q8S902) Syringolide-induced protein 19-1-5, partial (93%)	1,535
Nitrilases	Nitrile lyases	chr2_jgvv33_59_t01	similar to UP|Q3LRV4_LUPAN (Q3LRV4) Nitrilase 4B, partial (88%)	-1,054
		chr2_jgvv33_51_t01	similar to UP|Q3LRV4_LUPAN (Q3LRV4) Nitrilase 4B, partial (88%)	-1,167
		chr2_jgvv33_50_t01	similar to UP|Q3LRV4_LUPAN (Q3LRV4) Nitrilase 4B, partial (88%)	-1,261
		chr2_jgvv33_54_t01	similar to UP|Q3LRV4_LUPAN (Q3LRV4) Nitrilase 4B, partial (88%)	-1,151
		chr2_jgvv33_57_t01	similar to UP|Q3LRV4_LUPAN (Q3LRV4) Nitrilase 4B, partial (88%)	-1,054
		chr2_jgvv33_61_t01	similar to UP|Q3LRV4_LUPAN (Q3LRV4) Nitrilase 4B, partial (88%)	-1,111
		chr2_jgvv33_46_t01	similar to UP|Q3LRV4_LUPAN (Q3LRV4) Nitrilase 4B, partial (88%)	-1,045

3.5.2.3 Specific transcriptional responses after elicitation with phosphate

After elicitation with phosphonate, PR-proteins that were differentially expressed (down-regulated) were chitinases (different classes) (Table 3.22) while PR-proteins that were differentially expressed after elicitation with phosphate were PR-1, PR-4 and NtPRp27 (up-regulated) and SCUTL1 (down-regulated) (Table 3.25). Genes encoding for biotic stress receptors were down-regulated (Table 3.25).

In the isoprenoid and flavonoid biosynthesis pathway, isopentenyl pyrophosphate isomerase and cinnamoyl-CoA were up-regulated (Table 3.25), while in the phenylpropanoids pathway, phenylalanine ammonium lyase was down-regulated. Genes that code for receptor protein kinases were mostly down-regulated. DEGs involved in abscisic acid, auxin and gibberellin metabolism were down-regulated, while DEGs involved in cytokinin were up-regulated. However, a gene implicated in gibberellin metabolism was down-regulated (Table 3.26). Genes coding for lipoxygenase, an enzyme involved in jasmonate synthesis, were mostly up-regulated (Table 3.26).

Table 3.25: Examples of specific DEGs (in selected sub-bins from stress pathways and secondary metabolism) in plants treated with phosphate (compared to control)

Pathway	Bin name	Gene ID	Annotation	Fold change
Stress pathway	Biotic stress receptors	chr9_jgvv96_57_t01	weakly similar to UP|Q8H6R0_PONTR (Q8H6R0) NBS-LRR type disease resistance protein, partial (19%)	-1.063
		chr9_random_pdvv162_7_t01	weakly similar to GP|24461865|gb|AAN62352.1 NBS-LRR type disease resistance protein {Poncirus trifoliata}, partial (4%)	-1.237
		chrun_jgvv160_8_t01	weakly similar to GP|15787905|gb|AAL07544.1 resistance gene analog NBS9 {Helianthus annuus}, partial (49%)	-1.205
		chr18_jgvv1_415_t01	similar to UP|Q71RI4_VITVI (Q71RI4) Resistance protein (Fragment), partial (67%)	-1.196
		chr19_jgvv27_17_t01	weakly similar to UP|Q8H6R0_PONTR (Q8H6R0) NBS-LRR type disease resistance protein, partial (19%)	1.019
		chr18_jgvv75_5_t01	weakly similar to UP|Q947E3_HELAN (Q947E3) Resistance gene analog NBS5 (Fragment), partial (46%)	-1.027
		chr13_jgvv158_17_t01	GB|AF365880.1|AAQ15192.1 resistance protein [Vitis vinifera]	-1.206
		chr13_jgvv158_19_t01	weakly similar to GB|BAB01339.1|11994217|AB028617 disease resistance comples protein {Arabidopsis thaliana} (exp=0; wgp=1; cg=0), partial (11%)	-1.228
		chr13_jgvv139_28_t01	weakly similar to GB|BAB01339.1|11994217|AB028617 disease resistance comples protein {Arabidopsis thaliana} (exp=0; wgp=1; cg=0), partial (11%)	-1.668
		chr19_jgvv27_44_t01	GB|AF365880.1|AAQ15192.1 resistance protein [Vitis vinifera]	-1.115
	PR-proteins	chr3_jgvv91_104_t01	similar to UP|Q9XIY9_TOBAC (Q9XIY9) NtPRp27, partial (83%)	1.279
		chr3_jgvv88_50_t01	GB|AJ536326.1|CAD60273.1 putative pathogenesis related protein 1 (PR-1) precursor [Vitis vinifera]	1.406
		chr14_jgvv81_68_t01	homologue to UP|O81228_VITVI (O81228) PR-4 type protein, complete	1.609
		chr18_jgvv1_1085_t01	similar to UP|Q9SNY1_VITVI (Q9SNY1) SCUTL1 (Fragment), partial (73%)	-1.515
	Heat shock protein	chr2_jgvv25_377_t01	similar to UP|Q43455_SOYBN (Q43455) Heat shock transcription factor 29 (Fragment), partial (51%)	-1.302
		chr15_jgvv46_88_t01	homologue to UP|Q9M4E8_CUCSA (Q9M4E8) Heat shock protein 70, complete	-1.519
		chr18_jgvv166_3_t01	similar to UP|HSP81_ORYSA (P33126) Heat shock protein 81, partial (12%)	-1.38
		chr4_jgvv8_138_t01	similar to UP|Q9XGS6_PRUDU (Q9XGS6) Cytosolic class II low molecular weight heat shock protein, complete	1.038
		chr13_jgvv19_241_t01	similar to UP|HSP11_PEA (P19243) 18.1 kDa class I heat shock protein (HSP 18.1), partial (86%)	1.187
Secondary metabolism	Isoprenoids	chrun_jgvv768_3_t01	similar to GB|CAA57947.1|572635|CBIPISOM isopentenyl pyrophosphate isomerase {Clarkia breweri} (exp=-1; wgp=0; cg=0), partial (84%)	1.071
		chr11_jgvv206_2_t01	similar to GB|CAA57947.1|572635|CBIPISOM isopentenyl pyrophosphate isomerase {Clarkia breweri} (exp=-1; wgp=0; cg=0), partial (84%)	1.028
	Phenylpropanoids	chr9_jgvv18_102_t01	similar to UP|Q3ZPN4_9ROSI (Q3ZPN4) Anthraniloyal-CoA: methanol anthraniloyal transferase, partial (83%)	1.125
		chr16_jgvv39_33_t01	similar to UP|Q3ZPN4_9ROSI (Q3ZPN4) Anthraniloyal-CoA: methanol anthraniloyal transferase, partial (83%)	-2.169
	Phenylpropanoids. lignin biosynthesis	chr16_jgvv39_75_t01	phenylalanine ammonium lyase [Vitis vinifera]	-2.845
		chr17_jgvv0_765_t01	similar to UP|Q9M0X9_ARATH (Q9M0X9) 4-coumarate-CoA ligase-like protein, partial (64%)	-1.043
	Flavonoids. dihydroflavonols	chr18_jgvv122_69_t01	similar to RF|NP_180917.1|15226134|NM_128919 cinnamoyl-CoA reductase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (15%)	1.012
		chr18_jgvv1_929_t01	UP|P93799_VITVI (P93799) Dihydroflavonol 4-reductase , complete	-1.096

Table 3.26: Examples of specific DEGs (in selected sub-bins from signalling pathways and hormone metabolism) in plants treated with phosphate (compared to control)

Pathway	Bin name	Gene ID	Annotation	Fold change
Signalling pathway	Receptor kinases	chr12_jgvv55_47_t01	similar to UP|RIPK3_MOUSE (Q9QZL0) Receptor-interacting serine/threonine-protein kinase 3 (RIP-like protein kinase 3) (Receptor-interacting protein 3) (RIP-3) (mRIP3) , partial (6%)	1.236
		chr19_jgvv14_391_t01	similar to UP|Q9ZT07_ARATH (Q9ZT07) Receptor-like protein kinase, partial (19%)	1.16
		chr15_jgvv24_14_t01	similar to UP|O81906_ARATH (O81906) Serine/threonine kinase-like protein, partial (20%)	1.106
		chr3_jgvv132_28_t01	weakly similar to RF|NP_177131.1|15222427|NM_105641 kinase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (4%)	-1.574
		chr18_jgvv1_503_t01	similar to UP|Q56X19_ARATH (Q56X19) Leucine-rich repeat receptor-like kinase At1g09970, partial (55%)	-1.388
		chr18_jgvv1_126_t01	similar to UP|O04086_ARATH (O04086) Ser/Thr protein kinase isolog; 46094-44217, partial (37%)	-1.275
		chr1_jgvv11_606_t01	similar to UP|Q9SCZ4_ARATH (Q9SCZ4) Receptor-protein kinase-like protein, partial (27%)	-1.513
		chr18_jgvv1_628_t01	weakly similar to UP|Q9SN81_ARATH (Q9SN81) Receptor-kinase like protein, partial (13%)	-1.473
	Signalling.calcium	chr11_jgvv206_8_t01	similar to UP|Q9FJI9_ARATH (Q9FJI9) Similarity to calmodulin-binding protein, partial (46%)	-2.253
Hormone metabolism	Abscisic acid metabolism	chr2_jgvv87_11_t01	similar to UP|Q2PHF8_LACSA (Q2PHF8) Carotenoid cleavage dioxygenase 1, partial (34%)	-1,496
	Auxin.induced-regulated-responsive-activated	chr9_jgvv2_309_t01	similar to GB|AAM65282.1|21593333|AY087745 phytochrome-associated protein 1 (PAP1) {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (65%)	-1,024
	Cytokinin.signal transduction	chr4_jgvv8_12_t01	similar to UP|Q94KS0_CATRO (Q94KS0) Histidine-containing phosphotransfer protein, complete	1,992
	Gibberelin.induced-responsive-activated	chr14_jgvv6_35_t01	similar to GB|AAY28970.1|63054405|DQ006269 GIA/RGA-like gibberellin response modulator {Gossypium hirsutum} (exp=-1; wgp=0; cg=0), partial (55%)	-1,573
	Jasmonate metabolism lipoxygenase	chr14_jgvv128_74_t01	UP|Q6YCG7_VITVI (Q6YCG7) Lipoxygenase (Fragment), complete	-1,188
		chr6_jgvv4_644_t01	similar to UP|Q96573_LYCES (Q96573) Lipoxygenase , partial (54%)	1,842
		chr6_jgvv4_645_t01	similar to UP|Q96573_LYCES (Q96573) Lipoxygenase , partial (54%)	1,056

3.5.3 Changes in the gene expression pattern upon elicitation with phosphonate and phosphate and subsequent inoculation

Elicitation and subsequent inoculation had a different impact on the gene expression than elicitation alone (Table 3.5). This may be because the plant is under two stresses; abiotic (elicitation) and biotic (inoculation). Moreover, the effect of inoculation after elicitation is different from the effect of inoculation alone (Table 3.5). This indicates that treatments with elicitors have an impact on the infection process and disease development. Genes involved in stress pathways that were up-regulated after inoculation (60%) (Table 3.7) and found to be down-regulated after treatment with phosphonate and phosphate (75%) were up-regulated after elicitation and subsequent inoculation (66% and 62% respectively) (Table 3.27). Genes involved in secondary metabolism that were mostly repressed after inoculation (69%) (Table 3.7) and were found to be up-regulated after treatment with phosphonate and phosphate with ratios of 73% and 70% respectively (Table 3.15) were even more up-regulated after elicitation and subsequent inoculation (92% and 84% respectively) (Table 3.27). Elicitation with phosphonate and phosphate and subsequent inoculation led to up-regulation of cell wall related genes (85 % and 79% respectively).

Interestingly, some of the pathways that were repressed under elicitation with phosphonate and phosphate such as signalling (67% and 74% respectively) (Table 3.15) were up-regulated after elicitation and subsequent inoculation with ratios of 79% and 66%, respectively (Table 3.27). Although elicitation with phosphonate led to up-regulation of hormone related genes and elicitation with phosphate led to down-regulation of hormone related genes (Table 3.15), elicitation with phosphonate and phosphate and subsequent inoculation led to up-regulation of hormone related genes (83% and 80% respectively) (Table 3.27).

Table 3.27: Differential regulation of genes grouped to 'bins' using the MapMan software after elicitation and inoculation. Up-, down-regulated genes and total no. of genes are shown. Gray shades represent the pathways discussed in text. **(P1):** elicitation and inoculation

Bin	Bin Name	Phosphate (P1) – inoculated					Phosphonate (P1) – inoculated			
		Down	Up	Σ	No. of clones in the BIN	% of DEGs in the BIN	Down	Up	Σ	% of DEGs in the BIN
1	Photosynthesis	31 (82%)	7 (18%)	38	494	8%	25 (74%)	9 (26%)	34	7%
2	Major carbohydrate metabolism	8 (53%)	7 (47%)	15	165	9%	4 (27%)	11 (73%)	15	9%
3	Minor carbohydrate metabolism	4 (40%)	6 (60%)	10	162	6%	2 (18%)	9 (82%)	11	7%
4	Glycolysis	-	4 (100%)	4	123	3%	-	5 (100%)	5	4%
5	Fermentation	1 (20%)	4 (80%)	5	52	10%	1 (17%)	5 (83%)	6	12%
6	Gluconeogenesis	4 (80%)	1 (20%)	5	22	23%	1 (50%)	1 (50%)	2	9%
7	Oxidative pentose phosphate pathway	1 (25%)	3 (75%)	4	42	10%	-	3 (100%)	3	7%
8	TCA – organic transformation	1 (8%)	11 (92%)	12	123	10%	1 (9%)	10 (91%)	11	9%
9	Electron transport / ATP synthesis	9 (60%)	6 (40%)	15	156	10%	10 (77%)	3 (23%)	13	8%
10	Cell wall	13 (21%)	49 (79%)	62	595	10%	8 (15%)	45 (85%)	53	9%
11	Lipid metabolism	11 (31%)	25 (69%)	36	459	8%	8 (28%)	21 (72%)	29	6%
12	N-metabolism	4 (27%)	11 (73%)	15	59	25%	3 (75%)	1 (25%)	4	7%
13	Amino acid metabolism	6 (25%)	18 (75%)	24	459	5%	2 (8%)	23 (92%)	25	5%
14	S-assimilation	-	1 (100%)	1	15	7%	-	1 (100%)	1	7%
15	Metal handling	3 (37%)	5 (33%)	8	142	6%	1 (20%)	4 (80%)	5	4%
16	Secondary metabolism	13 (16%)	69 (84%)	82	543	15%	6 (8%)	72 (92%)	78	14%
17	Hormone metabolism	17 (20%)	69 (80%)	86	502	17%	10 (17%)	50 (83%)	60	12%
18	Vitamine metabolism	3 (100%)	-	3	45	7%	1 (100%)	-	1	2%
19	Tetrapyrrole synthesis	2 (67%)	1 (33%)	3	56	5%	-	1 (100%)	1	2%
20	Stress	75 (38%)	124 (62%)	199	948	21%	49 (34%)	94 (66%)	143	15%
21	Redox	-	14 (100%)	14	282	5%	1 (7%)	14 (93%)	15	5%
22	Polyamine metabolism	-	1 (100%)	1	18	6%	1 (50%)	1 (50%)	2	11%

Table 3.27: Continued…

Bin	Bin Name	Phosphate (P1) – inoculated					Phosphonate (P1) – inoculated			
		Down	Up	∑	No. of clones in the BIN	% of DEGs in the BIN	Down	Up	∑	% of DEGs in the BIN
23	Nucleotide metabolism	-	3 (100%)	3	147	2%	1 (25%)	3 (50%)	4	3%
24	Biodegradation of Xenobiotics	2 (100%)	-	2	24	8%	-	-	0	0%
25	C1-metabolism	1 (100%)	-	1	33	3%	-	-	0	0%
26	Miscellaneous	59 (28%)	150 (72%)	209	1219	17%	29 (15%)	166 (85%)	195	16%
27	RNA processing and regulation	106 (61%)	67 (39%)	173	2296	8%	66 (54%)	57 (46%)	123	5%
28	DNA synthesis and repair	11 (52%)	10 (48%)	21	422	5%	8 (50%)	8 (50%)	16	4%
29	Protein metabolism	85 (39%)	131 (61%)	216	3628	6%	50 (25%)	153 (75%)	203	6%
30	Signalling	56 (34%)	107 (66%)	163	1157	14%	28 (21%)	103 (79%)	131	11%
31	Cell cycle and organization	23 (30%)	53 (70%)	76	655	12%	17 (31%)	38 (69%)	55	8%
33	Development	-	-	0	405	0%	-	-	0	0%
34	Transport	8 (32%)	17 (68%)	25	951	3%	6 (30%)	14 (70%)	20	2%
35	Not assigned.no ontology	39 (35%)	73 (65%)	112	3276	3%	24 (23%)	80 (77%)	104	3%
∑	Total no. of genes in all bins	758 (64%)	429 (36%)	1187	19675	6%	519 (55%)	422 (45%)	941	5%

3.5.3.1 Similar changes in the gene expression pattern upon elicitation with phosphonate and phosphate and subsequent inoculation

Again it was noticed that phosphonate and phosphate had similar effect on the gene expression even under biotic stress condition (inoculation with *P. viticola*), where 2118 DEGs, which represent 74% of the DEGs after phosphonate treatment and subsequent inoculation and 62% of the DEGs after phosphate treatment and subsequent inoculation (Table 3.28), were common between both conditions (Figure 3.17). Therefore, in the following parts, the DEGs will be divided again into common DEGs (between phosphonate treatment and phosphate treatment) and specific DEGs (for each condition separately) to eliminate the redundancy in the results and to make the discussion easier.

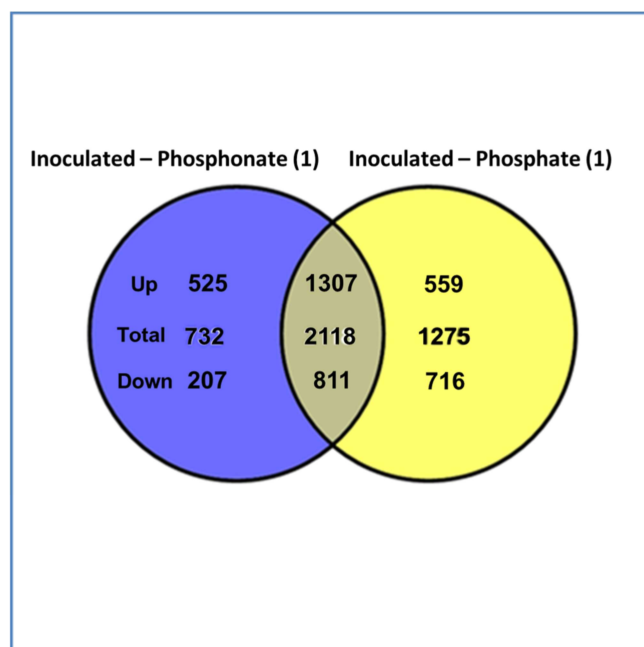

Figure 3.17: Common and specific DEGs after elicitation with phosphonate and phosphate and subsequent inoculation; **Control:** DEGs in control condition (no elicitation, no inoculation); **Phosphonate (1):** DEGs after elicitation with phosphonate and subsequent inoculation; **Phosphate (1):** DEGs after elicitation with phosphate and subsequent inoculation

Table 3.28: Common and specific DEGs after elicitation with phosphonate and phosphate

	Total DEGs	Common DEGs		Specific DEGs
Phosphonate Pac (1)	2848	2118	74%	732 (26%)
Phosphate Pate (1)	3390		62%	1275 (38%)

Common transcriptional responses between plants treated with phosphonate and plants treated with phosphate and subsequent inoculation

Differentially expressed genes in plants treated with phosphonate and plants treated with phosphate and subsequently inoculated followed similar trends but with slightly different fold changes. Common DEGs involved in stress pathways, that were down-regulated after elicitation with phosphate and phosphonate (Table 2.17), were up-regulated after elicitation and subsequent inoculation (Table 3.29). Among these up-regulated DEGs were genes that code for PR-proteins such as class IV endochitinase (PR-3), PR-10 and PR-1. Most of the genes that code for heat shock proteins were also up-regulated (Table 3.29). However, most of the genes coding for biotic stress receptors such as R-proteins, which are involved in effector triggered immunity, were down-regulated (Table 3.29).

Most of the DEGs involved in secondary metabolites pathways were up-regulated. For example, genes coding for enzymes involved in synthesis of isoprenoids such as laccase and in the synthesis of terpenoids such as terpene synthase were up-regulated (Table 3.30). Moreover, genes involved in synthesis of anthocyanins such as flavonol 3-O-glucosyltransferase, synthesis of dihydroflavonols such as cinnamoyl-CoA reductase and synthesis of isoflavonols such as isoflavone reductase were also up-regulated (Table 3.30).

Most of the DEGs involved in signalling pathways were up-regulated. Most notable were genes coding for receptor kinases (e.g. S-locus glycoprotein like) (Table 3.31). However, genes coding for the leucine rich repeat class VIII.2 and class XII receptor kinases, which are involved in PAMP-triggered immunity, were mostly down-regulated (Table 3.31). DEGs involved in hormone metabolism were mostly up-regulated. For example, genes coding for enzymes involved in ABA metabolism such as ABA-responsive protein and enzymes involved in synthesis of brassinosteroids such as squalene monooxygenase were up-regulated (Table 3.32). Genes involved in auxin metabolism such as oxidoreductase, cytokinin metabolism such as cytokinin dehydrogenase and gibberellin metabolism such as gibberellin responsive proteins were also up-regulated (Table 3.32). Most of the genes involved in jasmonate- and salicylic acid-signalling such as lipoxygenase and salicylic acid carboxyl methyltransferase were also up-regulated (Table 3.32).

Most of the DEGs involved in photosynthesis were down-regulated. These were genes coding for Photosystem II such as PSII proteins, cytochrome b and photosystem I P700. Genes coding for enzymes involved in light reaction such as NADH dehydrogenase and NAD(P)H-quinone oxidoreductase were also down-regulated (Table 3.33). Moreover, RuBisCO and genes involved in chlororespiration such as ribosomal protein S7 were down-regulated (Table 3.33).
Most of the DEGs involved in carbohydrate metabolism were up-regulated. These were genes coding for enzymes involved in starch synthesis such as granule-bound starch synthase and synthesis of trehaloses such as trehalose-phosphate phosphatase (Table 3.34). However, a gene involved in synthesis of callose such as 1,3-beta-glucan synthase and genes involved in starch degradation such as vacuolar invertase 2 and sucrose synthase were down-regulated (Table 3.34).
Most of the DEGs involved in cell wall synthesis and modification were up-regulated. These genes were involved in cellulose synthesis such as cellulose synthase, while others were involved in cell wall modification and coded for cell wall modifying enzymes such as expansin and syringolide-induced protein (Table 3.34). However, most of the genes involved in cell wall degradation such as polygalacturonase and pectate lyase were also up-regulated (Table 3.34).
DEGs coding for transcription factors such as C2H2 zinc finger family and MADS box family were up-regulated, while genes coding for MYB transcription family were down-regulated (Table 3.35). Genes involved in lipid metabolism were up-regulated. Genes involved in fatty acid synthesis and elongation such as pyruvate kinase isozymes were up-regulated, while genes coding for enzymes involved in lipid degradation such as lipase were down-regulated (Table 3.35).
Nitrilases, enzymes that have a significant impact on the outcome of plant–microbe interactions by catalysing the hydrolysis of toxic nitrile compounds, that were found down-regulated after elicitation (Table 3.24) were up-regulated after elicitation and subsequent inoculation (Table 3.36). DEGs coding for glutathione-S-transferases, enzyme that are involved in detoxification of endogenous compounds and toxins as well as breakdown of xenobiotics were also up-regulated (Table 3.36).

Table 3.29: Common DEGs (selected sub-bins from stress pathways) between plants treated with phosphonate and plants treated with phosphate and subsequently inoculated

Pathway	Bin name	Gene ID	Annotation	Fold change	
				Phosphonate	Phosphate
Stress pathways	Biotic stress receptors	chr14_jgvv36_113_t01	GB|AF369831.1|AAM21288.1 resistance gene analog [Vitis vinifera]	1,043	1,185
		chrun_jgvv144_11_t01	GB|AF365880.1|AAQ15192.1 resistance protein [Vitis vinifera]	1,072	1,06
		chrun_jgvv144_16_t01	GB|AF365880.1|AAQ15192.1 resistance protein [Vitis vinifera]	1,633	1,272
		chr18_jgvv72_6_t01	GB|AF365879.1|AAQ15191.1 resistance protein [Vitis vinifera]	-1,27	-1,034
		chr13_jgvv139_3_t01	GB|AF369833.1|AAM21290.1 resistance gene analog [Vitis vinifera]	-3,023	-3,436
		chr19_jgvv27_46_t01	GB|AF365880.1|AAQ15192.1 resistance protein [Vitis vinifera]	-2,003	-3,495
		chr19_jgvv27_54_t01	GB|AF365880.1|AAQ15192.1 resistance protein [Vitis vinifera]	-2,002	-1,708
		chr12_jgvv35_155_t01	GB|AF365881.1|AAQ15193.1 resistance protein [Vitis vinifera]	-1,638	-1,92
	PR-proteins	chr3_jgvv88_52_t01	pathogenesis-related protein [Vitis vinifera]	-1,157	-1,841
		chr3_jgvv91_105_t01	similar to UP|Q9XIY9_TOBAC (Q9XIY9) NtPRp27, partial (83%)	1,588	2,108
		chr3_jgvv88_50_t01	GB|AJ536326.1|CAD60273.1 putative pathogenesis related protein 1 precursor [Vitis vinifera]	1,902	2,574
		chr5_jgvv94_29_t01	UP|O24530_VITVI (O24530) Class IV endochitinase , complete	2,3	2,18
		chr5_jgvv77_137_t01	UP|Q9FS42_VITVI (Q9FS42) Pathogenesis-related protein 10, complete	1,727	2,096
		chr5_jgvv94_25_t01	similar to UP|Q7XAU6_VITVI (Q7XAU6) Class IV chitinase, partial (98%)	2,923	2,44
	Heat shock protein	chr7_jgvv95_48_t01	homologue to UP|Q6UJX6_NICBE (Q6UJX6) Molecular chaperone Hsp90-1, partial (59%)	2,181	2,209
		chr5_jgvv29_87_t01	similar to UP|HSF8_LYCES (Q40152) Heat shock factor protein HSF8 (Heat shock transcription factor 8) (HSTF 8) (Heat stress transcription factor), partial (24%)	-1,258	-1,405
		chr7_pdvv5_557_t01	homologue to UP|Q5QHT3_9FABA (Q5QHT3) 70 kDa heat shock protein 2, partial (86%)	2,012	2,141
		chr14_pdvv83_107_t01	similar to UP|HSP81_ORYSA (P33126) Heat shock protein 81-1 (HSP81-1), partial (12%)	2,498	2,703
		chr2_jgvv12_67_t01	weakly similar to RF|NP_181097.1|15227436|NM_129106 heat shock protein binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (10%)	-2,073	-2,426
		chr13_jgvv19_375_t01	homologue to UP|Q9M4E6_CUCSA (Q9M4E6) Heat shock protein 70, complete	1,81	1,567
		chr3_jgvv180_2_t01	UP|Q3L1D0_VITVI (Q3L1D0) Heat shock protein 101, partial (48%)	1,099	1,059
		chr13_jgvv19_207_t01	similar to UP|Q9SWE4_TOBAC (Q9SWE4) Low molecular weight heat-shock protein, partial (94%)	2,093	1,535
		chr7_jgvv95_13_t01	similar to UP|HSP81_ORYSA (P33126) Heat shock protein 81-1, partial (12%)	-2,872	-2,271
		chr7_jgvv5_582_t01	similar to UP|HSP7M_SOLTU (Q08276) Heat shock 70 kDa protein, partial (25%)	1,986	1,291
		chrun_jgvv198_8_t01	similar to UP|HSP7M_PEA (P37900) Heat shock 70 kDa protein, partial (48%)	1,13	1,153
		chrun_jgvv131_20_t01	homologue to UP|Q6UJX6_NICBE (Q6UJX6) Molecular chaperone Hsp90-1, partial (59%)	1,388	2,218
		chrun_jgvv279_1_t01	similar to UP|HSP81_ORYSA (P33126) Heat shock protein 81-1 (HSP81-1), partial (12%)	-3,89	-3,32
	Germin-like proteins	chr14_jgvv60_240_t01	similar to GB|AAB51752.1|1934730|ATU95036 germin-like protein {Arabidopsis thaliana} partial (96%)	2,994	2,724

Table 3.30: Common DEGs (selected sub-bins from secondary metabolism) between plants treated with phosphonate and plants treated with phosphate and subsequently inoculated

Pathway	Bin name	Gene ID	Annotation	Fold change	
				Phosphonate	Phosphate
Secondary metabolites	Isoprenoids	chr18_random_pdvv152_2_t01	weakly similar to UP|Q6TDS6_GOSAR (Q6TDS6) Secretory laccase , partial (25%)	2,202	1,59
		chr18_jgvv117_1_t01	similar to UP|Q38757_ACEPS (Q38757) Laccase , partial (34%)	2,177	2,205
		chr18_jgvv75_63_t01	weakly similar to RF|NP_199621.2|30695378|NM_124184 copper ion binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (42%)	2,225	2,004
		chr16_jgvv100_111_t01	similar to UP|Q6QLW8_HEVBR (Q6QLW8) HMG-CoA synthase 2, complete	2,385	2,058
		chrun_jgvv768_3_t01	similar to GB|CAA57947.1|572635|CBIPISOM isopentenyl pyrophosphate isomerase {Clarkia breweri} (exp=-1; wgp=0; cg=0), partial (84%)	1,581	1,784
		chr11_jgvv206_2_t01	similar to GB|CAA57947.1|572635|CBIPISOM isopentenyl pyrophosphate isomerase {Clarkia breweri} (exp=-1; wgp=0; cg=0), partial (84%)	1,323	1,601
	Isoprenoids terpenoids	chr9_jgvv54_60_t01	similar to UP|Q8W3Z4_9ROSI (Q8W3Z4) Cycloartenol synthase , partial (52%)	2,239	1,366
		chr4_jgvv8_281_t01	similar to GP|3688598|dbj|BAA33460.1 Cycloartenol Synthase {Panax ginseng}, partial (36%)	-1,593	-1,946
		chr19_jgvv14_425_t01	homologue to UP|Q6Q3H3_VITVI (Q6Q3H3) Terpene synthase, complete	2,179	1,37
	Flavonoids anthocyanins	chr3_pdvv17_160_t01	similar to SP|Q40285|UFO2_MANES Flavonol 3-O-glucosyltransferase 2 (EC 2.4.1.91) (UDP-glucose flavonoid 3-O-glucosyltransferase 2), partial (41%)	1,429	1,388
	Flavonoids dihydroflavonols	chr18_jgvv122_69_t01	similar to RF|NP_180917.1|15226134|NM_128919 cinnamoyl-CoA reductase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (15%)	1,438	1,744
		chr18_jgvv122_70_t01	similar to RF|NP_180917.1|15226134|NM_128919 cinnamoyl-CoA reductase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (36%)	1,348	1,209
		chr4_jgvv23_171_t01	UP|P93799_VITVI (P93799) Dihydroflavonol 4-reductase , complete	1,606	1,65
		chr15_jgvv48_191_t01	weakly similar to UP|Q9SDZ2_SOYBN (Q9SDZ2) 2\'-hydroxy isoflavone/dihydroflavonol reductase homolog (Fragment), partial (82%)	1,984	1,402
		chr8_jgvv7_125_t01	UP|Q3C210_VITVI (Q3C210) Flavonoid 3\',5\'-hydroxylase, complete	-1,925	-1,669
	Flavonoids Flavonols	chr3_jgvv17_40_t01	weakly similar to RF|XP_507337.1|51965106|XM_507337 P0562A06.31 gene product {Oryza sativa (japonica cultivar-group)} (exp=-1; wgp=0; cg=0), partial (28%)	1,251	1,214
		chr2_jgvv12_220_t01	similar to UP|Q39224_ARATH (Q39224) SRG1 protein (F6I1.30/F6I1.30) (At1g17020/F6I1.30), partial (26%)	2,246	1,516
		chr10_jgvv3_220_t01	similar to UP|Q9SB32_ARATH (Q9SB32) SRG1-like protein (At4g25310), partial (32%)	1,461	1,217
	Flavonoids isoflavonols	chr17_jgvv53_37_t01	UP|Q3KN72_VITVI (Q3KN72) Isoflavone reductase-like protein 1, complete	1,066	1,175
		chr3_pdvv38_10_t01	UP|Q3KN67_VITVI (Q3KN67) Isoflavone reductase-like protein 6, partial (81%)	3,283	2,927
		chr2_jgvv33_22_t01	UP|Q3KN72_VITVI (Q3KN72) Isoflavone reductase-like protein 1, complete	1,408	1,299

Table 3.31: Common DEGs (selected sub-bins from signalling pathways) between plants treated with phosphonate and plants treated with phosphate and subsequently inoculated

Pathway	Bin name	Gene ID	Annotation	Fold change	
				Phosphonate	Phosphate
Signalling pathways	Receptor kinases LRR.VIII.2	chr10_jgvv3_168_t01	weakly similar to UP|O22579_ARATH (O22579) Receptor-like serine/threonine kinase, partial (19%)	-2,392	-1,279
		chr19_jgvv140_3_t01	weakly similar to UP|Q6EQG8_ORYSA (Q6EQG8) Leucine-rich repeat family protein /protein kinase family protein-like, partial (16%)	-1,171	-1,345
		chr10_jgvv3_181_t01	weakly similar to UP|O22579_ARATH (O22579) Receptor-like serine/threonine kinase, partial (19%)	-1,944	-2,077
		chrun_jgvv125_18_t01	weakly similar to UP|O22579_ARATH (O22579) Receptor-like serine/threonine kinase, partial (19%)	-1,143	-1,061
	Receptor kinases LRR.XII	chrun_jgvv125_11_t01	weakly similar to UP|Q9ZTK0_LYCES (Q9ZTK0) Hcr2-0A, partial (22%)	-1,387	-1,163
		chr12_jgvv34_1_t01	weakly similar to UP|Q9ZTK0_LYCES (Q9ZTK0) Hcr2-0A, partial (22%)	2,72	3,015
	Receptor kinases S-locus glycoprotein like	chrun_jgvv425_1_t01	weakly similar to UP|Q84K89_AVESA (Q84K89) Receptor kinase LRK10 (Receptor kinase LRK14), partial (18%)	2,107	1,786
		chr16_random_jgvv307_1_t01	weakly similar to UP|Q84K89_AVESA (Q84K89) Receptor kinase LRK10 (Receptor kinase LRK14), partial (18%)	1,727	1,657
		chr19_jgvv14_391_t01	similar to UP|Q9ZT07_ARATH (Q9ZT07) Receptor-like protein kinase, partial (19%)	1,607	1,777
		chr19_jgvv14_392_t01	weakly similar to UP|Q40096_IPOTF (Q40096) Receptor protein kinase, partial (9%)	2,548	2,508
		chrun_jgvv409_6_t01	similar to UP|Q9ZT07_ARATH (Q9ZT07) Receptor-like protein kinase, partial (19%)	1,385	1,184
		chrun_jgvv437_3_t01	similar to UP|O81906_ARATH (O81906) Serine/threonine kinase-like protein, partial (20%)	1,928	1,464
		chr19_jgvv14_397_t01	similar to UP|Q9ZT07_ARATH (Q9ZT07) Receptor-like protein kinase, partial (19%)	3,124	3,097
		chr12_jgvv134_26_t01	similar to UP|O81906_ARATH (O81906) Serine/threonine kinase-like protein, partial (20%)	1,502	1,541
	Receptor kinases miscelleanous	chr16_pdvv13_108_t01	similar to UP|Q53JL7_ORYSA (Q53JL7) Receptor-like protein kinase, partial (38%)	-1,497	-1,638
		chr12_jgvv34_2_t01	similar to UP|Q75WU3_POPNI (Q75WU3) Leucine-rich repeat receptor-like protein kinase 1, partial (28%)	2,193	2,193
		chr10_jgvv42_55_t01	similar to UP|Q75WU3_POPNI (Q75WU3) Leucine-rich repeat receptor-like protein kinase 1, partial (28%)	1,811	1,455
		chr12_random_jgvv99_27_t01	similar to UP|Q75WU3_POPNI (Q75WU3) Leucine-rich repeat receptor-like protein kinase 1, partial (28%)	1,503	1,564
		chr7_jgvv129_89_t01	similar to UP|Q8S519_CUCME (Q8S519) PTH-2 (Fragment), partial (88%)	-1,371	-1,327
		chr16_jgvv22_74_t01	similar to UP|Q9M574_ORYSA (Q9M574) Receptor-like protein kinase, partial (25%)	2,147	1,557
		chrun_jgvv258_6_t01	similar to UP|Q9M574_ORYSA (Q9M574) Receptor-like protein kinase, partial (25%)	2,014	1,898
		chr16_pdvv13_101_t01	weakly similar to UP|Q9FF29_ARATH (Q9FF29) Receptor serine/threonine kinase, partial (35%)	1,981	1,59
		chr16_jgvv13_86_t01	weakly similar to UP|Q9FF29_ARATH (Q9FF29) Receptor serine/threonine kinase, partial (35%)	1,848	1,551
		chr16_jgvv148_21_t01	weakly similar to UP|Q9FF29_ARATH (Q9FF29) Receptor serine/threonine kinase, partial (35%)	2,19	2,436
		chr6_jgvv80_20_t01	weakly similar to UP|Q9FF29_ARATH (Q9FF29) Receptor serine/threonine kinase, partial (35%)	2,077	1,955

Table 3.32: Common DEGs (selected sub-bins from hormone metabolism) between plants treated with phosphonate and plants treated with phosphate and subsequently inoculated

Pathway	Bin name	Gene ID	Annotation	Fold change	
				Phosphonate	Phosphate
Hormone metabolism	Abscisic acid Metabolism	chr13_jgvv64_95_t01	UP|Q3T4H1_VITVI (Q3T4H1) 9,10[9\',10\']carotenoid cleavage dioxygenase, complete	1,153	1,142
		chr3_jgvv132_8_t01	weakly similar to UP|Q2R1K3_ORYSA (Q2R1K3) AtHVA22a, partial (24%)	-2,654	-2,644
		chr4_jgvv23_328_t01	similar to UP|Q6H5X2_ORYSA (Q6H5X2) ABA-responsive protein-like, partial (58%)	1,401	1,647
	Brassinosteroid metabolism	chr1_jgvv11_272_t01	similar to UP|Q4ACU1_ZINEL (Q4ACU1) Delta7 sterol C-5 desaturase, partial (94%)	2,213	2,173
		chr7_jgvv5_520_t01	similar to UP|Q9ATR0_PEA (Q9ATR0) Brassinosteroid biosynthetic protein LKB, partial (34%)	2,683	2,54
		chr3_jgvv88_14_t01	similar to UP|Q506K3_DATIN (Q506K3) Squalene monooxygenase, partial (89%)	2,205	1,231
		chrun_jgvv525_2_t01	similar to UP|Q506K3_DATIN (Q506K3) Squalene monooxygenase, partial (89%)	1,969	1,73
		chrun_jgvv441_2_t01	similar to UP|Q506K3_DATIN (Q506K3) Squalene monooxygenase, partial (89%)	1,757	1,721
	Auxin metabolism	chr14_jgvv30_26_t01	similar to UP|Q338B1_ORYSA (Q338B1) Oxidoreductase, aldo/keto reductase family, partial (90%)	1.627	1.493
		chr14_jgvv30_33_t01	similar to UP|Q338B1_ORYSA (Q338B1) Oxidoreductase, aldo/keto reductase family, partial (90%)	1.243	1.124
		chr14_jgvv30_30_t01	similar to UP|Q338B1_ORYSA (Q338B1) Oxidoreductase, aldo/keto reductase family, partial (90%)	1.559	1.394
		chr10_jgvv116_12_t01	similar to UP|Q2LAJ3_LYCES (Q2LAJ3) Auxin response factor 2, partial (13%)	2.199	2.19
	Cytokinin metabolism	chrun_jgvv2191_1_t01	similar to GB|AAG30909.1|11120516|AF303982 cytokinin oxidase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (46%)	1,672	1,471
		chrun_jgvv2520_1_t01	similar to UP|CKX1_ARATH (O22213) Cytokinin dehydrogenase 1 precursor (Cytokinin oxidase 1) (CKO 1) (AtCKX1) , partial (23%)	1,31	1,234
		chr7_jgvv5_533_t01	similar to UP|CKX1_ARATH (O22213) Cytokinin dehydrogenase 1 precursor (Cytokinin oxidase 1) (CKO 1) (AtCKX1) , partial (23%)	1,739	1,178
		chr3_jgvv63_116_t01	weakly similar to UP|Q86B83_DROME (Q86B83) CG33099-PA, partial (7%)	1,518	1,318
		chr3_jgvv63_115_t01	weakly similar to UP|Q86B83_DROME (Q86B83) CG33099-PA, partial (7%)	1,599	1,232
	Gibberelin signal transduction	chr14_jgvv36_50_t01	similar to UP|Q3EB84_ARATH (Q3EB84) Protein At3g11540, partial (44%)	1,317	1,279
		chr14_jgvv36_48_t01	similar to UP|Q3EB84_ARATH (Q3EB84) Protein At3g11540, partial (44%)	2,69	2,313
		chr9_jgvv2_100_t01	similar to GB|AAY28970.1|63054405|DQ006269 GIA/RGA-like gibberellin response modulator {Gossypium hirsutum} (exp=-1; wgp=0; cg=0), partial (55%)	-1,716	-2,03

Table 3.32: continued…

Pathway	Bin name	Gene ID	Annotation	Fold change	
				Phosphonate	Phosphate
Hormone metabolism	Jasmonate metabolism	chr6_jgvv4_642_t01	similar to UP|Q96573_LYCES (Q96573) Lipoxygenase , partial (54%)	1,28	1,054
		chr6_jgvv4_644_t01	similar to UP|Q96573_LYCES (Q96573) Lipoxygenase , partial (54%)	2,213	2,237
		chr6_jgvv4_645_t01	similar to UP|Q96573_LYCES (Q96573) Lipoxygenase , partial (54%)	1,071	1,411
		chr18_jgvv41_35_t01	similar to UP|Q76DL0_LITER (Q76DL0) LEDI-5c protein, partial (96%)	2,597	2,713
		chr18_jgvv41_31_t01	similar to UP|Q76DL0_LITER (Q76DL0) LEDI-5c protein, partial (96%)	1,191	1,13
	Salicylic acid metabolism	chr12_jgvv57_95_t01	weakly similar to UP|Q53L40_ORYSA (Q53L40) SAM dependent carboxyl methyltransferase, partial (29%)	-1,253	-1,946
		chr4_jgvv23_152_t01	similar to UP|Q9SPV4_CLABR (Q9SPV4) S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase, partial (76%)	1,59	2,186
		chr1_jgvv11_73_t01	weakly similar to UP|Q9XI57_ARATH (Q9XI57) F9L1.6, partial (29%)	1,251	1,879
		chr1_jgvv11_75_t01	weakly similar to UP|Q9XI57_ARATH (Q9XI57) F9L1.6, partial (29%)	1,759	1,872
		chr1_jgvv11_76_t01	weakly similar to UP|Q9XI57_ARATH (Q9XI57) F9L1.6, partial (29%)	3,718	3,795

Table 3.33: Common DEGs (selected sub-bins from photosynthesis pathway) between plants treated with phosphonate and plants treated with phosphate and subsequently inoculated

Pathway	Bin name	Gene ID	Annotation	Fold change	
				Phosphonate	Phosphate
Photosynthesis	Lightreaction photosystem II	chr11_jgvv37_105_t01	homologue to emb|X70938.1|CHNPTRNVI N.plumbaginifolia chloroplast 16SrDNA, trnV and trnI genes for 16S ribosomal RNA, partial (94%)	1,681	1,711
		chr13_pdvv101_15_t01	homologue to PRF|1011228A|224159|1011228A cytochrome b559. {Spinacia oleracea}, complete	-3,503	-3,262
		chrun_jgvv2608_1_t01	homologue to PRF|1011228A|224159|1011228A cytochrome b559. {Spinacia oleracea}, complete	-1,215	-1,48
		chrun_jgvv246_13_t01	homologue to UP|NU4C_PANGI (Q68RV6) NAD(P)H-quinone oxidoreductase chain 4, chloroplast (NAD(P)H dehydrogenase, chain 4) (NADH-plastoquinone oxidoreductase chain 4) , complete	-1,2	-1,392
		chrun_jgvv246_14_t01	homologue to UP|NU4C_PANGI (Q68RV6) NAD(P)H-quinone oxidoreductase chain 4, chloroplast (NAD(P)H dehydrogenase, chain 4) (NADH-plastoquinone oxidoreductase chain 4) , complete	-1,279	-1,573
		chrun_jgvv396_1_t01	homologue to UP|Q3V538_ACOCL (Q3V538) PSII 43 kDa protein, complete	-2,896	-2,994
		chrun_jgvv275_1_t01	homologue to UP|Q3V538_ACOCL (Q3V538) PSII 43 kDa protein, complete	-1,213	-1,816
		chr11_jgvv103_59_t01	homologue to UP|Q3V538_ACOCL (Q3V538) PSII 43 kDa protein, complete	-1,599	-2,23
		chrun_jgvv629_1_t01	homologue to UP|Q8M8A9_BETVU (Q8M8A9) NADH dehydrogenase subunit 4, partial (34%)	-3,632	-4,171
	Lightreaction NADH DH	chr14_pdvv6_38_t01	homologue to UP|Q8M8A9_BETVU (Q8M8A9) NADH dehydrogenase subunit 4, partial (34%)	-1,443	-1,829
		chr13_random_jgvv112_7_t01	similar to UP|Q9M0G6_ARATH (Q9M0G6) Photosystem II protein W-like, partial (52%)	1,296	1,369
		chrun_jgvv505_6_t01	UP|Q2L933_GOSHI (Q2L933) Cytochrome b6, partial (50%)	-1,763	-2,431
		chr7_jgvv151_46_t01	UP|Q2MII8_SOLBU (Q2MII8) Photosystem I P700 apoprotein A2, complete	-2,331	-1,902
	Lightreaction chlororespiration	chr9_jgvv70_75_t01	ribosomal protein S7 [Vitis vinifera]	-1,625	-1,705
		chr9_jgvv70_74_t01	ribosomal protein S7 [Vitis vinifera]	-2,032	-1,788
		chrun_jgvv198_4_t01	ribosomal protein S7 [Vitis vinifera]	-1,028	-1,39
		chr14_jgvv108_152_t01	ribosomal protein S7 [Vitis vinifera]	-1,821	-2,032
		chrun_jgvv173_14_t01	ribosomal protein S7 [Vitis vinifera]	-1,714	-1,517
		chr5_jgvv62_111_t01	ribosomal protein S7 [Vitis vinifera]	2,527	1,855
		chr2_pdvv12_11_t01	ribosomal protein S7 [Vitis vinifera]	-3,354	-3,102
	Calvin cycle rubisco large subunit	chr12_pdvv55_46_t01	UP|Q9MVF6_9ROSI (Q9MVF6) Ribulose-1,5-bisphosphate carboxylase/oxygenase, large subunit, complete	1,9	1,035
		chr14_pdvv68_174_t01	UP|Q9MVF6_9ROSI (Q9MVF6) Ribulose-1,5-bisphosphate carboxylase/oxygenase, large subunit, complete	-2,322	-2,373
		chr7_jgvv129_76_t01	UP|Q9MVF6_9ROSI (Q9MVF6) Ribulose-1,5-bisphosphate carboxylase/oxygenase, large subunit, complete	-1,553	-1,769

Table 3.34: Common DEGs (selected sub-bins from carbohydrate and cell wall metabolism) between plants treated with phosphonate and plants treated with phosphate and subsequently inoculated

Pathway	Bin name	Gene ID	Annotation	Fold change	
				Phosphonate	Phosphate
Carbohydrate metabolism	Starch synthesis	chr16_jgvv22_151_t01	similar to UP|Q9FR03_PERFR (Q9FR03) Granule-bound starch synthase, partial (41%)	1,533	1,092
		chr10_random_jgvv168_13_t01	similar to UP|O49447_ARATH (O49447) ADP, ATP carrier-like protein, partial (94%)	1,257	1,393
		chr16_random_jgvv300_3_t01	similar to UP|O49447_ARATH (O49447) ADP, ATP carrier-like protein, partial (30%)	2,314	1,458
	Carbohydrate degradation sucrose	chrun_jgvv233_1_t01	homologue to UP|Q9S943_VITVI (Q9S943) Vacuolar invertase 2, GIN2, partial (46%)	-2,914	-2,845
		chr12_jgvv57_12_t01	similar to UP|Q9SLS2_CITUN (Q9SLS2) Sucrose synthase, partial (85%)	1,198	1,4
		chrun_jgvv1562_1_t01	similar to PIR|S19125|YUMU sucrose synthase (EC 2.4.1.13), partial (18%)	3,206	2,41
	Trehalose	chr11_jgvv37_65_t01	similar to UP|Q3ZTF5_TOBAC (Q3ZTF5) Trehalose-phosphate phosphatase, partial (54%)	1,791	2,545
		chr14_jgvv36_22_t01	similar to UP|Q9LMI0_ARATH (Q9LMI0) T2D23.11 protein, partial (33%)	1,784	1,59
	Callose	chr17_jgvv0_40_t01	similar to RF|NP_187372.1|15231404|NM_111596 ATGSL10 (glucan synthase-like 10); 1,3-beta-glucan synthase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (4%)	-2,7	-2,956
Cell wall	Cellulose synthesis	chrun_pdvv2628_1_t01	weakly similar to GP|16519227|gb|AAL25130.1 cellulose synthase-like protein }, partial (14%)	-1,813	-2,178
		chr2_jgvv25_180_t01	homologue to UP|P93156_GOSHI (P93156) Cellulose synthase (Fragment), partial (82%)	4,403	3,7
		chr14_jgvv6_202_t01	homologue to UP|Q6XP46_SOLTU (Q6XP46) Cellulose synthase, partial (39%)	1,609	1,436
		chr2_jgvv25_174_t01	similar to UP|Q3Y6V1_TOBAC (Q3Y6V1) Cellulose synthase-like protein CslG, partial (33%)	2,607	1,896
		chr12_jgvv59_91_t01	homologue to UP|P93156_GOSHI (P93156) Cellulose synthase (Fragment), partial (82%)	2,312	2,024
		chr5_jgvv49_68_t01	homologue to UP|Q6QGY1_VITVI (Q6QGY1) Merlot proline-rich protein 2, partial (68%)	1,223	2,118
		chr5_jgvv20_175_t01	similar to UP|O18465_HIRME (O18465) Tractin, partial (6%)	1,208	1,559
		chr3_jgvv63_126_t01	similar to GP|9279698|dbj|BAB01255.1 extensin protein-like, partial (49%)	-1,491	-1,387
		chr14_jgvv83_96_t01	homologue to UP|Q8VWN8_GOSHI (Q8VWN8) Reversibly glycosylated polypeptide, partial (95%)	1,134	1,376
	Cell wall degradation pectate lyases and polygalacturonases	chr12_jgvv57_31_t01	weakly similar to UP|Q9MBB8_9ROSI (Q9MBB8) Polygalacturonase, partial (50%)	1,978	1,94
		chr1_jgvv10_247_t01	homologue to UP|Q94FT5_FRAAN (Q94FT5) Pectate lyase (Fragment), complete	1,635	1,918
		chr12_jgvv57_34_t01	weakly similar to UP|Q9MBB8_9ROSI (Q9MBB8) Polygalacturonase, partial (50%)	-1,24	-1,853
		chrun_pdvv2630_1_t01	similar to UP|Q9SMT3_ARATH (Q9SMT3) Endo-polygalacturonase-like protein (Glycoside hydrolase family 28 protein), partial (50%)	-1,171	-2,04
		chr12_jgvv28_256_t01	weakly similar to UP|Q9MBB8_9ROSI (Q9MBB8) Polygalacturonase, partial (50%)	1,524	1,407
	Cell wall modification	chr11_jgvv52_64_t01	similar to UP|Q8S902_SOYBN (Q8S902) Syringolide-induced protein 19-1-5, partial (93%)	2,157	1,723
		chr5_jgvv77_13_t01	UP|Q84UT0_9ROSI (Q84UT0) Expansin, complete	2,056	2,055
		chr11_jgvv52_55_t01	similar to UP|Q8S902_SOYBN (Q8S902) Syringolide-induced protein 19-1-5, partial (93%)	1,881	1,764
		chr11_jgvv52_62_t01	similar to UP|Q8S902_SOYBN (Q8S902) Syringolide-induced protein 19-1-5, partial (91%)	2,111	1,272

Table 3.35: Common DEGs (selected sub-bins from RNA and lipid metabolism) between plants treated with phosphonate and plants treated with phosphate and subsequently inoculated

Pathway	Bin name	Gene ID	Annotation	Fold change	
				Phosphonate	Phosphate
RNA metabolism	C2H2 zinc finger family	chr10_jgvv92_46_t01	homologue to UP|ZFP4_ARATH (Q39263) Zinc finger protein 4, partial (27%)	1,447	1,573
		chr14_jgvv36_91_t01	weakly similar to UP|Q2HW14_MEDTR (Q2HW14) Zinc finger, CCCH-type; Sugar transporter superfamily, partial (16%)	1,067	1,104
		chr13_jgvv64_74_t01	homologue to UP|Q9SVY1_ARATH (Q9SVY1) Zinc finger-like protein (WIP2 protein) (At3g57670), partial (50%)	1,216	1,432
	MADS box transcription factor family	chr15_jgvv46_264_t01	weakly similar to UP|Q9ATE2_PETHY (Q9ATE2) MADS-box transcription factor FBP29, partial (42%)	1,46	1,347
		chr15_jgvv24_1_t01	similar to UP|Q6V0J8_BRACM (Q6V0J8) Short vegetative phase protein, partial (69%)	-1,901	-2,506
		chr15_jgvv46_258_t01	weakly similar to UP|Q9ATE2_PETHY (Q9ATE2) MADS-box transcription factor FBP29, partial (42%)	1,776	2,051
	MYB transcription factor family	chr19_jgvv15_120_t01	homologue to UP|Q9XHV0_ARATH (Q9XHV0) Atmyb103 (MYB transcription factor), partial (53%)	-1,372	-1,742
		chr14_jgvv36_82_t01	homologue to UP|Q8W149_MAIZE (Q8W149) CDC5 protein, partial (21%)	-4,085	-4,22
		chr4_jgvv8_174_t01	MYB-like protein [Vitis vinifera]	1,124	1,576
Lipid metabolism	Fatty acid synthesis and elongation	chr12_pdvv34_186_t01	homologue to UP|Q4JIJ4_9ROSI (Q4JIJ4) Stearoyl-ACP desaturase , complete	2.449	2.028
		chr5_jgvv94_57_t01	similar to GB|AAA74692.1|951427|RCCSACPD stearoyl-acyl-carrier protein desaturase {Ricinus communis} (exp=-1; wgp=0; cg=0), partial (41%)	1.999	1.435
		chr5_jgvv94_52_t01	homologue to UP|Q4JIJ4_9ROSI (Q4JIJ4) Stearoyl-ACP desaturase , complete	-1.202	-1.25
		chr7_jgvv141_4_t01	similar to UP|Q8VWP9_GOSHI (Q8VWP9) Fiddlehead-like protein, partial (66%)	-1.368	-1.199
		chr19_jgvv93_31_t01	similar to UP|Q9LN49_ARATH (Q9LN49) F18O14.21, partial (67%)	2.186	2.093
		chr7_jgvv5_42_t01	similar to UP|KPYG_TOBAC (Q40546) Pyruvate kinase isozyme G, chloroplast precursor , partial (84%)	2.951	2.793
		chrun_pdvv184_7_t01	similar to UP|KPYG_TOBAC (Q40546) Pyruvate kinase isozyme G, chloroplast precursor , partial (84%)	2.439	2.456
		chrun_jgvv184_9_t01	similar to UP|KPYG_TOBAC (Q40546) Pyruvate kinase isozyme G, chloroplast precursor , partial (84%)	2.798	2.509
	Lipid degradation lipases	chr16_jgvv98_143_t01	weakly similar to UP|Q8W0F0_ORYSA (Q8W0F0) Lipase class 3-like, partial (32%)	1.468	1.153
		chr9_jgvv18_162_t01	similar to RF|NP_181773.2|42569869|NM_129806 triacylglycerol lipase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (49%)	-1.407	-1.262
		chr7_jgvv141_19_t01	similar to GB|AAL11566.1|15983396|AF424572 At1g51440/F5D21_19 {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (27%)	-1.764	-1.553

Table 3.36: Common DEGs (selected sub-bins from different pathways) between plants treated with phosphonate and plants treated with phosphate and subsequently inoculated

Pathway	Bin name	Gene ID	Annotation	Fold change	
				Phosphonate	Phosphate
Miscelleanous	Nitrile lyases	chr6_jgvv4_293_t01	similar to RF|NP_180489.1|15227060|NM_128482 oxidoreductase {Arabidopsis thaliana}, partial (79%)	1.247	1.493
		chr6_jgvv4_287_t01	similar to RF|NP_180489.1|15227060|NM_128482 oxidoreductase {Arabidopsis thaliana}, partial (79%)	1.244	1.323
		chr6_jgvv4_282_t01	similar to RF|NP_180489.1|15227060|NM_128482 oxidoreductase {Arabidopsis thaliana}, partial (79%)	1.372	1.029
		chr2_jgvv109_1_t01	similar to UP|Q3LRV4_LUPAN (Q3LRV4) Nitrilase 4B, partial (88%)	1.091	1.153
		chr6_jgvv80_29_t01	similar to UP|Q3LRV4_LUPAN (Q3LRV4) Nitrilase 4B, partial (88%)	1.868	1.004
	Glutathione S transferases	chr19_jgvv93_18_t01	glutathione-S-transferase [Vitis vinifera]	1.143	1.091
		chr19_jgvv15_204_t01	glutathione-S-transferase [Vitis vinifera]	1.891	1.431
		chr19_jgvv93_11_t01	glutathione-S-transferase [Vitis vinifera]	2.336	2.695
		chrun_jgvv240_3_t01	glutathione-S-transferase [Vitis vinifera]	1.691	1.663
		chrun_jgvv240_2_t01	glutathione-S-transferase [Vitis vinifera]	2.585	2.098
		chr19_jgvv93_19_t01	similar to UP|O49235_SOYBN (O49235) 2,4-D inducible glutathione S-transferase , complete	2.577	2.419
		chr19_jgvv15_220_t01	similar to UP|O49235_SOYBN (O49235) 2,4-D inducible glutathione S-transferase , complete	1.223	1.251
		chr19_jgvv15_206_t01	similar to UP|O49235_SOYBN (O49235) 2,4-D inducible glutathione S-transferase , complete	1.863	1.572
		chr19_jgvv15_210_t01	similar to UP|O49821_CARPA (O49821) Glutathione transferase , partial (67%)	2.374	1.724
		chr19_jgvv93_26_t01	similar to UP|Q5GMM5_CAPCH (Q5GMM5) Glutathione S-transferase/peroxidase, complete	1.289	1.028
		chr12_jgvv34_77_t01	similar to UP|Q84VH0_MALPU (Q84VH0) Glutathione S-transferase Z1, partial (97%)	1.054	1.032
		chr19_jgvv15_211_t01	similar to UP|Q84VH2_MALPU (Q84VH2) Glutathione S-transferase U1, partial (80%)	1.651	2.023
		chr1_jgvv26_126_t01	similar to UP|Q9M6R4_GOSHI (Q9M6R4) Glutathione S-transferase, partial (76%)	1.789	1.704
		chr6_pdvv4_259_t01	weakly similar to UP|Q9FQE5_SOYBN (Q9FQE5) Glutathione S-transferase GST 13 , partial (62%)	2.713	1.617
		chr6_jgvv4_263_t01	weakly similar to UP|Q9FQE5_SOYBN (Q9FQE5) Glutathione S-transferase GST 13 , partial (62%)	1.037	1.546

3.5.3.2 Specific transcriptional responses after treatment with phosphonate and subsequent inoculation

Most of DEGs involved in stress pathways that are specific to treatment with phosphonate were up-regulated (Table 3.37). These genes code for biotic stress receptors such as resistance genes and R-proteins and PR-proteins such as chitinases and PR-10. Most of genes involved in abiotic stress response such as heat shock proteins were also up-regulated. Genes implicated in secondary pathways were up-regulated (Table 3.38). These included genes in the isoprenoid and terpenoid pathway such as laccase and cycloartenol synthase, respectively. Genes involved in phenylpropanoids and lignin biosynthesis such as sinapyl alcohol dehydrogenase were up-regulated. Resveratrol synthase and stilbene synthase, genes coding for enzymes responsible for the biosynthesis of chalcones, were also up-regulated (Table 3.38).

Genes coding for proteins working as receptors kinases such as serine/threonine kinases and leucine-rich repeat receptor-like proteins kinase were up-regulated (Table 3.39). A gene involved in calcium signalling such as calmodulin binding protein was also up-regulated. Most of genes coding for enzymes involved in hormone metabolism were up-regulated (Table 3.40). Genes implicated in ethylene, gibberellin, jasmonate and salicylic acid metabolism were up-regulated. However, genes implicated in auxin metabolism were mostly down-regulated, while a gene implicated in abscisic acid was down-regulated (Table 3.40).

Genes participating in cell wall metabolism, such as synthesis of cellulose and cell wall proteins, were up-regulated (Table 3.41). However, genes coding for enzymes involved in cell wall degradation such as beta -1,4-glucanase, pectate lyases and polygalacturonases were also up-regulated. Genes coding for enzymes involved in cell wall modification such as syringolide-induced protein and expansin were also down-regulated (Table 3.41). Genes implicated in carbohydrate metabolism were up-regulated (Table 3.41). These were genes coding for enzymes responsible for the synthesis of trehaloses, sucrose and raffinose. Genes coding for WRKY and MYB transcription factors were down-regulated (Table 3.41).

Table 3.37: Specific DEGs (in selected sub-bins from stress pathways) in plants treated with phosphonate and subsequently inoculated (compared to inoculated plants)

Pathway	Bin Name	Gene ID	Annotation	Fold change
Stress pathways	Stress.biotic.receptors	chr12_jgvv34_139_t01	weakly similar to UP|Q84ZU6_SOYBN (Q84ZU6) R 1 protein, partial (4%)	1,016
		chr12_jgvv34_11_t01	GB|AF365881.1|AAQ15193.1 resistance protein [Vitis vinifera]	1,762
		chr7_random_jgvv224_1_t01	weakly similar to UP|Q3A3E7_PELCD (Q3A3E7) Peptide ABC transporter, permease protein, partial (6%)	2,048
		chr8_jgvv58_124_t01	weakly similar to UP|Q93V91_LYCES (Q93V91) Verticillium wilt disease resistance protein Ve2, partial (5%)	1,044
		chrun_jgvv2381_1_t01	GB|AF369833.1|AAM21290.1 resistance gene analog [Vitis vinifera]	-2,132
		chr5_jgvv51_17_t01	weakly similar to RF|NP_192939.2|30681996|NM_117272 WRKY19; transcription factor {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (4%)	1,406
		chr5_jgvv20_465_t01	similar to UP|Q2CML8_9EURY (Q2CML8) AAA ATPase, central region:ATPase associated with various cellular activities, AAA_3, partial (8%)	-1,078
		chr18_jgvv117_41_t01	weakly similar to UP|Q947D9_HELAN (Q947D9) Resistance gene analog NBS9 (Fragment), partial (44%)	-1,123
		chr6_pdvv4_440_t01	weakly similar to UP|Q947E6_HELAN (Q947E6) Resistance gene analog NBS2 (Fragment), partial (50%)	1,097
		chr12_jgvv57_67_t01	weakly similar to GP|3894385|gb|AAC78592.1| Hcr2-0A {Lycopersicon esculentum}, partial (16%)	-1,098
		chr16_jgvv50_225_t01	weakly similar to UP|Q6JN46_LYCES (Q6JN46) EIX receptor 2, partial (11%)	1,279
		chr16_jgvv50_94_t01	weakly similar to UP|Q6JN46_LYCES (Q6JN46) EIX receptor 2, partial (11%)	1,088
	Stress.biotic. PR-proteins	chr15_jgvv48_273_t01	UP|Q9SNY0_VITVI (Q9SNY0) SCUTL2, complete	-1,124
		chr5_jgvv77_133_t01	UP|Q9FS43_VITVI (Q9FS43) Pathogenesis-related protein 10, partial (70%)	1,024
		chr5_jgvv94_24_t01	similar to UP|Q7XB39_VITVI (Q7XB39) Class IV chitinase, partial (95%)	1,112
	Stress.abiotic.heat	chr17_jgvv0_825_t01	similar to UP|Q3E7E4_ARATH (Q3E7E4) Protein At5g35753, partial (26%)	-1,186
		chr4_pdvv43_40_t01	homologue to UP|Q6UJX6_NICBE (Q6UJX6) Molecular chaperone Hsp90-1, partial (59%)	2,082
		chr18_jgvv86_38_t01	similar to UP|Q8S0V9_ORYSA (Q8S0V9) DnaJ-like protein, partial (44%)	1,078
		chr7_jgvv95_27_t01	homologue to UP|O22329_SOLCO (O22329) Heat shock cognate protein, partial (28%)	1,014
		chr13_jgvv19_229_t01	similar to UP|Q40510_TOBAC (Q40510) Nthsp18p, partial (94%)	1,6
	Stress.abiotic.unspecified	chr1_jgvv11_622_t01	weakly similar to UP|Q9SIJ8_ARATH (Q9SIJ8) Expressed protein (RD2 protein), partial (11%)	-1,287

Table 3.38: Specific DEGs (in selected sub-bins from secondary metabolism) in plants treated with phosphonate and subsequently inoculated (compared to inoculated plants)

Pathway	Bin Name	Gene ID	Annotation	Fold change
Secondary metabolism	Isoprenoids	chr8_jgvv7_794_t01	similar to GP|1621467|gb|AAB17194.1| laccase {Liriodendron tulipifera}, partial (47%)	-1,231
		chr18_random_jgvv152_12_t01	weakly similar to GP|9955523|emb|CAC05462.1 laccase-like protein {Arabidopsis thaliana}, partial (10%)	2,233
	Isoprenoids.terpenoids	chr9_jgvv54_56_t01	similar to UP|Q8W3Z4_9ROSI (Q8W3Z4) Cycloartenol synthase , partial (52%)	1,19
		chr9_jgvv54_62_t01	similar to UP|Q8W3Z4_9ROSI (Q8W3Z4) Cycloartenol synthase , partial (52%)	1,348
		chrun_jgvv208_5_t01	similar to GP|3688598|dbj|BAA33460.1 Cycloartenol Synthase {Panax ginseng}, partial (36%)	1,557
	Phenylpropanoids	chr10_jgvv3_43_t01	weakly similar to UP|Q8GU24_ROSCH (Q8GU24) Orcinol O-methyltransferase 1 , partial (62%)	2,002
		chr11_jgvv37_53_t01	weakly similar to UP|Q2Z1Y0_PRUMU (Q2Z1Y0) Alcohol acyl-transferase, partial (24%)	1,193
		chr18_pdvv1_715_t01	similar to UP|Q8LLM2_TOBAC (Q8LLM2) AER, partial (30%)	1,852
	Phenylpropanoids. lignin biosynthesis	chr2_pdvv25_251_t01	homologue to UP|Q9M560_VITVI (Q9M560) Caffeic acid O-methyltransferase, complete	1,406
		chr2_jgvv25_286_t01	similar to UP|Q2R114_ORYSA (Q2R114) Oxidoreductase, zinc-binding dehydrogenase family, partial (65%)	1,238
		chrun_jgvv346_12_t01	similar to UP|Q5I6D6_9ROSI (Q5I6D6) Sinapyl alcohol dehydrogenase-like protein, complete	1,468
		chrun_jgvv371_7_t01	similar to UP|Q5I6D6_9ROSI (Q5I6D6) Sinapyl alcohol dehydrogenase-like protein, complete	1,466
		chrun_jgvv371_3_t01	similar to UP|Q5I6D6_9ROSI (Q5I6D6) Sinapyl alcohol dehydrogenase-like protein, complete	1,572
	Flavonoids.chalcones	chr16_jgvv22_124_t01	homologue to UP|Q2HY10_VITVI (Q2HY10) Resveratrol synthase, complete	1,764
		chr16_jgvv100_17_t01	UP|THS1_VITVI (P28343) Stilbene synthase 1 (Resveratrol synthase 1) (Trihydroxystilbene synthase 1) (PSV25) , complete	1,893
		chr16_jgvv100_19_t01	UP|THS1_VITVI (P28343) Stilbene synthase 1 (Resveratrol synthase 1) (Trihydroxystilbene synthase 1) (PSV25) , complete	2,423
	Flavonoids.dihydroflavonols	chr6_jgvv9_76_t01	UP|Q3C210_VITVI (Q3C210) Flavonoid 3\',5\'-hydroxylase, complete	1,002

Table 3.39: Specific DEGs (in selected sub-bins from signalling pathways) in plants treated with phosphonate and subsequently inoculated (compared to inoculated plants)

Pathway	Bin Name	Gene ID	Annotation	Fold change
Signalling pathways	Receptor kinases. S-locus glycoprotein like	chr7_jgvv95_10_t01	weakly similar to UP|O49974_MAIZE (O49974) KI domain interacting kinase 1, partial (21%)	1,088
		chr19_jgvv14_393_t01	weakly similar to UP|Q40100_IPOTF (Q40100) Secreted glycoprotein 3, partial (55%)	1,058
		chrun_jgvv743_3_t01	weakly similar to RF|NP_194459.1|15237045|NM_118863 ATP binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (24%)	1,713
		chrun_jgvv484_1_t01	similar to UP|Q9ZT07_ARATH (Q9ZT07) Receptor-like protein kinase, partial (19%)	1,192
		chr19_jgvv14_379_t01	weakly similar to UP|Q40100_IPOTF (Q40100) Secreted glycoprotein 3, partial (55%)	2,309
	Receptor kinases. Miscellaneous	chr18_jgvv41_202_t01	similar to RF|NP_195827.1|15241674|NM_120285 kinase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (39%)	1,268
		chr16_jgvv98_182_t01	weakly similar to UP|Q9FF29_ARATH (Q9FF29) Receptor serine/threonine kinase, partial (35%)	2,373
		chr16_jgvv98_171_t01	weakly similar to UP|Q9FF29_ARATH (Q9FF29) Receptor serine/threonine kinase, partial (35%)	1,382
		chr7_jgvv5_383_t01	similar to UP|Q75WU3_POPNI (Q75WU3) Leucine-rich repeat receptor-like protein kinase 1, partial (28%)	1,092
		chr3_jgvv63_227_t01	similar to UP|Q6ZIG4_ORYSA (Q6ZIG4) Receptor protein kinase PERK1-like protein, partial (82%)	-1,041
		chr16_jgvv22_71_t01	similar to UP|Q9M574_ORYSA (Q9M574) Receptor-like protein kinase, partial (25%)	1,284
		chr9_jgvv18_72_t01	weakly similar to UP|Q9SN81_ARATH (Q9SN81) Receptor-kinase like protein, partial (13%)	1,02
		chr6_jgvv9_158_t01	weakly similar to RF|NP_180463.1|15227015|NM_128456 ATP binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (19%)	-1,127
		chr18_jgvv1_92_t01	similar to RF|NP_195827.1|15241674|NM_120285 kinase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (39%)	1,438
		chr12_jgvv35_176_t01	similar to UP|Q75WU3_POPNI (Q75WU3) Leucine-rich repeat receptor-like protein kinase 1, partial (28%)	1,269
		chr12_jgvv35_180_t01	similar to UP|Q75WU3_POPNI (Q75WU3) Leucine-rich repeat receptor-like protein kinase 1, partial (28%)	1,541
	Calcium signalling	chr14_jgvv30_23_t01	similar to RF|NP_188931.1|15228891|NM_113191 calcium-transporting ATPase/ calmodulin binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0) , partial (24%)	1,118
	G-proteins	chr3_jgvv17_27_t01	similar to UP|O65744_CICAR (O65744) GDP dissociation inhibitor, complete	1,312
		chr11_jgvv16_509_t01	similar to UP|Q5SMT0_ORYSA (Q5SMT0) GTPase activating protein-like, partial (13%)	1,469

Table 3.40: Specific DEGs (in selected sub-bins from hormone metabolism) in plants treated with phosphonate and subsequently inoculated (compared to inoculated plants)

Pathway	Bin Name	Gene ID	Annotation	Fold change
Hormone metabolism	Abscisic acid. synthesis-degradation	chr13_jgvv64_96_t01	UP|Q3T4H1_VITVI (Q3T4H1) 9,10[9\',10\']carotenoid cleavage dioxygenase, complete	-1,231
	Auxin.synthesis-degradation	chr16_jgvv98_113_t01	weakly similar to UP|Q2I747_BRACM (Q2I747) IAA-amino acid hydrolase 3, partial (77%)	1,093
		chr18_jgvv1_1172_t01	similar to UP|Q8LKH1_9ROSI (Q8LKH1) PIN1-like auxin transport protein, partial (46%)	-1,365
		chr18_random_jgvv152_13_t01	similar to UP|Q6YZX7_ORYSA (Q6YZX7) Auxin efflux carrier protein-like, partial (48%)	1,066
		chr1_jgvv127_2_t01	similar to RF|NP_563915.1|18391439|NM_101152 IPS1; ubiquitin-protein ligase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (12%)	-1,000
		chr10_jgvv116_55_t01	similar to UP|Q9LSE7_ARATH (Q9LSE7) Emb|CAB45497.1 (AT3g25290/MJL12_25), partial (51%)	-1,308
		chr9_jgvv54_97_t01	similar to UP|Q8H6T6_PHAVU (Q8H6T6) Auxin-regulated protein, partial (72%)	1,577
	Ethylene.synthesis-degradation	chr3_jgvv63_107_t01	weakly similar to UP|Q86B83_DROME (Q86B83) CG33099-PA, partial (7%)	1,633
		chr3_jgvv63_117_t01	weakly similar to UP|Q86B83_DROME (Q86B83) CG33099-PA, partial (7%)	1,348
		chr5_jgvv49_23_t01	weakly similar to UP|Q4W8D2_LYCES (Q4W8D2) 2-oxoglutarate-dependent dioxygenase, partial (40%)	1,58
		chr3_jgvv63_122_t01	weakly similar to UP|Q86B83_DROME (Q86B83) CG33099-PA, partial (7%)	1,651
	Gibberelin.synthesis-degradation	chr10_jgvv3_315_t01	similar to UP|Q6TN17_9ROSI (Q6TN17) Gibberellin 2-oxidase, partial (82%)	1,243
		chr1_jgvv26_193_t01	weakly similar to UP|O24040_9ROSI (O24040) LTCOR11, partial (61%)	-1,27
	Jasmonate.synthesis-degradation	chr18_jgvv41_33_t01	similar to UP|Q76DL0_LITER (Q76DL0) LEDI-5c protein, partial (96%)	1,167
		chr18_jgvv41_34_t01	similar to UP|Q76DL0_LITER (Q76DL0) LEDI-5c protein, partial (96%)	1,049
	Salicylic acid.synthesis-degradation	chr4_jgvv23_160_t01	similar to UP|Q9SPV4_CLABR (Q9SPV4) S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase, partial (76%)	1,686
		chr17_jgvv0_671_t01	weakly similar to UP|Q9XI57_ARATH (Q9XI57) F9L1.6, partial (29%)	1,423

Table 3.41: Specific DEGs (in selected sub-bins from cell wall, carbohydrate and RNA metabolism) in plants treated with phosphonate and subsequently inoculated (compared to inoculated plants)

Pathway	Bin Name	Gene ID	Annotation	Fold change
Cell wall	Cellulose synthesis	chrun_jgvv414_4_t01	weakly similar to UP|Q944E3_ORYSA (Q944E3) Cellulose synthase-like protein OsCslE2, partial (32%)	1,145
		chrun_jgvv469_4_t01	weakly similar to UP|Q944E3_ORYSA (Q944E3) Cellulose synthase-like protein OsCslE2, partial (32%)	1,559
		chrun_jgvv469_5_t01	weakly similar to UP|Q944E3_ORYSA (Q944E3) Cellulose synthase-like protein OsCslE2, partial (32%)	1,318
		chr2_jgvv25_169_t01	similar to UP|Q3Y6V1_TOBAC (Q3Y6V1) Cellulose synthase-like protein CslG, partial (33%)	1,431
		chrun_jgvv414_1_t01	weakly similar to UP|Q944E3_ORYSA (Q944E3) Cellulose synthase-like protein OsCslE2, partial (32%)	1,12
	Cell wall proteins.AGPs	chr14_jgvv6_54_t01	similar to UP|Q6J192_9ROSI (Q6J192) Fasciclin-like AGP 12, partial (72%)	2,452
	Cell wall proteins.LRR	chr2_jgvv12_141_t01	similar to UP|GRP2_PHAVU (P10496) Glycine-rich cell wall structural protein 1.8 precursor (GRP 1.8), partial (9%)	-1,023
	Degradation.cellulases and beta -1,4-glucanases	chr19_jgvv85_53_t01	homologue to UP|Q6QLN2_POPTM (Q6QLN2) Endo-1,4-beta-glucanase , partial (76%)	1,548
	Degradation.pectate lyases and polygalacturonases	chr4_jgvv8_381_t01	weakly similar to UP|Q4VT47_VITVI (Q4VT47) RD22-like protein, partial (48%)	3,191
		chr4_jgvv8_383_t01	weakly similar to UP|Q4VT47_VITVI (Q4VT47) RD22-like protein, partial (48%)	2,357
	Cell wall.modification	chr11_jgvv52_52_t01	similar to UP|Q8S902_SOYBN (Q8S902) Syringolide-induced protein 19-1-5, partial (94%)	1,702
		chr4_jgvv79_1_t01	similar to UP|Q49QW6_9ROSI (Q49QW6) Expansin, partial (96%)	1,897
		chr11_jgvv52_50_t01	similar to UP|Q8S902_SOYBN (Q8S902) Syringolide-induced protein 19-1-5, partial (94%)	1,545
		chr11_jgvv52_65_t01	homologue to UP|Q8S902_SOYBN (Q8S902) Syringolide-induced protein 19-1-5, partial (65%)	1,234
Carbohydrate metabolism	Raffinose family	chr14_jgvv60_184_t01	similar to UP|Q7XZ08_SOYBN (Q7XZ08) Galactinol synthase, partial (41%)	1,45
		chr7_jgvv5_191_t01	similar to UP|Q9XEJ7_BRANA (Q9XEJ7) Galactinol synthase (Fragment), partial (87%)	1,309
	Trehalose	chr7_jgvv5_514_t01	similar to UP|Q9LMI0_ARATH (Q9LMI0) T2D23.11 protein, partial (33%)	1,182
	Synthesis.sucrose	chr18_jgvv75_27_t01	UP|Q5EEP9_VITVI (Q5EEP9) Sucrose-phosphate synthase 1 , partial (22%)	-1,183
	Synthesis.starch.transporter	chr16_random_jgvv196_8_t01	similar to UP|O49447_ARATH (O49447) ADP, ATP carrier-like protein, partial (30%)	1,01
		chr19_jgvv177_35_t01	similar to UP|ADT1_GOSHI (O22342) ADP,ATP carrier protein 1, mitochondrial precursor (ADP/ATP translocase 1) (Adenine nucleotide translocator 1) (ANT 1), partial (92%)	1,023
	Degradation.sucrose.Susy	chr10_jgvv71_91_t01	similar to UP|Q9SLS2_CITUN (Q9SLS2) Sucrose synthase, partial (85%)	1,264
	Degradation.starch.starch cleavage	chr2_jgvv87_91_t01	similar to GB|AAP68250.1|31711788|BT008811 At3g23640 {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (25%)	1,026
RNA metabolism	Transcription factor Family MYB	chr17_pdvv0_648_t01	similar to GP|9294065|dbj|BAB02022.1 contains similarity to myb proteins~gene_id:MRC8.8 {Arabidopsis thaliana}, partial (8%)	1.194
		chr13_jgvv64_82_t01	similar to GP|28628949|gb|AAO49411.1 MYB2 {Dendrobium sp. XMW-2002-2}, partial (36%)	-1.091
		chr3_pdvv63_238_t01	similar to UP|Q6NNN0_ARATH (Q6NNN0) At4g36570, partial (91%)	-1.157
	Transcription factor Family WRKY	chr4_jgvv69_71_t01	homologue to UP|WRK71_ARATH (Q93WV4) Probable WRKY transcription factor 71 (WRKY DNA-binding protein 71), partial (32%)	-2.07
		chr8_jgvv58_10_t01	similar to UP|Q9M6E1_TOBAC (Q9M6E1) DNA-binding protein 3, partial (26%)	-1.058
		chr7_jgvv31_152_t01	similar to UP|Q2PJR6_SOYBN (Q2PJR6) WRKY54, partial (47%)	-1.933

3.5.3.3 Specific transcriptional responses after treatment with phosphate and subsequent inoculation

There were more genes that were differentially expressed specifically upon elicitation with phosphate and subsequent inoculation than those differentially expressed specifically upon elicitation with phosphonate and subsequent inoculation.

50% of genes coding for biotic stress receptors such as resistance proteins and nucleotide binding sequence leucin rich repeat (NBS-LRR) type disease resistance protein in stress pathways were down-regulated (Table 3.42). However, genes coding for PR-proteins were up-regulated. Genes involved in abiotic stress responses such as cytosolic class II low molecular weight heat shock proteins and germin-like proteins were up-regulated (Table 3.42). Some of the genes involved in secondary metabolism were up- and down- regulated (Table 3.43). Genes coding for enzymes involved in isoprenoid synthesis such as laccase and in terpenoid synthesis such as terpenoid synthase were up-regulated, while genes coding for phenylalanine ammonium lyase, the enzyme responsible for the synthesis of phenylpropanoids, were down-regulated. A gene coding for stilbene synthase was down-regulated and isoflavone reductase was up-regulated, both enzymes involved in the synthesis of phytoalexins such as chalcones and isoflavonol (Table 3.43).

As for signalling, some genes coding for receptor kinases with leucine rich repeats were up-regulated, while others were down-regulated (Table 3.44). Also genes coding for receptor kinases such as serine/threonine kinase-like proteins were up- and down-regulated. However, genes coding for proteins involved in calcium signalling were down-regulated (Table 3.44).

Genes involved in abscisic acid metabolism were down-regulated, while genes involved in abscisic acid signal transduction, that code for ABA-responsive proteins, were up-regulated (Table 3.45). Genes coding for the auxin-induced SAUR-like proteins, small auxin-up RNA (SAUR), whose expressions are early auxin-responsive, were up-regulated (Table 3.45). Most of genes implicated in ethylene metabolism were down-regulated, while those implicated in jasmonate metabolism were up-regulated (Table 3.45). Genes involved in gibberellin metabolism were up-regulated, while genes involved in salicylic acid were up- and down-regulated (Table 3.45).

Genes involved in cell wall metabolism were up-regulated (Table 3.46). Genes coding for cellulose synthase, an enzyme that is involved in cellulose synthesis, were up-regulated. Genes implicated in the synthesis of hemicellulose and cell wall proteins were also up-regulated. However, genes coding for enzymes that degrade the cell wall such as polygalacturonase, pectate lyase, pectin methylesterase and enzymes that modify the cell wall such as expansin were also up-regulated (Table 3.46).

Genes coding for transcription family WRKY and AP2 (ethylene responsive element) were down-regulated, while MYB genes coding for transcription family MYB members were up-regulated (Table 3.47).

Table 3.42: Specific DEGs (in selected sub-bins from stress pathways) in plants treated with phosphate and subsequently inoculated (compared to inoculated plants)

Pathway	Bin Name	Gene ID	Annotation	Fold change
Stress pathways	Biotic stress-receptors	chr18_random_jgvv82_19_t01	GB|AF365879.1|AAQ15191.1 resistance protein [Vitis vinifera]	-2,142
		chr18_jgvv89_119_t01	GB|AF365879.1|AAQ15191.1 resistance protein [Vitis vinifera]	-1,6
		chr19_jgvv14_350_t01	GB|AF365880.1|AAQ15192.1 resistance protein [Vitis vinifera]	-1,367
		chr12_jgvv121_6_t01	GB|AF365880.1|AAQ15192.1 resistance protein [Vitis vinifera]	-1,219
		chr17_jgvv0_674_t01	GB|AF365880.1|AAQ15192.1 resistance protein [Vitis vinifera]	-1,803
		chr13_jgvv158_17_t01	GB|AF365880.1|AAQ15192.1 resistance protein [Vitis vinifera]	-1,093
		chr12_jgvv34_85_t01	GB|AF365880.1|AAQ15192.1 resistance protein [Vitis vinifera]	-2,082
		chr19_jgvv27_44_t01	GB|AF365880.1|AAQ15192.1 resistance protein [Vitis vinifera]	-1,515
		chr19_jgvv27_40_t01	GB|AF365880.1|AAQ15192.1 resistance protein [Vitis vinifera]	-2,064
		chr12_jgvv34_27_t01	GB|AF365881.1|AAQ15193.1 resistance protein [Vitis vinifera]	-1,647
		chr13_jgvv139_12_t01	GB|AF369833.1|AAM21290.1 resistance gene analog [Vitis vinifera]	-2,021
		chr7_jgvv95_1_t01	weakly similar to GB|BAB01339.1|11994217|AB028617 disease resistance comples protein {Arabidopsis thaliana} (exp=0; wgp=1; cg=0), partial (11%)	-1,626
		chr13_jgvv158_19_t01	weakly similar to GB|BAB01339.1|11994217|AB028617 disease resistance comples protein {Arabidopsis thaliana} (exp=0; wgp=1; cg=0), partial (11%)	-1,278
		chr13_jgvv139_28_t01	weakly similar to GB|BAB01339.1|11994217|AB028617 disease resistance comples protein {Arabidopsis thaliana} (exp=0; wgp=1; cg=0), partial (11%)	-1,91
		chr18_jgvv89_103_t01	weakly similar to GP|15787905|gb|AAL07544.1 resistance gene analog NBS9 {Helianthus annuus}, partial (49%)	-1,037
		chr18_random_jgvv82_109_t01	weakly similar to GP|29725485|gb|AAO89158.1 NBS-type resistance protein {Gossypium barbadense}, partial (42%)	-1,1
		chr18_jgvv89_68_t01	weakly similar to GP|3894385|gb|AAC78592.1| Hcr2-0A {Lycopersicon esculentum}, partial (16%)	2,426
		chr13_jgvv84_65_t01	weakly similar to UP|Q3A3E7_PELCD (Q3A3E7) Peptide ABC transporter, permease protein, partial (6%)	-1,329
		chrun_jgvv276_5_t01	weakly similar to UP|Q3A3E7_PELCD (Q3A3E7) Peptide ABC transporter, permease protein, partial (6%)	-1,807
		chr13_jgvv139_21_t01	weakly similar to UP|Q3A3E7_PELCD (Q3A3E7) Peptide ABC transporter, permease protein, partial (6%)	-1,23
		chr16_jgvv50_235_t01	weakly similar to UP|Q6JN46_LYCES (Q6JN46) EIX receptor 2, partial (11%)	1,268
		chr19_pdvv27_13_t01	weakly similar to UP|Q8H6R0_PONTR (Q8H6R0) NBS-LRR type disease resistance protein, partial (19%)	1,007
		chr9_jgvv96_13_t01	weakly similar to UP|Q8H6R0_PONTR (Q8H6R0) NBS-LRR type disease resistance protein, partial (19%)	-1,947
		chr19_jgvv27_14_t01	weakly similar to UP|Q8H6R0_PONTR (Q8H6R0) NBS-LRR type disease resistance protein, partial (19%)	1,066
		chrun_jgvv222_6_t01	weakly similar to UP|Q93VS9_PHAVU (Q93VS9) NBS-LRR resistance-like protein B8, partial (7%)	-1,632
		chr18_jgvv41_188_t01	weakly similar to UP|Q947D9_HELAN (Q947D9) Resistance gene analog NBS9 (Fragment), partial (44%)	-1,003
		chr12_random_jgvv99_20_t01	weakly similar to UP|Q947D9_HELAN (Q947D9) Resistance gene analog NBS9 (Fragment), partial (45%)	-1,321

Table 3.42: Continued…

Pathway	Bin Name	Gene ID	Annotation	Fold change
Stress pathways		chr1_jgvv11_545_t01	similar to UP|Q2CML8_9EURY (Q2CML8) AAA ATPase, central region:ATPase associated with various cellular activities, AAA_3, partial (8%)	1,052
		chr9_jgvv2_352_t01	weakly similar to GP|24461865|gb|AAN62352.1 NBS-LRR type disease resistance protein {Poncirus trifoliata}, partial (4%)	-1,005
	stress.biotic.signaling	chr12_jgvv35_68_t01	similar to UP|Q5EEY5_NICBE (Q5EEY5) SGT1, partial (62%)	1,229
		chr7_jgvv31_217_t01	weakly similar to UP|Q2TNK3_SOLTU (Q2TNK3) Phytoalexin-deficient 4-1 protein, partial (30%)	-1,124
	PR-proteins	chr3_jgvv88_43_t01	weakly similar to UP|Q6WHB9_CAPFR (Q6WHB9) Cytoplasmic small heat shock protein class I, partial (89%)	1,253
		chr3_jgvv88_45_t01	pathogenesis-related protein [Vitis vinifera]	1,325
		chr3_jgvv91_104_t01	similar to UP|Q9XIY9_TOBAC (Q9XIY9) NtPRp27, partial (83%)	1,923
		chr14_jgvv81_68_t01	homologue to UP|O81228_VITVI (O81228) PR-4 type protein, complete	1,778
		chr15_jgvv46_191_t01	similar to UP|CHIA_TOBAC (P29060) Acidic endochitinase precursor , partial (91%)	1,365
	Miscellaneous	chr12_jgvv34_132_t01	homologue to UP|Q5GI04_CAPAN (Q5GI04) Hypersensitive-induced reaction protein, partial (98%)	1,054
	Stress.abiotic.heat	chr1_jgvv10_29_t01	homologue to UP|Q6UJX6_NICBE (Q6UJX6) Molecular chaperone Hsp90-1, partial (50%)	1,496
		chr13_jgvv106_30_t01	homologue to UP|Q6UJX6_NICBE (Q6UJX6) Molecular chaperone Hsp90-1, partial (50%)	1,513
		chr2_jgvv154_42_t01	weakly similar to UP|O80432_LYCES (O80432) Mitochondrial small heat shock protein, partial (68%)	1,078
		chr4_jgvv8_145_t01	similar to UP|Q9XGS6_PRUDU (Q9XGS6) Cytosolic class II low molecular weight heat shock protein, complete	1,135
		chr4_jgvv8_148_t01	similar to UP|Q9XGS6_PRUDU (Q9XGS6) Cytosolic class II low molecular weight heat shock protein, complete	1,35
		chr4_jgvv8_147_t01	similar to UP|Q9XGS6_PRUDU (Q9XGS6) Cytosolic class II low molecular weight heat shock protein, complete	1,569
		chr4_jgvv8_146_t01	similar to UP|Q9XGS6_PRUDU (Q9XGS6) Cytosolic class II low molecular weight heat shock protein, complete	1,915
		chr4_jgvv8_142_t01	similar to UP|Q9XGS6_PRUDU (Q9XGS6) Cytosolic class II low molecular weight heat shock protein, complete	1,389
		chr4_jgvv8_144_t01	similar to UP|Q9XGS6_PRUDU (Q9XGS6) Cytosolic class II low molecular weight heat shock protein, complete	1,635
		chr4_jgvv8_138_t01	similar to UP|Q9XGS6_PRUDU (Q9XGS6) Cytosolic class II low molecular weight heat shock protein, complete	1,12
		chr4_jgvv8_139_t01	similar to UP|Q9XGS6_PRUDU (Q9XGS6) Cytosolic class II low molecular weight heat shock protein (96%)	2,119
		chr13_jgvv19_205_t01	similar to UP|Q9SWE4_TOBAC (Q9SWE4) Low molecular weight heat-shock protein, partial (94%)	1,087
		chr18_random_jgvv126_9_t01	weakly similar to UP|Q40865_PENAM (Q40865) Heat shock protein 16.9, partial (65%)	1,251
		chr13_jgvv19_243_t01	similar to UP|HSP12_MEDSA (P27880) 18.2 kDa class I heat shock protein, partial (86%)	1,24
		chr13_jgvv19_236_t01	similar to UP|HSP12_MEDSA (P27880) 18.2 kDa class I heat shock protein, partial (86%)	1,735
		chr13_jgvv19_241_t01	similar to UP|HSP11_PEA (P19243) 18.1 kDa class I heat shock protein (HSP 18.1), partial (86%)	1,765
		chr13_jgvv19_234_t01	similar to UP|Q6WHC0_CAPFR (Q6WHC0) Chloroplast small heat shock protein class I, partial (89%)	1,326

Table 3.42: Continued…

Pathway	Bin Name	Gene ID	Annotation	Fold change
Stress pathways	Stress.abiotic.unspecified	chr16_jgvv39_194_t01	GB|AY298727.1|AAQ63185.1 germin-like protein 3 [Vitis vinifera]	1,099
		chr18_jgvv86_41_t01	weakly similar to UP|Q9AR81_PEA (Q9AR81) Germin-like protein precursor, partial (96%)	2,988
		chr18_jgvv86_27_t01	weakly similar to UP|Q9AR81_PEA (Q9AR81) Germin-like protein precursor, partial (96%)	2,48
		chr7_jgvv5_528_t01	weakly similar to UP|Q9AR81_PEA (Q9AR81) Germin-like protein precursor, partial (96%)	2,061
		chr12_jgvv59_69_t01	weakly similar to UP|Q9AR81_PEA (Q9AR81) Germin-like protein precursor, partial (96%)	1,845
		chr10_pdvv3_429_t01	GB|AY298727.1|AAQ63185.1 germin-like protein 3 [Vitis vinifera]	2,143
		chr10_pdvv3_427_t01	GB|AY298727.1|AAQ63185.1 germin-like protein 3 [Vitis vinifera]	1,891

Table 3.43: Specific DEGs (in selected sub-bins from secondary metabolism) in plants treated with phosphate and subsequently inoculated (compared to inoculated plants)

Pathway	Bin Name	Gene ID	Annotation	Fold change
Secondary metabolism	Isoprenoids	chr18_jgvv117_12_t01	weakly similar to GP|9955523|emb|CAC05462.1 laccase-like protein {Arabidopsis thaliana}, partial (10%)	1,812
		chr6_jgvv4_410_t01	similar to GP|1621467|gb|AAB17194.1| laccase {Liriodendron tulipifera}, partial (47%)	1,52
		chr6_jgvv9_178_t01	homologue to UP|Q9ZS34_TOBAC (Q9ZS34) Geranylgeranyl reductase, partial (35%)	-1,091
		chr18_jgvv89_78_t01	similar to UP|Q2L8A7_9LAMI (Q2L8A7) Acetoacetyl-CoA thiolase, partial (92%)	-1,8
	Terpenoids	chr13_random_jgvv112_10_t01	UP|Q6PWU2_VITVI (Q6PWU2) Terpenoid synthase, complete	1,001
		chr18_random_jgvv82_31_t01	UP|Q6Q3H2_VITVI (Q6Q3H2) Terpenoid synthetase, complete	1,182
	Phenylpropanoids	chr12_jgvv28_119_t01	weakly similar to UP|Q8GU23_ROSCH (Q8GU23) Orcinol O-methyltransferase 2 , partial (52%)	2,085
		chr13_jgvv67_164_t01	weakly similar to UP|Q8H2B5_TAXCU (Q8H2B5) Phenylpropanoyltransferase, partial (18%)	-1,025
	Phenylpropanoids. lignin biosynthesis	chr16_jgvv39_75_t01	phenylalanine ammonium lyase [Vitis vinifera]	-2,638
		chr11_jgvv16_136_t01	phenylalanine ammonium lyase [Vitis vinifera]	-2,324
		chrun_jgvv2508_1_t01	phenylalanine ammonium lyase [Vitis vinifera]	-1,841
		chr16_jgvv39_134_t01	similar to UP|Q9LL50_RUBID (Q9LL50) 4-coumarate:coA ligase 2 , partial (97%)	-1,104
		chr1_jgvv10_257_t01	similar to UP|Q2YHM9_PLAMJ (Q2YHM9) Caffeoyl-CoA O-methyltransferase (Fragment), partial (86%)	1,028
		chr8_jgvv32_89_t01	similar to UP|Q6L8K4_ROSCH (Q6L8K4) Phloroglucinol O-methyltransferase, partial (92%)	1,083
		chrun_jgvv218_1_t01	similar to UP|Q5I6D6_9ROSI (Q5I6D6) Sinapyl alcohol dehydrogenase-like protein, complete	1,227
	Flavonoids.chalcones	chr10_jgvv42_65_t01	UP|Q9S982_9ROSI (Q9S982) Stilbene synthase , partial (46%)	-1,038
	Flavonoids.isoflavonols	chr18_jgvv1_918_t01	UP|Q3KN69_VITVI (Q3KN69) Isoflavone reductase-like protein 4, complete	1,86
		chr2_jgvv33_20_t01	UP|Q3KN72_VITVI (Q3KN72) Isoflavone reductase-like protein 1, complete	1,343
		chr3_jgvv38_8_t01	UP|Q3KN67_VITVI (Q3KN67) Isoflavone reductase-like protein 6, complete	1,075

Table 3.44: Specific DEGs (in selected sub-bins from signalling pathways) in plants treated with phosphate and subsequently inoculated (compared to inoculated plants)

Pathway	Bin Name	Gene ID	Annotation	Fold change
Signalling pathways	Receptor kinases.leucine rich repeat II	chr12_jgvv121_21_t01	similar to GB|AAK68074.1|14573459|AF384970 somatic embryogenesis receptor-like kinase 3 {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (7%)	1,538
	Receptor kinases.leucine rich repeat III	chr1_jgvv11_106_t01	similar to UP|Q9LP77_ARATH (Q9LP77) T1N15.9, partial (52%)	-1,253
	Receptor kinases.leucine rich repeat III	chr15_jgvv21_106_t01	similar to UP|Q9LP77_ARATH (Q9LP77) T1N15.9, partial (52%)	1,328
	Receptor kinases.leucine rich repeat VIII.2	chr19_jgvv14_56_t01	weakly similar to UP|Q6EQG8_ORYSA (Q6EQG8) Leucine-rich repeat family protein /protein kinase family protein-like, partial (16%)	-1,543
	Receptor kinases.leucine rich repeat VIII.3	chrun_jgvv173_11_t01	weakly similar to UP|Q6EQG8_ORYSA (Q6EQG8) Leucine-rich repeat family protein /protein kinase family protein-like, partial (16%)	-1,256
	Receptor kinases.DUF 26	chrun_jgvv366_1_t01	similar to GP|13506747|gb|AAK28316.1 receptor-like protein kinase 5 {Arabidopsis thaliana}, partial (20%)	1,178
	Receptor kinases.S-locus glycoprotein like	chr15_jgvv24_14_t01	similar to UP|O81906_ARATH (O81906) Serine/threonine kinase-like protein, partial (20%)	1,133
		chr15_jgvv24_17_t01	similar to UP|O81906_ARATH (O81906) Serine/threonine kinase-like protein, partial (20%)	-1,216
		chr7_pdvv95_1_t01	similar to UP|O81906_ARATH (O81906) Serine/threonine kinase-like protein, partial (20%)	1,076
		chr19_jgvv14_395_t01	weakly similar to UP|Q40100_IPOTF (Q40100) Secreted glycoprotein 3, partial (55%)	1,471
		chr19_jgvv14_398_t01	weakly similar to UP|Q40100_IPOTF (Q40100) Secreted glycoprotein 3, partial (55%)	-2,182
		chr10_jgvv3_301_t01	weakly similar to UP|Q9ZT07_ARATH (Q9ZT07) Receptor-like protein kinase, partial (15%)	-1,345
		chr19_jgvv14_373_t01	similar to UP|Q9ZT07_ARATH (Q9ZT07) Receptor-like protein kinase, partial (19%)	-1,805
		chr17_jgvv53_32_t01	weakly similar to UP|O49974_MAIZE (O49974) KI domain interacting kinase 1, partial (21%)	1,295
		chrun_jgvv347_11_t01	weakly similar to RF|NP_172597.1|15220338|NM_101003 ATP binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (6%)	-1,358
		chrun_jgvv286_4_t01	weakly similar to UP|Q40100_IPOTF (Q40100) Secreted glycoprotein 3, partial (55%)	-1,246
		chr12_jgvv134_22_t01	similar to UP|Q9ZT07_ARATH (Q9ZT07) Receptor-like protein kinase, partial (19%)	1,032
	Receptor kinases.Miscellaneous	chr16_jgvv98_147_t01	weakly similar to UP|Q9SN81_ARATH (Q9SN81) Receptor-kinase like protein, partial (13%)	1,4
		chr16_random_jgvv307_2_t01	similar to UP|Q9M574_ORYSA (Q9M574) Receptor-like protein kinase, partial (25%)	1,163
		chr18_jgvv1_503_t01	similar to UP|Q56X19_ARATH (Q56X19) Leucine-rich repeat receptor-like kinase At1g09970, partial (55%)	-1,229
		chr18_jgvv1_126_t01	similar to UP|O04086_ARATH (O04086) Ser/Thr protein kinase isolog; 46094-44217, partial (37%)	-1,291
		chr14_jgvv6_164_t01	weakly similar to UP|Q9FF29_ARATH (Q9FF29) Receptor serine/threonine kinase, partial (35%)	1,06
		chr14_jgvv66_246_t01	similar to UP|Q75WU3_POPNI (Q75WU3) Leucine-rich repeat receptor-like protein kinase 1, partial (28%)	1,48
		chr9_jgvv2_261_t01	weakly similar to UP|Q39143_ARATH (Q39143) Light repressible receptor protein kinase, partial (15%)	1,157
		chr9_jgvv2_281_t01	weakly similar to UP|Q39143_ARATH (Q39143) Light repressible receptor protein kinase, partial (15%)	3,202
		chr16_jgvv39_123_t01	weakly similar to UP|Q9FF29_ARATH (Q9FF29) Receptor serine/threonine kinase, partial (35%)	-1,159

Table 3.44: Continued…

Pathway	Bin Name	Gene ID	Annotation	Fold change
Signalling pathways	Receptor kinases.Miscellaneous	chr10_jgvv3_438_t01	similar to RF|NP_195827.1|15241674|NM_120285 kinase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (39%)	1,105
		chr16_jgvv13_94_t01	weakly similar to UP|Q9FF29_ARATH (Q9FF29) Receptor serine/threonine kinase, partial (35%)	1,057
		chr1_jgvv11_606_t01	similar to UP|Q9SCZ4_ARATH (Q9SCZ4) Receptor-protein kinase-like protein, partial (27%)	-1,323
		chr11_jgvv37_60_t01	similar to UP|Q75WU3_POPNI (Q75WU3) Leucine-rich repeat receptor-like protein kinase 1, partial (28%)	-1,781
		chr4_jgvv8_272_t01	similar to GP|15215682|gb|AAK91387.1 AT3g49060/T2J13_100 {Arabidopsis thaliana}, partial (18%)	-1,241
		chr3_jgvv88_10_t01	similar to UP|Q56X19_ARATH (Q56X19) Leucine-rich repeat receptor-like kinase At1g09970, partial (55%)	-1,61
		chr3_jgvv132_35_t01	similar to RF|NP_195827.1|15241674|NM_120285 kinase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (39%)	1,133
		chrun_jgvv1542_1_t01	similar to UP|Q9LK35_ARATH (Q9LK35) Receptor-protein kinase-like protein, partial (23%)	1,169
	Signalling.calcium	chr11_jgvv206_8_t01	similar to UP|Q9FJI9_ARATH (Q9FJI9) Similarity to calmodulin-binding protein, partial (46%)	-2,825
		chr11_jgvv118_47_t01	weakly similar to RF|XP_473074.1|50926227|XM_473074 {Oryza sativa (japonica cultivar-group)} (exp=-1; wgp=0; cg=0), partial (15%)	-1,112
		chr18_jgvv122_103_t01	weakly similar to GB|AAP21364.1|30102892|BT006556 At1g76650 {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (66%)	-1,369
	Signalling.light	chr7_jgvv5_243_t01	similar to GP|13486760|dbj|BAB39994. P0498A12.24 {Oryza sativa (japonica cultivar-group)}, partial (9%)	-2,106
		chr12_jgvv35_81_t01	similar to GP|4914326|gb|AAD32874.1| F14N23.12 {Arabidopsis thaliana}, partial (34%)	-1,424

Table 3.45: Specific DEGs (in selected sub-bins from hormone metabolism) in plants treated with phosphate and subsequently inoculated (compared to inoculated plants)

Pathway	Bin Name	Gene ID	Annotation	Fold change
Hormone metabolism	Abscisic acid.synthesis-degradation	chr2_jgvv87_11_t01	similar to UP|Q2PHF8_LACSA (Q2PHF8) Carotenoid cleavage dioxygenase 1, partial (34%)	-1,638
		chr10_jgvv3_335_t01	UP|Q5SGD0_VITVI (Q5SGD0) 9-cis-epoxycarotenoid dioxygenase 2, complete	-1,561
	Abscisic acid.induced-regulated-responsive-activated	chr10_jgvv71_72_t01	similar to UP|Q6H5X2_ORYSA (Q6H5X2) ABA-responsive protein-like, partial (58%)	1,104
		chr10_jgvv71_63_t01	similar to UP|Q6H5X2_ORYSA (Q6H5X2) ABA-responsive protein-like, partial (58%)	1,049
		chr10_jgvv71_52_t01	similar to UP|Q6H5X2_ORYSA (Q6H5X2) ABA-responsive protein-like, partial (58%)	1,25
		chr10_jgvv71_56_t01	similar to UP|Q6H5X2_ORYSA (Q6H5X2) ABA-responsive protein-like, partial (58%)	-2,089
		chr4_pdvv23_331_t01	similar to UP|Q6H5X2_ORYSA (Q6H5X2) ABA-responsive protein-like, partial (58%)	1,175
	Auxin.induced-regulated-responsive-activated	chr3_jgvv38_325_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	1,457
		chr3_jgvv38_329_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	1,395
		chr3_jgvv38_328_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	1,541
		chr4_jgvv23_305_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	1,527
		chr4_jgvv23_304_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	1,734
		chr3_jgvv38_326_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	1,382
		chr3_jgvv38_336_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	1,283
		chr3_jgvv38_335_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	1,925
		chr3_jgvv38_337_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	1,625
		chr3_jgvv38_334_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	1,537
		chr3_jgvv38_322_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	1,928
		chr3_jgvv38_332_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	1,214
		chr3_jgvv38_333_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	2,168
		chr4_jgvv23_306_t01	similar to UP|Q8S351_CAPAN (Q8S351) Auxin-induced SAUR-like protein, partial (84%)	1,926
		chr18_jgvv1_489_t01	UP|Q84V38_VITVI (Q84V38) Aux/IAA protein, complete	-1,267
		chr10_jgvv3_289_t01	similar to UP|ARFE_ARATH (P93024) Auxin response factor 5 (Transcription factor MONOPTEROS) (Auxin-responsive protein IAA24), partial (18%)	1,44
	Ethylene.synthesis-degradation	chr9_jgvv2_475_t01	weakly similar to UP|Q945B6_ARATH (Q945B6) AOP1.2, partial (34%)	1,806
		chr5_jgvv77_104_t01	weakly similar to UP|Q48IK0_PSE14 (Q48IK0) Aldo-keto reductase family protein, partial (27%)	-1,643
		chrun_jgvv274_5_t01	similar to UP|Q5ZAK6_ORYSA (Q5ZAK6) BHLH transcription factor-like protein, partial (32%)	-1,679
	Gibberelin.synthesis-degradation	chr19_jgvv177_3_t01	weakly similar to GP|29825611|gb|AAO92303.1 gibberellin 2-oxidase 1 {Nicotiana sylvestris}, partial (24%)	1,329
		chr8_jgvv7_297_t01	similar to UP|Q2HRH3_MEDTR (Q2HRH3) Gibberellin regulated protein, partial (64%)	1,327
	Jasmonate.synthesis-degradation	chr9_jgvv2_89_t01	similar to UP|O24371_SOLTU (O24371) 13-lipoxygenase , partial (29%)	-1,6
		chr5_jgvv20_298_t01	similar to UP|Q7X9G5_FRAAN (Q7X9G5) Lipoxygenase , partial (31%)	1,11
		chr18_jgvv41_29_t01	similar to UP|Q76DL0_LITER (Q76DL0) LEDI-5c protein, partial (96%)	1,305
	Salicylic acid.synthesis-degradation	chr12_jgvv57_94_t01	weakly similar to UP|Q53L40_ORYSA (Q53L40) SAM dependent carboxyl methyltransferase, partial (29%)	-1,21
		chr4_jgvv23_150_t01	similar to UP|Q9SPV4_CLABR (Q9SPV4) S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase, partial (76%)	1,894

Table 3.46: Specific DEGs (in selected sub-bins from cell wall metabolism) in plants treated with phosphate and subsequently inoculated (compared to inoculated plants)

Pathway	Bin Name	Gene ID	Annotation	Fold change
Cell wall	Cell wall.precursor synthesis	chr12_jgvv59_48_t01	similar to UP|GALE1_ARATH (Q42605) UDP-glucose 4-epimerase (Galactowaldenase) (UDP-galactose 4-epimerase) , partial (96%)	1,137
	Cellulose synthesis	chr14_jgvv83_101_t01	similar to UP|COBL4_ARATH (Q9LFW3) COBRA-like protein 4 precursor, partial (93%)	1,651
		chr2_jgvv25_179_t01	similar to UP|O22989_ARATH (O22989) Cellulose synthase isolog, partial (14%)	1,198
	Hemicellulose synthesis	chr17_jgvv0_348_t01	similar to SP|Q9M5Q1|FUT1_PEA Galactoside 2-alpha-L-fucosyltransferase (EC 2.4.1.69) (Xyloglucan alpha-(1 2)-fucosyltransferase), partial (43%)	1,047
	Cell wall proteins.AGPs	chr8_jgvv40_121_t01	weakly similar to UP|Q7Y250_GOSHI (Q7Y250) Arabinogalactan protein, partial (68%)	1,085
	Cell wall proteins	chr18_jgvv1_673_t01	similar to RF|NP_188563.1|15230349|NM_112819 protein binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (49%)	1,272
		chr13_jgvv73_13_t01	similar to RF|NP_849414.1|30685110|NM_179083 SMB {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (27%)	-1,096
		chr3_jgvv63_2_t01	weakly similar to UP|Q2HTP6_MEDTR (Q2HTP6) Leucine-rich repeat, plant specific, partial (32%)	1,404
		chr5_jgvv49_62_t01	UP|Q8LGR5_VITVI (Q8LGR5) Proline rich protein 2, complete	1,466
		chr5_jgvv49_73_t01	homologue to UP|Q6QGY1_VITVI (Q6QGY1) Merlot proline-rich protein 2, partial (68%)	1,657
		chr5_jgvv49_58_t01	homologue to UP|Q8LGR5_VITVI (Q8LGR5) Proline rich protein 2, partial (73%)	1,315
		chr1_jgvv11_614_t01	homologue to UP|Q8VWN8_GOSHI (Q8VWN8) Reversibly glycosylated polypeptide, complete	1,067
	Cellulases and beta -1,4-glucanases	chr2_jgvv25_56_t01	weakly similar to UP|Q9SAE6_ARATH (Q9SAE6) F3F19.15, partial (50%)	1,231
	Degradation.mannan-xylose-arabinose-fucose	chr5_jgvv77_101_t01	similar to GB|AAS17751.1|42495032|AY486104 beta xylosidase {Fragaria x ananassa} (exp=-1; wgp=0; cg=0), partial (54%)	-1,444
	Degradation.pectate lyases and polygalacturonases	chr19_jgvv27_47_t01	weakly similar to UP|Q9MBB8_9ROSI (Q9MBB8) Polygalacturonase, partial (50%)	1,129
		chr12_jgvv57_32_t01	weakly similar to UP|Q9MBB8_9ROSI (Q9MBB8) Polygalacturonase, partial (50%)	1,145
		chr17_pdvv0_63_t01	similar to UP|Q4JLV6_GOSHI (Q4JLV6) Pectate lyase, partial (96%)	1,806
		chr14_jgvv66_95_t01	polygalacturonase [Vitis vinifera]	-1,055
	Cell wall.modification	chr14_jgvv108_94_t01	similar to UP|Q8L5J6_MALDO (Q8L5J6) Expansin 3, complete	1,457
		chr5_jgvv62_45_t01	homologue to UP|BRU1_SOYBN (P35694) Brassinosteroid-regulated protein BRU1 precursor, partial (82%)	1,068
	Pectin.esterases	chr11_jgvv16_14_t01	UP|Q94B16_VITVI (Q94B16) Pectin methylesterase PME1, complete	1,499
		chr16_jgvv50_201_t01	similar to UP|Q9FF93_ARATH (Q9FF93) Pectinacetylesterase, partial (79%)	-1,323

Table 3.47: Specific DEGs (in selected sub-bins from RNA metabolism) in plants treated with phosphate and subsequently inoculated (compared to inoculated plants)

Pathway	Bin Name	Gene ID	Annotation	Fold change
RNA metabolism	Transcription factor Family WRKY	chr10_jgvv3_484_t01	similar to UP|Q3SAJ9_CAPAN (Q3SAJ9) WRKY-A1244, partial (57%)	-1,155
		chr12_jgvv59_82_t01	homologue to UP|Q6B6R1_ORYSA (Q6B6R1) Transcription factor WRKY10, partial (24%)	-1,201
		chr4_jgvv23_308_t01	similar to UP|Q40090_IPOBA (Q40090) SPF1 protein, partial (65%)	-1,01
	Transcription factor Family MYB	chr17_jgvv0_689_t01	similar to UP|O49021_GOSHI (O49021) MYB-like DNA-binding domain protein (Myb-like transcription factor 5), partial (56%)	1,673
		chr11_jgvv16_109_t01	MYB-like protein [Vitis vinifera]	1,145
		chr15_jgvv46_313_t01	MYB-like protein [Vitis vinifera]	2,099
	Transcription factor AP2.Ethylene responsive element	chr19_jgvv138_4_t01	UP|Q3ZNL6_VITRI (Q3ZNL6) CBF-like transcription factor, complete	-2,71
		chr17_jgvv0_217_t01	UP|Q3ZNL6_VITRI (Q3ZNL6) CBF-like transcription factor, complete	-2,525
		chr3_jgvv63_43_t01	similar to UP|Q67U00_ORYSA (Q67U00) Ethylene-binding protein-like, partial (28%)	-2,008
		chr2_jgvv234_13_t01	similar to GB|BAA32418.1|3434967|AB008103 ethylene responsive element binding factor 1 {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (35%)	-2,003
		chr18_pdvv89_41_t01	weakly similar to RF|NP_177844.1|15223860|NM_106369 DNA binding {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (36%)	-1,451
		chr12_jgvv59_27_t01	similar to UP|Q4FH87_SOYBN (Q4FH87) Dehydration responsive element-binding protein 3, partial (54%)	-2,018
		chr16_jgvv13_67_t01	similar to GB|BAA32418.1|3434967|AB008103 ethylene responsive element binding factor 1 {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (35%)	-1,48
		chr2_jgvv25_404_t01	UP|Q3ZNL6_VITRI (Q3ZNL6) CBF-like transcription factor, complete	-2,545
		chr19_jgvv14_197_t01	similar to UP|Q5S004_CUCSA (Q5S004) Ethylene response factor 3, partial (39%)	-1,029
		chr11_jgvv16_478_t01	similar to GB|AAO63284.1|28950721|BT005220 At1g15360 {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (72%)	1,407

3.5.4 Changes in the gene expression pattern upon elicitation with Frutogard®

Elicitation with Frutogard® had less impact on the gene expression compared to elicitation with phosphonate and phosphate (Table 3.48). This was reflected in the absence of the DEGs in many pathways such as photosynthesis, glycolysis and polyamine metabolism to name a few (Table 3.48). However, elicitation with Frutogard® led to repression of 63% of the DEGs.

Stress related DEGs were mostly up-regulated. These involved genes coding for biotic stress receptors and abiotic stress proteins such as heat shock proteins. Genes coding for germin proteins were also up-regulated. However, genes coding for PR-proteins were down-regulated. (Table 3.49). Genes involved in secondary metabolite production were also up-regulated such as methanol anthraniloyal transferase, betaine aldehyde dehydrogenase and chalcone synthase (Table 3.49).

Most of the genes involved in hormone metabolism such as ABA-responsive proteins, cytokinin- and auxin-metabolism were down-regulated, while genes implicated in ethylene- -metabolism were up-regulated (Table 3.50). Copalyl pyrophosphate synthase, a gene involved in gibberellin metabolism, was strongly up-regulated, a Gip1-like protein, a protein involved in gibberelin metabolism was down-regulated.

No DEGs were observed in photosynthesis pathway, while only five genes involved in carbohydrate metabolism were differentially expressed most notably hexokinase and inositol oxygenase, which were down-regulated and beta-amylase and trehalose-phosphate phosphatase, which were up-regulated (Table 3.51).

Most of DEGs involved in cell wall synthesis were down-regulated such as cellulose synthase and pectinacetylesterase (Table 3.51). Nitrilases, enzymes that have a significant impact on the outcome of plant–microbe interactions were down-regulated (Table 3.51).

3.5.5 Changes in the gene expression pattern upon elicitation with Frutogard® and subsequent inoculation

Although elicitation with Frutogard® resulted in 462 DEGs, elicitation with Frutogard® and subsequent inoculation resulted in only 47 DEGs when compared to inoculated plants (Table 3.5). Interestingly, inoculation resulted in 3466 DEGs. It is not known why elicitation and inoculation together (compared to inoculated plants) resulted in only 47 DEGs while elicitation with phosphonate or phosphate and subsequent inoculation resulted in 2848 and 3390 DEGs, respectively (Table 3.5). Most of the pathways had no DEGs (Table 3.48). Among the DEGs that were up-regulated were gibberellin 2-oxidase in (hormone pathway), phosphoenolpyruvate carboxylase (glycolysis), lipid transfer protein (lipid synthesis) and multidrug resistance-associated protein-like protein (transport), while among the DEGs that were down-regulated were disease resistance protein (biotic stress receptors) and expressed proteins that belong to signalling pathways (Table 3.52).

Table 3.48: Differential regulation of genes grouped to 'bins' using the MapMan software after elicitation with Frutogard® and elicitation with Frutogard® and inoculation. Up-, down-regulated genes and total no. of genes are shown

Bin	Bin Name	Frutogard (P0) – control			Frutogard (P1) – inoculated		
		Down	**Up**	∑	**Down**	**Up**	∑
1	Photosynthesis	-	-	0	-	-	0
2	Major carbohydrate metabolism	1 (50%)	1 (50%)	2	-	-	0
3	Minor carbohydrate metabolism	2 (67%)	1 (33%)	3	-	-	0
4	Glycolysis	-	-	0	-	2 (100%)	2
5	Fermentation	-	-	0	-	-	0
6	Gluconeogenesis	-	-	0	-	-	0
7	Oxidative pentose phosphate pathway	-	-	0	-	-	0
8	TCA – organic transformation	-	1 (100%)	1	-	1 (100%)	1
9	Mitochondrial electron transport / ATP synthesis	-	1 (100%)	1	-	-	0
10	Cell wall	7 (78%)	21 (22%)	9	-	1 (100%)	1
11	Lipid metabolism	2 (50%)	2 (50%)	4	-	1 (100%)	1
12	N-metabolism	1 (100%)	-	1	-	-	0
13	Amino acid metabolism	3 (75%)	1 (25%)	4	-	-	0
14	S-assimilation	-	-	0	-	-	0
15	Metal handling	-	-	0	-	-	0
16	Secondary metabolism	2 (25%)	6 (75%)	8	-	1 (100%)	1
17	Hormone metabolism	9 (75%)	3 (25%)	12	-	1 (100%)	1
18	Vitamine metabolism	-	-	0	-	-	0
19	Tetrapyrrole synthesis	-	1 (100%)	1	-	-	0
20	Stress	2 (10%)	18 (90%)	20	2 (100%)	-	2
21	Redox	8 (89%)	1 (11%)	9	-	-	0
22	Polyamine metabolism	-	-	0	-	-	0

Table 3.48: continued…

Bin	Bin Name	Frutogard (P0) – control			Frutogard (P1) – inoculated		
		Down	Up	∑	Down	Up	∑
23	Nucleotide metabolism	1 (25%)	3 (75%)	4	-	-	0
24	Biodegradation of Xenobiotics	-	-	0	-	-	0
25	C1-metabolism	-	-	0	-	-	0
26	Miscellaneous	33 (70%)	14 (30%)	47	3 (75%)	1 (25%)	4
27	RNA processing and regulation	15 (68%)	7 (32%)	22	-	2 (100%)	2
28	DNA synthesis and repair	5 (71%)	2 (29%)	7	-	-	0
29	Protein metabolism	24 (77%)	7 (23%)	31	-	2 (100%)	2
30	Signalling	5 (56%)	4 (44%)	9	1 (100%)	-	1
31	Cell cycle and organization	6 (67%)	3 (33%)	9	-	-	0
33	Development	6 (86%)	1 (14%)	7	-	-	
34	Transport	9 (45%)	11 (55%)	20	1 (50%)	1 (50%)	2
35	Not assigned.no ontology	100 (65%)	54 (35%)	154	-	3 (100%)	3
∑	Total no. of genes in all bins	241 (63%)	144 (37%)	385	4 (22%)	14 (78%)	18

Table 3.49: DEGs (in selected sub-bins from stress pathways and secondary metabolism) in plants treated with Frutogard®

Pathway	Bin Name	Gene ID	Annotation	Fold change
Stress pathways	Stress.biotic.receptors	chr13_jgvv64_2_t01	similar to UP|Q71RI4_VITVI (Q71RI4) Resistance protein (Fragment), partial (67%)	1,483
		chr13_jgvv64_8_t01	weakly similar to UP|Q84TR2_PHAVU (Q84TR2) Truncated NBS-LRR resistance-like protein isoform JA88, partial (12%)	1,532
		chr5_jgvv51_22_t01	weakly similar to RF|NP_192939.2|30681996|NM_117272 WRKY19; transcription factor {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (4%)	1,013
		chr13_random_ jgvv221_3_t01	weakly similar to UP|Q84TR2_PHAVU (Q84TR2) Truncated NBS-LRR resistance-like protein isoform JA88, partial (12%)	1,482
		chr13_random_ jgvv112_29_t01	similar to UP|Q71RI4_VITVI (Q71RI4) Resistance protein (Fragment), partial (67%)	1,445
		chr13_random_ jgvv112_37_t01	weakly similar to UP|Q84TR2_PHAVU (Q84TR2) Truncated NBS-LRR resistance-like protein isoform JA88, partial (12%)	1,819
		chr18_jgvv75_1_t01	weakly similar to UP|Q9SCZ3_ARATH (Q9SCZ3) Disease resistance-like protein, partial (3%)	1,247
		chr18_jgvv75_26_t01	similar to GB|BAD82812.1|56790017|AB182389 CLV1-like LRR receptor kinase {Oryza sativa (japonica cultivar-group)} (exp=-1; wgp=0; cg=0), partial (3%)	1,519
		chr13_jgvv156_48_t01	weakly similar to UP|Q84TR2_PHAVU (Q84TR2) Truncated NBS-LRR resistance-like protein isoform JA88, partial (12%)	1,171
		chrun_jgvv335_5_t01	weakly similar to GB|BAB01339.1|11994217|AB028617 disease resistance comples protein {Arabidopsis thaliana} (exp=0; wgp=1; cg=0), partial (11%)	1,179
		chr13_jgvv101_20_t01	weakly similar to GB|BAB01339.1|11994217|AB028617 disease resistance comples protein {Arabidopsis thaliana} (exp=0; wgp=1; cg=0), partial (11%)	1,058
	PR-proteins	chr19_jgvv14_100_t01	similar to UP|Q9ZV96_ARATH (Q9ZV96) F9K20.18 protein, partial (71%)	-2,141
		chr15_jgvv46_183_t01	UP|CHIT3_VITVI (P51614) Acidic endochitinase precursor , complete	-1,838
	Stress.abiotic.heat	chr14_pdvv83_107_t01	similar to UP|HSP81_ORYSA (P33126) Heat shock protein 81-1 (HSP81-1) (Heat shock protein 82), partial (12%)	1,519
		chrun_jgvv131_20_t01	homologue to UP|Q6UJX6_NICBE (Q6UJX6) Molecular chaperone Hsp90-1, partial (59%)	1,636
	Stress.abiotic.unspecified	chr10_pdvv3_429_t01	GB|AY298727.1|AAQ63185.1 germin-like protein 3 [Vitis vinifera]	1,962
		chr10_pdvv3_427_t01	GB|AY298727.1|AAQ63185.1 germin-like protein 3 [Vitis vinifera]	1,804
Secondary metabolism	Phenylpropanoids	chr9_jgvv18_102_t01	similar to UP|Q3ZPN4_9ROSI (Q3ZPN4) Anthraniloyal-CoA: methanol anthraniloyal transferase, partial (83%)	1,12
	Alkaloid-like	chr4_jgvv210_5_t01	similar to UP|Q9FWE6_ORYSA (Q9FWE6) Mucin-like protein, partial (28%)	-1,412
	Betaine	chr14_jgvv36_75_t01	similar to UP|Q6JSK3_PANGI (Q6JSK3) Betaine aldehyde dehydrogenase, complete	1,188
	Flavonoids.chalcones	chr5_jgvv136_15_t01	UP|Q8W3P6_VITVI (Q8W3P6) Chalcone synthase , complete	1,491
	Flavonoids.dihydroflavonols	chr4_jgvv8_458_t01	homologue to UP|Q2QCX5_GOSHI (Q2QCX5) Gibberellin 20-oxidase 1, partial (94%)	1,634

Table 3.50: DEGs (in selected sub-bins from signalling pathways and hormone metabolism) in plants treated with Frutogard®

Pathway	Bin Name	Gene ID	Annotation	Fold change
Signalling pathways	Sugar and nutrient physiology	chr5_jgvv51_62_t01	similar to GP|30013669|gb|AAP03877.1 Avr9/Cf-9 rapidly elicited protein 141 {Nicotiana tabacum}, partial (2%)	1,233
	Receptor kinases.misc	chr10_jgvv3_442_t01	similar to RF|NP_195827.1|15241674|NM_120285 kinase {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (39%)	1,165
	Receptor kinases.misc	chr18_jgvv41_109_t01	homologue to UP|Q75UP2_IPOBA (Q75UP2) Leucine-rich repeat receptor-like kinase, partial (58%)	1,648
	Signalling.calcium	chr11_jgvv118_47_t01	weakly similar to RF|XP_473074.1|50926227|XM_473074 {Oryza sativa (japonica cultivar-group)} (exp=-1; wgp=0; cg=0), partial (15%)	-1,584
	Signalling.G-proteins	chr11_jgvv16_189_t01	weakly similar to UP|Q6DE71_XENLA (Q6DE71) MGC80037 protein, partial (13%)	1,448
Hormone metabolism	Abscisic acid metabolism	chr10_pdvv71_81_t01	similar to UP|Q6H5X2_ORYSA (Q6H5X2) ABA-responsive protein-like, partial (58%)	-1.586
		chr10_jgvv71_73_t01	similar to UP|Q6H5X2_ORYSA (Q6H5X2) ABA-responsive protein-like, partial (58%)	-1.944
	Auxin metabolism	chr19_jgvv14_276_t01	similar to UP|Q9LE80_ARATH (Q9LE80) Arabidopsis thaliana genomic DNA, chromosome 3, P1 clone: MJK13 (AT3g15450/MJK13_11) (MJK13.11 protein), partial (94%)	-3.16
	Cytokinin metabolism	chr7_jgvv5_525_t01	similar to UP|CKX1_ARATH (O22213) Cytokinin dehydrogenase 1 precursor (Cytokinin oxidase 1) (CKO 1) (AtCKX1) , partial (23%)	-2.378
		chr11_jgvv16_179_t01	similar to UP|CKX1_ARATH (O22213) Cytokinin dehydrogenase 1 precursor (Cytokinin oxidase 1) (CKO 1) (AtCKX1) , partial (23%)	-2.334
	Ethylene metabolism	chr10_jgvv116_16_t01	similar to UP|Q84RC3_NICSY (Q84RC3) Gibberellin 2-oxidase 1, partial (33%)	1.826
		chr5_jgvv49_31_t01	weakly similar to UP|Q948K9_CUCME (Q948K9) CmE8 protein, partial (51%)	1.061
	Gibberelin metabolism	chr7_jgvv151_5_t01	similar to UP|O22667_STERE (O22667) Copalyl pyrophosphate synthase, partial (20%)	3.333
		chr17_jgvv0_371_t01	similar to UP|Q49RB3_9ROSI (Q49RB3) Gip1-like protein, complete	-1.547

Table 3.51: DEGs (in selected sub-bins from carbohydrate, cell wall and nitrilase pathways) in plants treated with Frutogard®

Pathway	Bin Name	Gene ID	Annotation	Fold change
Carbohydrate metabolism	Carbohydrate degradation	chrun_jgvv2422_1_t01	similar to UP|Q9FVD3_CITSI (Q9FVD3) Hexokinase, partial (24%)	-1,028
		chr12_jgvv59_253_t01	similar to UP|Q5F305_SOYBN (Q5F305) Beta-amylase , partial (85%)	1,317
	Trehalose	chr11_jgvv37_65_t01	similar to UP|Q3ZTF5_TOBAC (Q3ZTF5) Trehalose-phosphate phosphatase, partial (54%)	1,214
		chr17_jgvv0_210_t01	similar to UP|Q9LMI0_ARATH (Q9LMI0) T2D23.11 protein, partial (33%)	-2,234
	Inositol oxygenases	chr11_jgvv16_244_t01	similar to UP|MIOX1_ARATH (Q8L799) Inositol oxygenase 1 (Myo-inositol oxygenase 1) (AtMIOX1) , partial (91%)	-3,884
Cell wall	Cell wall.precursor synthesis	chr2_jgvv25_381_t01	similar to UP|GALE1_ARATH (Q42605) UDP-glucose 4-epimerase (Galactowaldenase) (UDP-galactose 4-epimerase) , partial (96%)	-1,762
	Cellulose synthesis	chr2_jgvv25_181_t01	similar to UP|Q3Y6V1_TOBAC (Q3Y6V1) Cellulose synthase-like protein CslG, partial (33%)	-1,404
		chr14_jgvv6_202_t01	homologue to UP|Q6XP46_SOLTU (Q6XP46) Cellulose synthase, partial (39%)	1,101
		chr19_jgvv15_67_t01	weakly similar to UP|Q944E3_ORYSA (Q944E3) Cellulose synthase-like protein OsCslE2, partial (32%)	-1,51
	Cell wall proteins	chr15_jgvv46_216_t01	similar to RF|NP_566070.3|42569970|NM_130196 AGP16 (ARABINOGALACTAN PROTEIN 16) {Arabidopsis thaliana} (exp=-1; wgp=0; cg=0), partial (64%)	-1,394
		chr5_jgvv20_175_t01	similar to UP|O18465_HIRME (O18465) Tractin, partial (6%)	1,456
	Cell wall.degradation	chr5_jgvv77_101_t01	similar to GB|AAS17751.1|42495032|AY486104 beta xylosidase {Fragaria x ananassa} (exp=-1; wgp=0; cg=0), partial (54%)	-2,591
	Cell wall.modification	chr4_jgvv8_490_t01	similar to GP|20338421|gb|AAM18791.1 immuno-reactant natriuretic peptide-like protein {Erucastrum strigosum}, partial (26%)	-2,546
	Pectin*esterases	chr16_jgvv50_201_t01	similar to UP|Q9FF93_ARATH (Q9FF93) Pectinacetylesterase, partial (79%)	-1,442
Nitrilases	Nitrile lyases	chr2_jgvv33_59_t01	similar to UP|Q3LRV4_LUPAN (Q3LRV4) Nitrilase 4B, partial (88%)	-1.265
		chr2_jgvv33_51_t01	similar to UP|Q3LRV4_LUPAN (Q3LRV4) Nitrilase 4B, partial (88%)	-1.487
		chr2_jgvv33_50_t01	similar to UP|Q3LRV4_LUPAN (Q3LRV4) Nitrilase 4B, partial (88%)	-1.291
		chr2_jgvv33_54_t01	similar to UP|Q3LRV4_LUPAN (Q3LRV4) Nitrilase 4B, partial (88%)	-1.31
		chr2_jgvv33_57_t01	similar to UP|Q3LRV4_LUPAN (Q3LRV4) Nitrilase 4B, partial (88%)	-1.22
		chr2_jgvv33_60_t01	similar to UP|Q3LRV4_LUPAN (Q3LRV4) Nitrilase 4B, partial (88%)	-1.054
		chr2_jgvv33_61_t01	similar to UP|Q3LRV4_LUPAN (Q3LRV4) Nitrilase 4B, partial (88%)	-1.276
		chr2_jgvv33_46_t01	similar to UP|Q3LRV4_LUPAN (Q3LRV4) Nitrilase 4B, partial (88%)	-1.165

Table 3.52: DEGs (in selected sub-bins from different pathways) in plants treated with Frutogard® and subsequently inoculated (compared to inoculated plants)

Pathway	Bin Name	Gene ID	Annotation	Fold change
Glycolysis	Glycolysis	chr1_jgvv10_177_t01	homologue to UP|Q7FAH2_ORYSA (Q7FAH2) OJ000223_09.15 protein, partial (98%)	1.623
		chr1_jgvv11_381_t01	similar to UP|Q6Q2Z9_SOYBN (Q6Q2Z9) Phosphoenolpyruvate carboxylase , partial (23%)	1.013
TCA cycle	TCA – organic transformation	chr14_jgvv60_92_t01	homologue to UP|FUM1_ARATH (P93033) Fumarate hydratase 1, mitochondrial precursor (Fumarase 1) , partial (31%)	1.056
Lipid metabolism	Llipid transfer proteins etc	chr14_jgvv108_51_t01	weakly similar to UP|Q6EV47_CITSI (Q6EV47) Lipid transfer protein (Fragment), partial (90%)	2.819
Secondary metabolism	Flavonoids.anthocyanins	chr2_jgvv25_429_t01	UP|LDOX_VITVI (P51093) Leucoanthocyanidin dioxygenase (LDOX) (Leucocyanidin oxygenase) (Leucoanthocyanidin hydroxylase) , complete	1.344
Hormone metabolism	Gibberelin metabolism	chr19_jgvv177_3_t01	weakly similar to GP|29825611|gb|AAO92303.1 gibberellin 2-oxidase 1 {Nicotiana sylvestris}, partial (24%)	1.453
Stress	Stress.biotic.receptors	chr13_jgvv47_22_t01	weakly similar to GB|BAB01339.1|11994217|AB028617 disease resistance comples protein {Arabidopsis thaliana} (exp=0; wgp=1; cg=0), partial (11%)	-1.405
	Stress.abiotic.heat	chr17_jgvv0_825_t01	similar to UP|Q3E7E4_ARATH (Q3E7E4) Protein At5g35753, partial (26%)	-1.164
RNA metabolism	Regulation of transcription	chr10_jgvv42_99_t01	weakly similar to UP|Q3E8G9_ARATH (Q3E8G9) Protein At5g45113, partial (27%)	1.312
Signalling	Signalling.G-proteins	chr4_jgvv69_24_t01	weakly similar to UP|Q6ATR5_ORYSA (Q6ATR5) Expressed protein, partial (45%)	-1.612
Development	Development.storage proteins	chr7_jgvv31_75_t01	similar to UP|Q9FZ09_TOBAC (Q9FZ09) Patatin-like protein 1, partial (42%)	1.251
	Development.unspecified	chr15_jgvv46_291_t01	UP|Q9XGC2_VITVI (Q9XGC2) SINA1p, complete	-1.235
Transport	Transport.potassium	chr1_jgvv11_355_t01	similar to UP|HAK13_ORYSA (Q652J4) Probable potassium transporter 13 (OsHAK13), partial (35%)	2.22
	ABC transporters and multidrug resistance systems	chr9_jgvv2_219_t01	multidrug resistance-associated protein-like protein [Vitis vinifera]	1.301
		chr10_jgvv3_229_t01	similar to UP|Q9SDM5_GOSHI (Q9SDM5) P-glycoprotein, partial (20%)	1.101

4. DISCUSSION

4.1 Control of *Plasmopara viticola* in the greenhouse using resistance inducers

The greenhouse experiments were carried out with the aim of finding a sustainable alternative to copper-containing fungicides used to control *Plasmopara viticola* or at least to reduce the use of these fungicides. Some products (Frutogard®, Algin Biovital, ß-1,3-Glucan, Myco-Sin® VIN, phosphonate solo (a constituent of Frutogard®) and phosphate solo (a constituent of Algin Biovital®)) known as resistance inducers, plant strengtheners or plant activators were tested for their ability to control *P. viticola* under greenhouse conditions on potted vines of the variety Riesling, Müller-Thurgau, Solaris and Regent.

4.1.1 Efficiency of the elicitors in susceptible grapevine varieties (Riesling and Müller-Thurgau)

Strobilurin (BASF F500) (Cabrio®, **i.e.** ***Pyraclostrobin)***

Pyraclostrobin is active against fungal development stages both on the plant surface and within the tissues. It has protective as well as a curative action (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, Pyraclostrobin - Assessment Report). Moreover, there are evidences in the literature that Strobilurin can have some elicitor activity that may enhance plant resistance. Strobilurin had a protective action on Riesling, and both a protective and curative action on Müller-Thurgau (Figuer 3.1 and 3.2). The reasons for this are not very clear. However, plant variety might play a role in the efficiency of Strobilurin. Indeed, there are evidences for direct influences of strobilurins on plant physiology (Koehle et al., 2002). The so-called greening effect, in which disease free plants treated with strobilurins are intense green and look healthier than non-treated plants (Koehle et al., 2002). Therefore, it is suggested that strobilurins might also enhance the capability of plants to ward off pathogens (Herms et al., 2002).

Normally Strobilurin fungicides exert their effect on fungal pathogens by inhibiting the mitochondrial respiration (quinone outside inhibiting, QoI) through binding to the ubiquinol oxidation center of the mitochondrial bc1 complex (complex III) (Becker et al., 1981; Gisi et al., 2002), thereby blocking electron transfer (Sauter et al., 1999; Ammermann et al., 2000). However, it was found that Pyraclostrobin also possesses some elicitor activity that enhances the resistance (tolerance) of tobacco plants against tobacco mosaic virus and wildfire disease caused by *Pseudomonas syringae*

pv. tabaci possibly by priming the plants prior to subsequent attack (Herms et al., 2002). This was explained by acceleration of TMV-induced activation of PR-1 genes in tobacco plants. However, resistance (tolerance) to TMV generated by the strobilurin treatment was variable and cultivar dependent (Anderson et al., 2006).

Algin Biovital® and Frutogard®

Algin Biovital® and Frutogard® contain algae extracts (*Ascophyllum nodosum*, Laminaria spp.) as main ingredients. Algin Biovital® contains phosphate, while Frutogard® contains phosphonate.

Algin Biovital® showed better protection when used protectively, while Frutogard® provided better protection when applied curatively to Riesling (Figuer 3.1). Algin Biovital® provided moderate protection when applied protectively and curatively, while it showed high protection when applied protectively and curatively on Müller-Thurgau (Figuer 3.2). However, when the experiment was repeated in 2011, Algin Biovital® demonstrated again better protection when used protectively and Frutogard® provided better protection when applied protectively or curatively to Riesling (Figuer 3.3), while Algin Biovital® provided moderate protection when applied protectively and curatively and Frutogard® showed high protection when applied protectively and curatively to Müller-Thurgau (Figuer 3.4). There are evidences in the literature that Frutogard® induced defense responses against a broad spectrum of oomycetes and fungi such as *Plasmopara viticola, Phytophthora infestans*, *Botrytis cinerea* and *Erysiphe* spp. (Neuhoff et al., 2002). In a study to evaluate some resistance inducers against downy mildew in susceptible grapevines, it was found that susceptible grapevine cv. Grüner Veltliner (*Vitis vinifera* L. sativa) treated with Frutogard®, protectively, had no disease symptoms and the efficiency reached 100% in the greenhouse and outdoors (Harm et al., 2011). In another study in which Frutogard® was used to control *Peronospora destructor*, *Peronospora parasitica*, *Bremia lactucae* and *Pseudoperonospora cubensis* in lettuce and cucumber it was found that disease severity was reduced significantly by applying Frutogard® protectively (Kofoet and Fischer 2006). A combination of copper hydroxide at low rates with two or three applications of potassium-phosphonate (Frutogard®) at pre flowering to fruit set achieved a very good control of *P. viticola*. These results are in agreement with some long term studies on research trials (Kast 1996; Kauer 2003; Tamm et al., 2004; Tamm et al., 2006) and farm (Hofmann,

2003), while new copper-hydroxide formulation reduced the infection from 76 to 47% and was as successful as the combination with phosphonate (Frutogard®) (Berkelmann-Löhnertz et al., 2008).

The efficiency of Frutogard® and Algin Biovital® in the control of *P. viticola* was explained by the activation of the plant's defense responses (Heibertshausen, personal communication). These responses comprise formation of phytoalexins (e.g. resveratrol), PR-proteins, callose deposition at stomatal opening and production of hydrogen peroxide H_2O_2 close to the fungal infection (Hofmann, personal communication). Callose as well as H_2O_2 prevents the penetration of the fungus into the host, while phytoalexins also prevent the further spread and development of already penetrated fungal hyphae in the plant (Hofmann, personal communication).

Phosphates and phosphonates as resistance inducers

Phosphorus in the form of phosphate is one of the major plant nutrients influencing almost all biochemical processes and developmental phases of plants (Varadarajan et al. 2002). It is a constituent of cell membrane, nucleic acids, vitamins, proteins and ATP. In addition, it enhances the growth of shoot and root apex and leaves, whereas in grapes it improves the aroma (Bavaresco et al., 2010). Phosphates are usually used as fertilizers. Like phosphate, phosphonate is easily taken up and redistributed in the plant through the xylem and then the phloem (Street and Kidder 1989; Rickard, 2000). There are two types of phosphorus salts that are usually used in plant protection; phosphoric acid (H_3PO_4) and phosphorous acid (H_3PO_3). Phosphoric acid forms phosphate salts, while phosphorous acid dissociates to form the phosphonate ion ($HPO_3{}^{2-}$). Salts are termed phosphites when in dry powder form while in water they are converted to phosphonates (Anderson et al., 2006). They are used commercially as alternative to phosphate fertilizers, and increase plant growth (Anderson et al., 2006). However, when given as a foliar spray, disease resistance of plants is improved. Indeed, foliar spray of NPK fertilizers was shown to induce systemic protection against pathogens (Reuveni et al. 1996).

Phosphate-induced resistance

Phosphate provided better protection when applied protectively to Riesling (Figure 3.1 and 3.3), while on Müller-Thurgau it showed better protection when applied protectively and curativly (Figure 3.2 and 3.4). Phosphate-induced resistance was proven in many host-microbe interactions. Previous studies demonstrated the effectiveness of phosphate salts in inducing local and systemic protection against powdery mildew in cucumber plants (Gottstein and Kuc, 1989; Descalzo et al., 1990; Mucharromah and Kuc, 1991; Agapov et al., 1993; Reuveni et al. 1993; Orober et al. 2002), tomato (Ehret et al. 2002), broad bean (Walters and Murray, 1992), maize (Reuveni et al., 1994), pepper (Reuveni et al., 1998), rice (Manandhar et al., 1998) and in controlling powdery mildew on barley (Mitchell & Walter 2004) and on grapevines (Reuveni and Reuveni, 1995). Moreover, foliar sprays of phosphates have the potential for controlling powdery mildews in field-grown nectarine and mango trees, too (Reuveni and Reuveni, 1995). Moreover, it was shown that phosphate-induced resistance works against a broad spectrum of pathogens. In cucumber, application of phosphate led to systemic protection against eight diseases caused by fungi, bacteria and viruses (Mucharromah and Kuc, 1991). Therefore, it has been proposed that phosphate salts could be used either in rotation with fungicides or in a tank mix with reduced rates of fungicide in integrated disease-management programs (Reuveni et al., 1998a, b).

The mechanisms by which phosphates induce defense responses are not fully understood. However, phosphates are not toxic to pathogens, implying that the observed enhancement of resistance is due to the activation of plant defense. It is believed that phosphates sequester apoplastic calcium, altering membrane integrity and influencing the activity of apoplastic enzymes like polygalacturonases, thereby releasing elicitor-active oligogalacturonides from plant cell walls (Gottstein & Kuc, 1989; Walters and Murray, 1992). Indeed, later studies showed that phosphate-induced resistance in cucumber was associated with localized cell death, preceded by a rapid generation of superoxide and hydrogen peroxide (Orober et al., 2002). Moreover, it was found that salicylic acid was increased locally and systemically after phosphate application (Orober et al., 2002). Phosphates have shown efficacy mostly against powdery mildew fungi, which are sensitive even to free water and surfactants (Ehret et al., 2002). In barley, the application of phosphate, as K_3PO_4, to first leaves reduced powdery mildew infection by 89% in second leaves (Mitchell and Walters,

2004). This was explained by a significant increase in activities of phenylalanine ammonia-lyase (PAL), peroxidase and lipoxygenase in second leaves. Moreover, the activities of these enzymes were increased further after pathogen challenge (Mitchell and Walters, 2004).

Phosphates have also been shown to provide disease control under field conditions (Reignault and Walters, 2002). Indeed, foliar application of phosphate (K_3PO_4) on barley in a field trial reduced powdery mildew infection by up to 70% and gave an increase in grain yield of 12% compared to untreated controls (Mitchell and Walters, 2004). Whereas in cucumber grown hydroponically, phosphate applied to the hydroponic solution reduced powdery mildew infection by 80-92%, with reductions of up to 91% in numbers of conidia produced on infected leaves (Reuveni et al., 2000). Foliar application of mono-potassium phosphate fertilizer has been used successfully to manage powdery mildew in grapevine (Creasy and Creasy, 2009), and its alternation with organic fungicides such as demethylation inhibitors (DMIs) has resulted in control equivalent to the use of DMIs alone (Reuveni and Reuveni, 2002).

Phosphonate-induced resistance

Phosphonate showed better protection when applied protectively or curatively to Riesling (Figure 3.1). However, when the experiment was repeated in 2011 it exhibited better protection when applied protectively (Figure 3.3). On Müller-Thurgau the protection was moderated though slightly higher in protective treatment (Figure 3.2), while in 2011 protective treatment provided better protection (Figure 3.4).

Generally, phosphonates are well known to possess powerful antifungal activity (Ouimette and Coffey, 1989). They also have been shown to induce pathogen resistance in plants (Reignault and Walters, 2002). But interpretation of these findings is more complex because of debates on their mode of action (Anderson et al., 2006). Oxidation to phosphates is a presumed mechanism, while a direct fungicidal effect of phosphonates is also observed, especially for the fungal-like pathogens (oomycetes) such as *Pythium* spp., *Phytophthora* spp. and causal agents of downy mildews (Anderson et al., 2006). Phosphonates are believed to work through limiting polyphosphate formation in the fungi, a form in which fungi store phosphate reserves (Niere et al., 1994). Activation of plant defense is another proposed mode of action of the phosphonates (Smillie et al., 1989). For example, Phytogard® (a formulation containing 58% potassium phosphonate, K_2HPO_3), was

shown to provide protection against the downy mildew pathogen *P. parasitica* in cauliflower seedlings when used as a foliar spray or as a root treatment by reducing germination of *P. parasitica* spores (Becot et al., 2000). It was also argued that it induced resistance, since there was induction of β-1,3-glucanase and PR-2 protein, if only weak. Subsequent work showed that Phytogard® also induced resistance to *B. lactucae* in lettuce and led to inhibition of spore germination (Pajot et al., 2001). However, it had no effect on PR protein induction (Pajot et al., 2001). Interestingly, the fungicide Fosetyl-Al, marketed as Aliette® (active ingredient O-ethyl phosphonate), is known to exert both a direct effect on the pathogen and an indirect effect via stimulation of host defense reactions (Nemestothy & Guest, 1990). Moreover, it was found that it protects potatoes from *P. infestans* and grapevine from *Plasmopara viticola* (Dyakov et al., 2007). Inside the plant, it is ionized into phosphonate, and therefore belongs to the group of phosphorous acid compounds (Cohen and Coffey 1986; McGrath 2004). Phosphorous acid was demonstrated to be efficient against downy mildew on grapes (Förster et al., 1998). However, in BÖL-Project 514-43.10/03OE572 (Berkelmann-Löhnertz et al., 2008) it was shown that under high disease pressure the effect is not sufficient to ensure high quality grape production.

ß-1,3-Glucan

ß-1,3-glucan showed the lowest efficiencies of all when it was applied to Riesling (Figure 3.1). Surprisingly, when ß-1,3-glucan was applied to Müller-Thurgau protectively it had very high efficiency, while curative treatment had very low efficiency (Figure 3.2). One of main constituents of Algin Biovital® and Frutogard® is brown algae extracts (Laminaria spp.). Brown algae contain laminarin that is a water-soluble β-1,3-glucan (polysaccharide) (Read et al., 1996), which upon hydrolysis yields only glucose (Bavaresco et al., 2009). Laminarin was reported to induce defense responses in grapevine cells (Aziz et al., 2003). These responses included calcium influx, oxidative burst and activation of mitogen-activated protein kinases. Laminarin also induces the expression of defense genes associated with the octadecanoid, phenylpropanoid, stilbenoid pathways and PR proteins (Aziz et al., 2003). Moreover, in the *P. viticola* infected plants, treatment with laminarin led to a 75% reduction in lesion diameter (Bavaresco et al., 2009). However, defense responses triggered by β-1,3-glucan differ from one pathosystem to another. For

example, algal β-1,3-glucan induced the HSR gene in tobacco (Pontier et al., 1998), which is considered to be an HR-like marker gene of cell death, while it did not induce it in grapevine (Aziz et al., 2003). It is noteworthy to mention that the level of induced resistance is increased if laminarin is sulfated (laminarin sulfate, PS3) (Trouvelot et al., 2008; Menard et al., 2004).

Myco-Sin VIN®

Myco-Sin VIN® is a powder product based on acidified clay with high aluminium sulphate content, plant extracts (horsetail) and diatomaceous earth. Myco-Sin VIN® applied to Riesling showed a moderate efficiency when applied protectively and relatively low efficiency when applied curatively (Figure 3.1). When it was applied to Müller-Thurgau it showed rather no efficiency (Figure 3.2). However, in former studies it was found that even under extremely high infection pressure Myco-Sin VIN® had a very good effect in controlling *P. viticola* under greenhouse conditions (Heibertshausen, personal communication). Fischer (1996) found that Myco-Sin® (formulation not especially produced for vine application) under low infection pressure had good efficiency, while it was unsatisfactory under high infection pressure in the field. Other authors demonstrated that Myco-Sin® and its improved version Myco-Sin VIN® could have a good effect in controlling *P. viticola* under medium disease pressure (Patzwahl und Kopf, 1998; Hofmann, 2003a). Myco-Sin® (the older formulation) was also successful in controlling *P. infestans* in potato (Schüler, 1999). When Myco-Sin® was used in combination with Milsana® and a bacterial antagonist *(Brevibacillus brevis*) to control powdery mildew, downy mildew and Botrytis in grapevines, disease incidence of the mentioned pathogens was reduced (Schmitt et al., 2002).

Resistance inducers have the potential to amount defense responses gainst *Plasmopara viticola* in susceptible *V. vinifera*

V. vinifera is susceptible to many pathogens. However, it can defend itself against them, indicating that defense mechanisms are present, but not activated in response to the distinct pathogen (Polesani et al., 2010). Indeed, *V. vinifera* can react on *P. viticola* infection by activating the expression of defense-related genes (Busam et al., 1997; Hamiduzzaman et al., 2005; Kortekamp, 2006; Mzid et al., 2007; Chong et al., 2008; Trouvelot et al., 2008a), but this reaction is not sufficient to prevent or limit

pathogen spread (Gessler et al., 2011). Studies on early stages of *P. viticola* infection indicated the presence of a weak defense response in susceptible grapevines (Polesani et al., 2010). However, at the end of the incubation period (oil-spot stage) transcripts from all major functional categories, including defense processes, were strongly down-regulated (Polesani et al., 2008). It is assumed that compatible interaction between *V. vinifera* and *P. viticola* is probably achieved through a lack of recognition (Gessler et al., 2011) since *V. vinifera* did not co-evolve in the presence of *P. viticola* (Di Gaspero et al., 2007). Therefore, resistance inducers were used to enhance the tolerance in susceptible grapevines and reduce downy mildew growth and spread and, therefore, symptom development (Gessler et al., 2011). Mechanisms of induced resistance against *P. viticola* have been shown to involve stomatal closure (Allègre et al., 2009), the expression of defense genes (Hamiduzzaman et al., 2005; Trouvelot et al., 2008b; Harm et al., 2011; Perazzolli et al., 2011), increased enzymatic activity (Godard et al., 2009; Harm et al., 2011), callose deposits (Hamiduzzaman et al., 2005) and the accumulation of phytoalexins (Dercks and Creasy, 1989b; Slaughter et al., 2008; Ferri et al., 2009; Godard et al., 2009).

4.1.2 Efficiency of the elicitors in tolerant grapevine varieties (Regent and Solaris)

The best performing elicitors (Algin Biovital®, Frutogard®, phosphonate and phosphate) were applied protectively on tolerant varieties (Regent and Solaris).
As expected, the absence of symptoms on Regent and Solaris indicate their tolerance to *P. viticola*. Indeed, two genes, *VRP1-1* and *VRP1-2* (for Resistance to *P. viticola*) were identified. However, VRP1-1 and VRP1-2 sequences show nucleotide polymorphism when compared in the downy mildew resistant Vitis accession Regent (Kortekamp et al., 2008). Slaughter and colleagues (2008) also used Solaris for disease management studies under greenhouse conditions. They reported that BABA was able to induce additional tolerance in Solaris, were transcript levels of genes involved in transresveratrol, trans δ-viniferin and trans-pterostilbene pathways were up-regulated (Slaughter et al., 2008).

Accumulation of stilbenes in resistant varieties in response to inoculation and/or elicitation

Defense responses were measured in protectively treated one-year old potted vine plants. These responses were represented in real-time optical signatures by the content of constitutive and induced flavonols (phytoalexins), mainly stilbenes. These measurements were made possible due to the autofluorescent property of stilbenes (Hillis and Ishikura, 1968, Jeandet et al., 1997), since they display a violet-blue fluorescence under UV in leaves (Poutaraud et al., 2007). Stilbenes are one of the major induced polyphenolics produced in stressed grapevine. In general, Regent vines produced more stilbenes than Solaris under protected conditions in the greenhouse (Figure 3.7 a and b). Control plants (neither inoculated nor elicited) of Regent and Solaris showed the lowest level of stilbenes, which indicates that stilbene production is constitutive in Regent and Solaris vines. Moreover, Regent plants that were treated with Frutogard® and Algin Biovital® had the highest content of stilbenes followed by phosphate, then phosphonate. Inoculated, non-elicited vines showed a low content of stilbenes compared to elicitor-treated plants. Stilbene content in Solaris followed a similar pattern, where plants treated with Frutogard® and Algin Biovital® had the highest content followed by phosphate, then phosphonate. It is proved in the literature that stilbene content can be increased due to treatments with resistance inducers. Larronde and colleagues (2003) have shown that very low levels of atmospheric methyl jasmonate can enhance the synthesis of trans-resveratrol, cis-resveratrol, ε-viniferin and piceids in the leaves of grapevine. Treatment with chitosan oligomers triggers the accumulation of stilbenes in grapevine leaves (Aziz et al., 2006), while treatment with salicylic acid triggers the synthesis of resveratrol (Li et al., 2008). No studies so far tested the effect of the here investigated resistance inducers on stilbene biosynthesis. However, the effect of Frutogard® and Algin Biovital® was explained by the activation of different plant defense responses, among them phytoalexins (Hofmann, personal communication).

However, in case of Solaris, inoculated and non-elicited plants showed relatively high level of stilbene concentration. This was shown by several other working groups. They found that Stilbenes are also produced in response to infection with *P. viticola* (Langcake et al., 1981; Dercks et al., 1989; Pezet et la., 2003, 2004; Hammerschmidt, 2004). Their presence and specific mode of action in some tolerant varieties may be strong enough to stop the infection (Chong et al., 2009) but not in

susceptible varieties (Latouche et al., 2013). In susceptible varieties, the pathogen spreads despite an increasing content of stilbenes (Poutaraud et al., 2010; Latouche et al., 2013). Indeed, flavonols, e.g. stilbenes, are known to contribute to plant resistance (Harborne and Williams, 2000; Treutter, 2005; Pourcel et al., 2007. The correlation between *P. viticola* induced stilbenes and the resistance level of the individual cultivars is now well established in grapevine (Pezet et al., 2004; Chong et al., 2009; Alonso-Villaverde et al., 2011; Malacarne et al., 2011). Alonso-Villaverde and colleagues (2011) showed that resistant varieties (e.g. Solaris) react rapidly to *P. viticola* infections by producing high concentrations of stilbenes. However, the type of stilbene produced may differ according to the variety. For example, in Solaris viniferins are produced while in the cultivar IRAC 2091 pterostilbene is produced (Alonso-Villaverde et al., 2011).

Additionally, our results showed diurnal fluctuations in stilbene content in Regent and Solaris, where stilbene content was not stable during the day. It was observed that stilbene content in Regent increased at 3 hat (hour after treatment) then decreased at 6 hat and then increased again to reach its maximum at 24 hat (Figure 3.7 a and b), while it followed that same pattern in the second day. However, in Solaris, stilbene content suddenly increased to reach its maximum at 24 hai (hour after inoculation) or 48 hat (Figure 3.7, b). These fluctuations were also observed in other studies. The decreased stilbene content may be due either to a lower metabolism of damaged leaves (Malacarne et al., 2011) or the pathogen degrades stilbenes (Latouche et al., 2013). However, there is no data that support the second explanation (Latouche et al., 2013), but a laccase-mediated degradation of stilbenes (probably detoxification) by *Botrytis cinerea* has been shown (Adrian and Jeandet, 2006).

4.2 Defense related genes are induced after elicitation and/or inoculation

4.2.1 Stilbene synthase (STS)

The expression of STS was different between treatments. Plants that were only elicited with phosphate, phosphonate, Algin Biovital® and Frutogard® showed an early strong expression of STS at 24 h after elicitation, while Strobilurin showed a strong expression at 72h. Plants treated with ß-1,3-glucan and Myco-Sin® VIN did not exhibited any significant expression of STS. Control plants (neither treated nor inoculated) and plants treated with water exhibited a slight expression of STS in the

first two days (Figure 3.14). Elicitor treatments such as ergosterol (Laquitaine et al., 2006), BcPG1 (Poinssot et al., 2003), oligogalacturonates (Aziz et al., 2004) and ß-1,3-glucan sulfate (Trouvelot et al., 2008) were shown to induce STS expression. Moreover, abiotic stress factors such as UV light (Langcake and Pryce, 1976; Bonomelli et al., 2004), heavy metals (Adrian et al., 1997a) and ozone (Schubert et al., 1997) can also induce the expression of STS.

Inoculation had a strong impact on the expression of STS, where inoculated plants demonstrated a strong expression at 24 h after inoculation, which decreased at 96h, then strongly increased until the 7th day after inoculation. However, plants that were elicited and inoculated demonstrated a strong expression of STS in the early days after inoculation. This trend was observed in plants treated with Strobilurin, Algin Biovital®, Frutogard® and ß-1,3-glucan and Myco-Sin® VIN. Plants that were treated and inoculated with phosphonate and phosphate demonstrated a slight expression in the early days after elicitation and inoculation that decreased with time (Figure 3.14). It is known from previous studies that phytoalexins such as stilbenes are synthesized in response to stress factors such as pathogen attack (Jeandet et al., 2002). Indeed, phytoalexins are active against many pathogens such as *P. viticola*, *Botrytis cinerea* and *Erysiphe necator* (Langcake and Lovell, 1980; Hoos and Blaich, 1990; Celimene et al., 2001). Stilbenes are produced at one of the last steps of the phenylpropane pathway by STS which share same substrates with chalcone synthase (CHS), the key enzyme in flavonoid biosynthesis (Schroder et al., 1990; Ferrer et al., 1999). It was found that the expression of STS in grapevine can be induced by several pathogens (Jeandet et al., 1991; Douillet-Breuil et al., 1999; Adrian et al., 2000; Borie et al., 2004). Moreover, expressing grapevine STS in tobacco resulted in the production of resveratrol, where transformed plants showed enhanced resistance to *B. cinerea*, albeit at levels too low for commercial interest (Hain et al., 1993).

4.2.2 9-Lipoxygenase (9-LOX)

Elicitation with Strobilurin, phosphate, Algin Biovital®, Frutogard®, ß-1,3-glucan and Myco-Sin® VIN led to a moderate expression of 9-LOX. However, treatment with Algin Biovital® showed a relatively strong expression only at the beginning (Figure 3.15). Plants treated with phosphonate showed a high expression at the 5th day after elicitation followed by a decrease. Control plants and plants treated with water showed a steady low expression. Lipoxygenases are involved in the biosynthesis of

oxylipins, oxygenated fatty acids, which are believed to be involved in plant resistance strategies (Gerwick, 1991; Blée, 2002). The biosynthesis of plant oxylipins is initiated by the action of lipoxygenases (9-LOX and 13-LOX) or α-dioxygenase (α-DOX) (Feussner and Wasternack, 2002; Vellosillo et al., 2007). LOX-derived oxylipins are involved in physiological processes of plants such as growth and fertility (Creelman and Mullet, 1997; Sanders et al., 2000; Stintzi and Browse, 2000), adaptation of plants to adverse growth conditions (Staswick et al., 1992; Creelman and Mullet, 1995; Armengaud et al., 2004) infection with pathogens and upon elicitor treatments (Rusterucci et al., 1999; Porta et al., 2002). Previous studies showed that lipoxygenase pathway can be stimulated with elicitor treatment such as salicylic acid (Weichert et al., 1999), methyl jasmonate (Avdiushko et al., 1995; Kohlmann et al., 1999) and laminarin (Aziz et al. (2003). Biotic elicitors can also induce the lipoxygenase pathway (Rustérucci et al., 1999). In potato, the 9-LOX pathway was preferentially stimulated in elicitor-treated cells (Gobel et al., 2001). Treatment of single barley leaves with oxylipins reduced infection of that leaf by the powdery mildew fungus *Blumeria graminis* (Cowley and Walters, 2005).
Inoculated vines as well as vines elicited and inoculated with Algin Biovital®, Frutogard®, ß-1,3-glucan and Myco-Sin® VIN showed an early expression of 9-LOX that increased with the time course to reach its maximum at the 7th day after inoculation. Plants elicited with phosphonate, phosphate and Strobilurin and subsequently inoculated showed a moderate expression throughout the time course (Figure 3.15). Indeed, it was found that infection with pathogens can also stimulate the lipoxygenase pathway (Croft et al., 1993; Montillet et al., 2002; Rancé et al., 1998). Previous studies showed that bacterial and fungal pathogens can stimulate lipoxygenase pathway (Melan et al., 1993; Sanz et al., 1998; Jalloul et al., 2002; Turner et al., 2002, Vellosillo et al., 2007; Blée, 2002; Farmer et al., 2003), while non-pathogenic bacteria can also induce this pathway (Ongena et al., 2004). The role of the products made through 13-LOX pathway is known, since these include jasmonates, potent biological regulators (Reignault and Dale Walters, 2002). However, the products of the 9-LOX pathway have only recently gained attention as potential defense compounds (Reignault and Dale Walters, 2002). Moreover, it has been demonstrated that 9-LOX is important for resistance in tobacco and potato to *Phytophthora parasitica* and *Pseudomonas syringae*, repectively (Rance et al., 1998; Gobel et al. 2002). In grapevine it was shown that 9-LOX expression increased

following laminarin treatment (Aziz et al., 2003) and fungal elicitor treatment in cell suspension (Gomes et al., 2003; Laquitaine et al., 2006). Specific products of the 9-LOX pathway also accumulated in response to infection by *Phytophthora infestans* (Weber et al., 1999). Moreover, its expression starts earlier in incompatible plant-pathogen interactions than in compatible ones, thus supporting a role for this 9-LOX in plant defense against fungal infection (Rance et al., 1998).

4.2.3 Chitinase (CHIT_1b)

Chitinase, an antimicrobial protein, is a PR-3 basic class I chitinase. In our study treated and inoculated plants mainly showed an early strong expression of CHIT_1b that decreased afterwards. However, plants treated with ß-1,3-glucan and subsequently inoculated, showed a low level of expression that increased with time (Figure 3.16). Chitinase induction upon pathogen infection has been reported in many plants (Bowles 1990; Collinge et al., 1993; Graham and Sticklen 1994; Gomes and Coutos-Thevenot, 2009). Indeed, when the susceptible variety Riesling was inoculated with *Pseudoperonospora cubensis* (causal agent of downy mildew in cucumber), a non-host pathogen in grapevine, chitinases were largely accumulating in comparison with a host situation (*P. viticola)* (Gomès and Coutos-Thévenot, 2009). The type of chitinase produced is different in a compatible or incompatible interaction (Robert et al., 2002). Therefore, this selective expression of specific chitinases might be a reliable indicator of the SAR response in *V. vinifera* (Busam et al., 1997). *P. viticola* is an oomycete and contains mainly glucan in its cell wall (Ruiz-Herrera, 1992). However, recent studies showed that it contains some chitin in its cell wall as well (Agrios, 2005; Kortekamp, 2008). Previous studies showed that chitinases along with ß-1,3 glucanases form the natural defense mechanism in a number of plants upon attack by pathogenic fungi (Logemann et al., 1992). Even plant growth promoting rhizobacteria suppress infection by pathogens through a number of mechanisms among which chitinases play an important role (Van Loon et al., 1998).
Plants treated with elicitors showed an early strong expression of CHIT_1b that decreased afterwards (Figure 3.16). Indeed, plants treated with Algin Biovital® or Frutogard® exhibited a low chitinase expression. However the expression was lower compared to inoculated plants (Selim et al., 2012). Previous studies pointed that chitinase genes are known to be regulated under stress conditions such as elicitor treatment (Kombrink and Somssich, 1995). It was reported that BTH successfully

induces resistance to various pathogens (Vallad and Goodman, 2004). In most cases, this effect was associated with an increase in PR proteins such as chitinases (Heil, 2002). Treating wheat and pea with chitosan led to increased chitinase expression (Hofgaard et al., 2005; Mauch et al., 1984). BABA induced chitinase in tobacco, tomato and pepper (Cohen et al., 1999; Siegrist et al., 2000). It is worth mentioning that constitutive expression of chitinases in disease-tolerant cultivars has been noted in grapevine (Busam et al., 1997).

4.3 Microarrays as a method to investigate differentially expressed genes

In this work, the differences (transcriptional responses) between different treatments such as inoculation, elicitation and elicitation and inoculation were characterized in order to elucidate the molecular mechanisms underlying induced resistance in the susceptible grapevine (*Vitis vinifera* L. cv. Riesling) against *Plasmopara viticola*, the causal agent of downy mildew. Therefore, a transcriptome analysis was performed to highlight the genes involved in this pathosystem as well as the genes involved in induced resistance mechanisms against *P. viticola*. Many genes that belong to many different pathways were differentially expressed and discussing them all would be beyond the scope of this study (Table 3.5). Therefore, we focused on certain pathways, which are identified by MapMan, that are involved in plant resistance such as stress, secondary metabolism, hormone metabolism, signalling, carbohydrate metabolism, cell wall and RNA processing. In some cases, other pathways, that demonstrated a conspicuous trend, are also discussed. Several reports using microarray analysis to elucidate this specific pathosystem exist in the literature (Figueiredo et al., 2008; Kortekamp et al., 2008; Polesani et al., 2008; Polesani et al., 2010; Wu et al., 2010; Malacarne et al., 2011). However, none of them characterized the transcriptional responses of *Vitis vinifera* to elicitation with resistance elicitors or the response to the pathogen after elicitation of induced resistance.

4.3.1 Transcriptional changes during *P. viticola*-grapevine interaction

Up-regulation of stress pathways after inoculation with P. viticola

Most of the identified differentially expressed genes (DEGs) involved in stress pathways and signalling were up-regulated (Table 3.7 and 3.8). These comprised genes coding for biotic receptors and PR-proteins such as class IV chitinases and PR-10 as previously shown by other authors (Kortekamp, 2006; Polesani et al., 2010; Figueiredo et al., 2012, Robert et al., 2001). Inoculation of grapevine with *Pseudoperonospora cubensis* (downy mildew of cucumber) and *Erysiphe necator* (the causal agent of powdery mildew of grapevine) induced chitinases too. Interestingly, induction of chitinases as well as the type of chitinase in *V. vinifera* depends on the infecting pathogen (Robert et al., 2002). The function of PR-10 proteins is still not known (Kortekamp, 2006). However, many functions such as related to biotic as well as abiotic stress have been suggested (Fernandes et al., 2013). However, sequence analysis indicated that they are structurally related to

ribonucleases located in the cytosol (Moiseyev et al., 1997). Some PR-proteins such as ß-1,3-glucanases (PR-2) and germin-like proteins (PR-16) were down-regulated. Kortekamp (2006) reported that genes coding for ß-1,3-glucanases were slightly down-regulated, their expression being delayed in resistant grapevine and not detectable in susceptible varieties such as Riesling. This may be because the pathogen suppress the synthesis of genes coding for ß-1,3-glucanases since they are thought to provide defense against the pathogen by hydrolyzing components of the cell walls (Mauch et al., 1988). Genes coding for germin-like proteins were down-regulated. Legay et al. (2011) and Godfrey et al. (2007) also reported down-regulation of germin-like proteins after infection with *P. viticola*. Germin-like proteins are the last class of PR-proteins described in grapevine. They exhibit oxalate oxidase or superoxide dismutase activities, however, their exact role in plant defense is not yet elucidated (Gomès and Coutos-Thévenot, 2009). They are expressed in response to a number of biotic and abiotic stresses (Bernier and Berna, 2001), including challenge by *Erysiphe necator*, *P. viticola* and *B. cinerea*.

Two genes coding for dirigent proteins were down-regulated. The presence of dirigent proteins was suggested in grapevines (Gang et al., 1999; Barselo et al., 2003) long before the grapevine genome was sequenced. These proteins were proposed to play a role in lignin synthesis (Davin and Lewis, 2000). Moreover, they could be important in the disease resistance processes because they are supposed to be responsible for the synthesis of highly toxic γ-viniferins, the presence of which in stressed grapevine leaves is well correlated with resistance against *P. viticola* (Pezet et al., 2005). Similarly, it was found that powdery mildew induced dramatic up-regulation of genes coding for dirigent-like proteins in grapevine (compatible interaction) (Fung et al., 2008). Our data, however, showed that two dirigent-like proteins were down-regulated. This may indicate that grape-oomycete interaction (*P. viticola*) is different from that of grape-fungi (*U. necator*).

Down-regulation of secondary metabolism after inoculation with P. viticola

Production of phytoalexins is another plant physiological change upon oomycete infection (Hardham, 2007). Our results showed that most of the genes involved in secondary metabolism (mainly related to phytoalexins) were down-regulated such as genes implicated in isoflavonol and terpenoid synthesis (Table 3.9). Terpenoids are

produced by organisms in all kingdoms of life (Davis et al., 2000) and make up the largest class of plant secondary metabolites (Dudareva et al., 2006). They also play a role in plant defense (Arimura et al., 2005). Terpenoids are the precursors of primary plant products such as the plant hormones (Logan et al., 2000) gibberellins and abscisic acid (Kant et al., 2009). Therefore, we assume that *P. viticola* down-regulates the biosynthesis of terpenoids in order to block/manipulate hormone biosynthesis in the host. Indeed, our study shows that most of the genes involved in hormone metabolism were down-regulated (discussed under hormone metabolism). Genes implicated in stilbene biosynthesis such as stilbene synthase and resveratrol synthase were up-regulated. Stilbenes are stress-induced phenylpropanoids synthesized by stilbene synthase (Amâncio et al., 2009). In grapevine, resveratrol, a low molecular weight stilbene, acts as a phytoalexin (Bavaresco et al., 2009). Stilbene synthase was reported to be induced in response to filamentous pathogen infection such as *P. viticola*, *B. cinerea*, *Phomopsis viticola* and *E. necator* in *V. vinifera* (Kortekamp 2006; Chong et al., 2008; Melchior and Kindl, 1991; Bavaresco and Fregoni, 2001; Tassoni et al., 2005, Fung et al., 2008; Bavaresco et al., 2009).

Down-regulation of hormone metabolism after inoculation with P. viticola

Plant hormones are known to play a role as primary signals in the regulation of plant defense (Verhage et al., 2010). Our results show that hormone responsive genes were mainly down-regulated (Table 3.10). These were mainly genes involved in the biosynthesis of abscisic acid (ABA), auxin, brassinosteroids, jasmonic acid and salicylic acid. It has been reported that ABA plays important roles in plant defense responses (Mauch-Mani and Mauch, 2005; Mohr and Cahill, 2007; de Torres-Zabala et al., 2007; Adie et al., 2007). Its precise role in plant defense is fragmentary, controversial (Asselbergh et al., 2008a; Lopez et al., 2008), complex and varies among different types of plant-pathogen interactions (Bari et al., 2009). In general, ABA is thought to be involved in the negative regulation of plant defense responses against many biotrophic and necrotrophic pathogens (Bari et al., 2009). Several reports have shown a negative impact of ABA treatment on resistance to pathogens in several plant species, while others have shown a positive role of ABA on activation of resistance against pathogens (Mauch-Mani and Mauch, 2005). ABA activates stomatal closure as a barrier against biotrophic pathogens (Melotto et al., 2006) such as oomycetes. Therefore, it may be assumed that *P. viticola* supresses the

biosynthesis of ABA in order to keep the stomata open for its entry. Indeed, there were studies that have reported a transpiration increase in the case of oomycete diseases (Allègre et al., 2006) indicating a host manipulation of the stomatal opining/closure activity. In case of infection with *P. viticola,* stomatal deregulation occurs quite early before macroscopic symptoms appear and increases with leaf colonization by the pathogen (Allègre et al., 2006). Similar observations were reported in another oomycete-host interaction (*P. cubensis*–cucumber) (Lindenthal et al., 2005).

Previous studies have postulated that part of the invading strategy of pathogens is the stimulation of auxin signalling (Chen et al., 2007) because increased auxin levels could suppress plant defenses and alter host physiology to favor pathogen growth and establishment (Lopez et al., 2008). Therefore, plants repress auxin signalling as a component of basal resistance (Heil and Walters, 2009). In our study, a set of auxin-responsive genes were down-regulated. Similarly, a global down-regulation of auxin-responsive genes was reported during *B. cinerea* infection in Arabidopsis (Llorente et al., 2008).

In grapevine, brassinosteroids are mainly responsible for processes like accumulation of sugars, metabolism of organic acids, synthesis of aroma compounds and accumulation of anthocyanins in the berry skin (Symons et al., 2006). However, brassinosteroids have been shown to have roles in growth promotion and stress response (Haubrick and Assmann, 2006) in different plants, where they alleviate biotic challenges of bacterial, fungal and viral pathogens (Krishna, 2003; Ali et al., 2007; Jager et al., 2008). Our results showed that most of genes involved in the metabolism of brassinosteroids were down-regulated. This may be due to pathogen suppression of genes responsible for brassinosteroids synthesis or due to a cross-communication between plant hormones that allows the plant to fine-tune its defense responses. However, there are no reports on the role of brassinosteroids in grapevine defense response.

According to our results, all DEGs involved in ethylene metabolism were up-regulated. Polesani et al. (2010) reported an induction of a gene involved in ethylene metabolism during *P. viticola* infection of resistant and susceptible varieties, the induction being more important in resistant varieties. In another study, several genes involved in ethylene biosynthesis were also induced after *P. viticola* infection (Malacarne et al., 2011).

DEGs involved in gibberellin metabolism did not show a clear trend. Gibberellins are plant hormones that regulate many processes during plant development involving cell division and expansion. However, in grapevine they are thought to be involved in early berry expansion and fruit set (Davies and Böttcher, 2009). There are no reports on the role of gibberellins in *P. viticola/*grapevine interaction. However, it is tempting to assume that the pathogen is trying to maintain the plant health by keeping cell division and expansion going through induction of gibberellin-responsive genes so that it can get its nutrition from the cells, while the plant is trying to down-regulate these genes. In mutualistic association of barley roots with *Piriformospora indica* , gibberellin was identified as a factor of compatibility during manipulation of plant innate immunity (Schäfer, 2009). This may partly explain the unclear trend in DEGs in gibberellin pathways.

Inoculation with *P. viticola* led to down-regulation of genes involved in salicylic acid metabolism such as salicylic acid carboxyl methyltransferase. Salicylic acid plays a crucial role in plant defense against biotrophic and hemi-biotrophic pathogens as well as the establishment of systemic acquired resistance (Grant and Lamb, 2006). Infected plants show increased levels of salicylic acid in pathogen challenged tissues of plants and exogenous applications result in the induction of pathogenesis related (PR) genes and enhanced resistance to a broad range of pathogens (Bari and Jones, 2009). According to Fung et al. (2008), salicylic acid levels increased in *V. vinifera* 120 h after inoculation with powdery mildew. However, in our study we investigated samples that were taken 24 h after inoculation. Therefore, it is difficult to say if the plant activates SAR later, since it has been reported that SAR induction may take several days after infection to develop (Hammerschmidt, 2009). But we may hypothesize that *P. viticola* rapidly suppresses the salicylic acid pathway as a way to suppress SA-based local defense on one hand and to suppress SAR activation on the other hand. Methyl salicylate is believed to function by being converted back to salicylic acid (the active form) (Park et al., 2007; Vlot et al., 2008a, b), leading to the activation of defense-related genes in the target tissues (Shulaev et al., 1997; Seskar et al., 1998). It was demonstrated that methyl salicylate can act as a long-distance mobile signal for SAR (Dempsey and klessig, 2012).

Infection with *P. viticola* led to down-regulation of genes involved in jasmonic acid metabolism. Genes coding for lipoxygenases (LOXs) were mostly down-regulated. However, a gene coding for a 13-LOX was up-regulated. It is known that 13-LOX

pathway produces jasmonic acid (Halitschke and Baldwin, 2003). Several studies have shown that the expression of genes encoding the enzymes initiating the synthesis of oxylipins as jasmonate was induced in many pathosystems (Melan et al., 1993; Sanz et al., 1998; Jalloul et al., 2002; Turner et al., 2002). In grapevine, the induction of jasmonic acid upon inoculation with *P. viticola* depends on the species. There was a rapid increase in the levels of jasmonic acid and methyl jasmonate in leaves of the resistant variety *V. riparia* 48 h after inoculation, while in *V. vinifera* there was no change in the basal level of jasmonic acid after inoculation and only a limited increase in methyl jasmonate levels at 24 and 48 h after inoculation (Polesani et al., 2010). LOXs catalyze the dioxygenation of polyunsaturated fatty acids, and are classified as either 9-LOX or 13-LOX depending on the positional specificity of the enzyme towards linoleic acid (Feussner et al., 2002). Although they are believed to generate signal molecules such as jasmonate or methyl jasmonate (LOX-13) and to play a role in the initiation of lipid peroxidation leading to irreversible membrane damage, and thus, cell death (Mauch-Mani, 2002) not much is known about LOX genes or activity in grapes (Dunlevy et al., 2009). 13-LOX–oxylipins such as jasmonic acid are regulators of plant defense gene expression (Bate and Rothstein, 1998; Weichert et al., 1999; Farmer et al., 2003), while 9-LOX–derived oxylipins play a role in localized cell death during the hypersensitive reaction (Rusterucci et al., 1999; Vellosillo et al., 2007). Moreover, ethyl jasmonate has been shown to promote stilbene accumulation and natural defenses of grape (Krisa et al., 1999).

It is known that biotrophic pathogens such as oomycetes are more sensitive to salicylic acid-mediated defense (systemic acquired resistance, SAR) than necrotrophic pathogens that are generally affected by jasmonic acid/ethylene mediated defense (induced systemic resistance, ISR) (Thomma et al., 2001; Howe, 2004; Glazebrook, 2005; Howe and Jander, 2008). Hormones interact antagonistically or synergistically (Wang et al., 2007, Navarro et al., 2008 Jiang et al., 2010) and their impact on the defense response is greatly influenced by the composition of hormones produced during host-microbe interaction (Koornneef et al., 2008; Leon-Reyes et al., 2010). Although salicylic acid and jasmonic acid/ethylene defense pathways are mutually antagonistic, evidences of synergistic interactions have also been reported (Schenk et al., 2000; Kunkel and Brooks, 2002; Beckers and Spoel, 2006; Mur et al., 2006).

Up-regulation of signalling pathways after inoculation with P. viticola

Inoculation with *P. viticola* led to up-regulation of genes involved in signalling transduction pathways (Table 3.11). Most of these genes code for receptors such as receptor protein kinases, mostly serine/threonine kinases or with leucine rich repeat domain. Figueiredo et al. (2012) have reported that 6 and 12h after inoculation with *P. viticola,* genes that code for receptor protein kinases were up-regulated in resistant grapevine such as Regent but not in susceptible grapevine such as Trincadeira. Similarly, it was shown that 12 h post inoculation with *P. viticola,* many of the signal transduction genes that were up-regulated in *V. riparia* were not modulated in *V. vinifera* (Polesani et al., 2010). This may indicate that susceptible varieties such as Riesling need more time to initiate these genes or they are not activated as the plant is susceptible.

Most of the genes involved in calcium signalling such as genes coding for calmodulin, a calcium binding protein, were also up-regulated. Elevated concentrations of cytosolic free calcium are induced in response to various stimuli, including pathogen attack (Sanders et al., 2002; White and Broadley, 2003). Calcium is a ubiquitous second messenger in plants (Zhang and Lu, 2003; Lecourieux et al., 2006). It regulates diverse cellular processes, among which the response to pathogen attack, by conveying signals received at the cell surface to the inside of the cell (Karita et al., 2004) to trigger signalling mechanisms for defense responses (Ma and Berkowitz, 2007). Polesani et al. (2010) reported that resistant varieties such as *V. riparia* infected with *P. viticola* exhibited an increase of calmodulin and calmodulin-binding proteins. Moreover, Garcia-Brugger et al. (2006) reported that *B. cinerea* triggered defense reactions in grapevine including Ca^{2+} influx (Poinssot et al., 2003).

Down-regulation of cell wall metabolism after inoculation with P. viticola

Many genes involved in cell wall metabolism were down-regulated upon inoculation with *P. viticola* (Table 3.12). Unlike necrotrophic pathogens that kill the cell to recover the nutrients by toxins that push the host cell beyond the hypersensitivity response threshold and cause massive cell death (Glazebrook, 2005; Kliebenstein and Rowe, 2008), biotrophic pathogens such as *P. viticola* use a different strategy: they extract nutrients from viable cells and thus, they induce a complex reorganization of plant membranes to establish haustoria within plant cells, which allows nutrient recovery (Hueckelhoven, 2005, 2007; O'Connell and Panstruga, 2006). Figueiredo et al.

(2012) reported that inoculation with *P. viticola* led to down-regulation of all genes involved in cell wall metabolism in both a resistant (*V. riparia*) and a susceptible (*V. vinifera)* variety. However, down-regulation was more pronounced in *V. vinifera*. Our data pointed out that polygalacturonase-inhibiting proteins were up-regulated. Polygalacturonases are important virulence factors that are used by oomycetes to loosen plant cell walls to allow tissue penetration/ invagination (Pryce-Jones et al., 1999). To limit damage to cell walls, plants produce polygalacturonase-inhibiting proteins to inactivate pathogen-produced polygalacturonases (D'Ovidio et al., 2004). Indeed, mutants, unable to produce polygalacturonase-inhibiting proteins, were shown to be more susceptible to *B. cinerea* (Ferrari et al., 2006). However, it remains unclear to what extent polygalacturonase-inhibiting proteins take part in a successful defense against *P. viticola* (Kortekamp, 2006). Moreover, enzymes responsible for cell wall modification such as pectate lyase, polygalacturonase, xyloglucan endotransglycosylase and expansin were down-regulated. The same aforementioned genes were also down-regulated upon inoculation of *V. vinifera* with the fungus *Eutypa lata*, the causal agent of dieback disease (Camps et al., 2010).

Change in some transcription factors after inoculation with P. viticola

Our results showed that genes coding for members of the WRKY transcription family were up-regulated, while those coding for MYB transcription factors were down-regulated (Table 3.13). It was reported that many genes coding for WRKY transcription factors were induced upon infection with *P. viticola,* these genes being more induced in *V. riparia* than in *V. vinifera* (Polesani et al., 2010). Moreover, WRKY-responsive genes were induced in response to powdery mildew infection (Fung et al., 2008). WRKY factors bind to DNA motifs which are often found in defense genes. Thus they are regarded as important regulators of resistance (Pandey and Somssich, 2009), and several WRKY transcription factors play important roles in the regulation of SA-dependent defense responses in plants (Wang et al., 2006; Eulgem and Somssich, 2007). WRKY-responsive genes were also induced in response to infection with *Eutypa lata* (Camps et al., 2010). WRKY transcription factors can act both as negative and positive regulators in pathogen defense, and interference with the negatively functioning WRKY factors has been documented to promote plant defense (Shen et al., 2007). As for MYB transcription factors, they are involved in the regulation of the biosynthesis of secondary

metabolites that play a role in defense compounds against pathogens (Van Verk et al., 2009). Defense responses regulated by MYB transcription factors seem to cover all signalling pathways and act against many types of pathogens (Van Verk et al., 2009). However, most reports on MYB in grapevine are related to anthocyanin accumulation in grape berries and berry color (Fischer et al., 2004; Jeong et al., 2006; Walker et al., 2007).

Down-regulation of genes involved in photosynthesis and carbohydrate metabolism after infection with P. viticola

Our results showed that the few differentially expressed genes involved in photosynthesis are down-regulated (Table 3.14), among which mainly genes involved in light reaction such as genes coding for chlorophyll a/b-binding proteins. Leaves infected with obligate biotrophic fungal pathogens often exhibit reduced rates of net photosynthesis (Tang et al., 1996; Chou et al., 2000; Walters and Mc Roberts, 2006). Grapevine–downy mildew interaction shows a general down-regulation of photosynthesis later in the inoculation process (Polesani et. al., 2010; Wu et al., 2010; Legay et al., 2011). Down-regulation of grapevine chlorophyll a/b-binding proteins shortly after powdery mildew infection was reported (Fung et al., 2008; Legay et al., 2011). This down-regulation can be associated with a loss of chlorophyll, as observed in leaves infected with downy and powdery mildew (Ahmad et al., 1983; Scholes et al., 1994; Wright et al., 1995). However, recently an up-regulation of photosynthesis related transcripts has been reported in a resistant grapevine genotype at 12 h post-inoculation (Malacarne et al., 2011). Surprisingly, it was reported in another study that in the resistant grape variety Regent, photosynthesis was more affected (down-regulated) than in the susceptible variety Trincadeira after infection with *P. viticola* (Figueiredo et al., 2012). Other oomycetes such as *Phytophthora infestans* were reported to suppress many photosynthesis-related genes in susceptible potato (Restrepo et al., 2005). The reason for the reduction in photosynthesis during the compatible interaction between grapevine and *P. viticola* is unknown, but in the frame of the interaction of grapevine with powdery mildew, Fung et al. (2008) have suggested that there is an up-regulation of invertases, which are involved in degradation of carbon reserves into hexoses, resulting in the reduction of photosynthetic rates, while up-regulation of these genes in resistant varieties could be an alternative strategy adopted to gain energy for

defense response (Malacarne et al., 2011). However, it is not known whether the pathogens cause this or it is a response of the plant to infection (Chou et al., 2000; Walters and Mc Roberts, 2006).

Since plants produce carbohydrates in photosynthetic source organs (Gamm et al., 2011) one can expect a change in the carbohydrate metabolism as long as photosynthesis is affected. Indeed, our results showed that most differentially expressed genes involved in carbohydrate metabolism were down-regulated (Table 3.14). These included genes coding for galactinol synthase, ADP-glucose pyrophosphorylase, sucrose-phosphate synthase and sucrose synthase. In a similar study by Gamm et al. (2011), a dramatic alteration of carbohydrate metabolism correlated with later stages of *P. viticola* development in leaves was reported. The authors found that ADP-glucose pyrophosphorylase and sucrose-phosphate synthase were both repressed at the end of the infection period. However, since sugars accumulate just before sporulation, a role in plant defense seems uncertain but they certainly serve as nutrients for the pathogen (Gamm et al., 2011). In grapevine, Hayes et al. (2010) and Gamm et al. (2011) have reported the induction of a cell-wall invertase gene expression in downy and powdery mildew-infected leaves. Fung et al. (2008) hypothesized that up-regulation of invertases, during powdery mildew-grapevine interaction, could result in the reduction in net photosynthetic rate, as documented in other plant-biotrophic pathogen interactions (Scholes et al., 1994; Hahn and Mendgen, 2001; Walters and McRoberts, 2006).

Invertases are key enzymes for carbohydrate allocation because they catalyze the irreversible cleavage of sucrose, the major form of translocated sugars in plants, into glucose and fructose (Gamm et al., 2011). Sugars play several role as energy source to fuel the activation of defense reactions (Gamm et al., 2011), where they can also induce pathogenesis related proteins (Roitsch 1999). Moreover, photo-assimilates are believed to be used to fuel the activation of defense reactions (Bolton, 2009), whereas pathogens attempt to use them for their development, resulting in modifications of their production and partitioning within host tissues (Abood and Lösel, 2003; Hall and Williams, 2000).

Genes coding for enzymes involved in trehalose metabolism were down-regulated (Table 3.14). However, an increasing level of trehalose was observed in *P. viticola*-infected leaves, where glucose was diverted to trehalose in *P. viticola*-infected leaves. However, this was after the beginning of sporulation (Brem et al., 1986).

Trehalose is a non-reducing disaccharide, mostly found in trace amounts, that is associated with the protection of plants against different types of abiotic stresses (Drennan et al., 1993) and may play a role in signaling (Paul, 2007).

Grapevine cv. Riesling defense responses are not sufficient to stop P. viticola

To conclude, most of stress pathways were induced upon inoculation with *P. viticola*. However, this was not sufficient to stop the infection. This was clear from the disease severity results, showing that inoculated plants were severely infected. However, *V. vinifera* can defend itself against other pathogens, indicating that defense mechanisms are present, but not activated in response to *P. viticola* (Polesani et al., 2010) or not activated fast enough to stop the pathogen. Our study showed transcriptional responses associated with early stages of *P. viticola* infection indicating the presence of weak defenses response in the susceptible variety Riesling. Similar observations were reported by Polesani et al., (2010). On the other hand, control plants (neither inoculated nor elicited) of the tolerant varieties Regent and Solaris showed low level of stilbenes indicating that stilbene production is constitutive in Regent and Solaris, while inoculated plants showed relatively high level of stilbene concentration.

4.3.2 Elicitation with phosphonate and phosphate led to similar changes

Treatments with phosphate and phosphonate led to similar changes in gene expression (Figure 3.16 and Table 3.16): similar number of DEGs, similar fold changes, and mostly down-regulated DEGs. Although many DEGs were common between both treatments, some differences were observed and they will be discussed hereafter.

Different PR-proteins are induced by phosphate and phosphonate

It was reported that phosphite (phosphonate releasing salt) and phosphate salts induce systemic resistance against fungi, bacteria and viruses (Anderson et al., 2006; Mucharromah and Kuc, 1991; Gottstein and Kuc, 1989). Indeed, when applied as a foliar spray, phosphate salts induced resistance under field conditions in many crops, among which grapevine (Reuveni and Reuveni, 1998). As for phosphonate-based compounds, activation of plant defenses was also proposed. Chipco®, a phosphonate-based fungicide (Bayer, UK) was reported to enhance plant defenses including the production of antimicrobial phytoalexins (Anderson et al., 2006). There are many products formulated to produce inorganic phosphonates such as Nutri-Phite® (Biagro Western, USA), Ele-Max® (Helena Chemical Co, USA), Phytogard® (CATE, France) and Frutogard® (Tilco Biochemie, Germany). In our study, treatment with phosphonate led to down-regulation of chitinases e.g. PR-3 and PR-4 as well as thaumatin (PR-5), while treatment with phosphate led to up-regulation of PR-1 and PR-4 protein (Table 3.17). Moreover, there were no common DEGs that code for PR-proteins between both treatments. However, there were many common DEGs that code for biotic stress receptors (mainly resistance proteins) that were all down-regulated. These results indicate that, at this particular sampling point, phosphate, but not phosphonate, may have succeeded in inducing a systemic acquired resistance (SAR), since PR-1 was up-regulated. The expression of PR-1 in particular is used as a molecular marker for a successful induction of SAR (Pieterse and Val Loon, 2002; Durrant and Dong, 2004), although its biochemical function is not known (Mauch-Mani, 2002; Jayaraj et al., 2004). However, it is assumed that PR-1 induction is a general stress response in some grapevine culture systems (Wielgoss and Kortekamp 2006) with an antifungal activity, however, through unknown mechanisms (Alexander et al., 1993; Niderman et al., 1995). In grapevine, Harm et al. (2011) reported that β-amino butyric acid (BABA) and (benzo-(1,2,3)-thiadiazole-7-

carbothioic acid S-methyl ester; Bion®, Actigard®) (BTH) induced the expression of PR-1 in grapevine against *P. viticola*, while BTH in maize activated the expression of both PR-1 and PR-5 genes (Mauch-Mani, 2002). Other authors reported that BABA induced PR-1 expression in tomato (Cohen et al., 1999) and pepper (Siegrist et al., 2000) but not in Arabidopsis (Cohen, 1994), cauliflower (Jakab et al., 2001) or tobacco (Silue et al., 2002). Moreover, Sparla et al. (2004) reported an induction of resistance in pear to blight upon BTH treatment that was not associated with the expression of PR-1. Therefore, it is agreed that the expression of PR-1 depends on the nature of elicitor used (Repka, 2001b) and the species. Moreover, Phytogard®, a phosphonate-releasing product, protected lettuce against downy mildew in a systemic manner (Pajor et al., 2001). However, this protection did not involve an increase in the PR-1 protein (Becot et al., 2000). This may explain the different responses obtained upon treatment with phosphate and phosphonate in our study.
Elicitation with phosphate and phosphonate led to down-regulation of DEGs coding for biotic receptors (Table 3.17). Some of these genes were specific for each treatment, while the majority of them were common between both treatments. Most of these genes coded for resistance proteins. However, it is not known why elicitation led to down-regulation of these genes. Resistance in plants is often mediated by specific interactions between plant resistance genes, that code for resistance proteins, and corresponding avirulence genes of the pathogen (Dangl and Jones, 2001). These resistance proteins either directly or indirectly interact with pathogen-specific effector proteins, resulting in a superimposed layer of defense variably termed effector triggered immunity, gene-for-gene resistance, or R gene-dependent resistance (Jones and Dangl, 2006).

Phosphonate down-regulates phytoalexin metabolism, but enhances lignin biosynthesis

Both treatments led to up-regulation of genes involved in phenylpropanoid and flavonoid pathways such as caffeoyl-CoA-O-methyltransferase and flavonoid 3\',5\'-hydroxylase, respectively (Table 3.18). However, both resistance inducers led to a differential expression of different sets of genes in the secondary metabolite pathways. Treatment with phosphonate involved down-regulation of genes involved in phytoalexin biosynthesis such as stilbene synthases (Table 3.22). Induction of stilbenes by chemicals in grapevine has been known since many years (Barlass et

al., 1987). Recently, it was reported that the activity of BABA against *P. viticola* is due to the accumulation of phytoalexins (Slaughter et al., 2008), and chitosan was reported to increase levels of resveratrol, viniferins (stilbene oligomers) and piceids (stilbene glycosides) (Aziz et al., 2006). Moreover, SAR activators led to up-regulation of stilbene synthase (Busam et al., 1997a), which is responsible for the synthesis of stilbenes. Phytoalexins (stilbenes) have been shown to be fungitoxic against grape pathogens such as *Plasmopara viticola* (Langcake and Pryce 1976). Accumulation of stilbenes in grape leaves has been associated with resiatance (Schnee et al., 2008), where the capacity, intensity and rapidity in stilbene production have been proposed as indicators for resistance to fungal infection (Pezet et al., 1991). In grapevine, viniferins have been shown to be a reliable marker for resistance to powdery mildew and for assessing the defense potential of grapevine cultivars (Schnee et al., 2008). A gene coding for caffeoyl-CoA-O-methyltransferase, an enzyme involved in lignin biosynthesis (Martz et al., 1998), was reported to be up-regulated by the SAR activator BTH in grape and wheat and, therefore, was assumed to play a role in the disease-resistance response (Busam et al., 1997a; Pasquer et al., 2005). The linear β-1,3-glucan laminarin, from brown algae, also elicits the expression of caffeic acid O-methyl transferase in tobacco (Klarzynski et al., 2000).

Genes involved in lignin biosynthesis such as phenylalanine ammonia lyase (PAL) were up-regulated after phosphonate treatment (Table 3.22). In contrast, treatment with phosphate involved down-regulation of PAL (Table 3.25). Phenylalanine ammonia-lyase (PAL) is an enzyme of the phenylpropanoid pathway that gives rise to a large number of compounds such as lignins, flavonoids, coumarins, stilbenes, salicylic acid (Hahlbrock and Scheel, 1989). Many resistance inducers have been shown to induce this pathway. Indeed, cellodextrin, a predominant molecule from cellulose degradation in plant cell walls and fungi (Scheible and Pauly, 2004; Matthysse et al., 2005), was reported to induce expression of genes involved in phenylpropanoid pathway such as PAL in grapevine (Aziz et al., 2007). Harm et al. (2011) also reported that BTH induced the expression of PAL encoding genes in grapevine, while Hammerschmidt (1999) and Iriti et al. (2010) reported that chitosan-induced resistance is associated with SAR establishment through the stimulation of the phenylpropanoid pathway. Nutri-Phite® (Biagro Western, USA), a phosphonates-releasing compound, led to a rapid, strong, and lasting increased expression of

genes encoding PAL (Anderson et al., 2006). It was also reported that application of phosphate (as potassium phosphate, K_3PO_4) to first leaves led to induced expression of PAL in barley (Mitchell and Walters, 2004). The linear β- 1,3-glucan laminarin was also found to elicit a variety of defense reactions in tobacco plants, such as the stimulation of phenylalanine ammonia lyase (Klarzynski et al., 2000), while â-1,3 glucan sulfate led to up-regulation of PAL in susceptible grapevine (Trouvelot et al., 2008). Chitosan was also found to stimulate phenylalanine ammonia lyase against *B. cinerea* (Trotel-Aziz et al., 2006).

Phosphate and phosphonate down-regulate signalling pathways

Phosphonate treatment modulated the calcium signalling by down-regulating most DEGs in the calcium signalling pathway such as a gene coding for hypersensitive reaction associated Ca^{2+} binding protein and calcium-transporting ATPase 9 plasma membrane (Table 3.23), while phosphate down-regulated another set of genes coding for receptor kinases (Table 3.26). It was speculated that basic phosphates applied to plants could sequester apoplastic calcium (Gottstein and Kuc, 1989; Walters and Murray, 1992). Calcium influx from extracellular spaces (apoplast) and changes in free cytosolic Ca^{2+} concentration are crucial steps in the signalling cascade leading to defense responses and hypersensitivity response (HR) (cell death) (Blume et al., 2000; Jabs et al., 1997; Lecourieux et al., 2002) which is a hallmark of effector triggered immunity (ETI). Indeed, calcium works as a secondary messenger that binds to calcium-binding proteins to trigger signalling mechanisms for activation of ETI (Ma and Berkowitz, 2007). Orober et al. (2002) showed that phosphate mediated resistance in cucumber was associated with localized cell death along with local and systemic increases in levels of free and conjugated salicylic acid. In our study, it seems that phosphonate treatment in grapevine blocks the HR pathway, by down-regulating genes coding for hypersensitive reaction such as Ca^{2+} binding protein and calcium-transporting ATPase 9 plasma membrane. Indeed, blocking Ca^{2+} ion channels using calcium channel blockers was shown to inhibit HR in tobacco, Arabidopsis and soybean systems (Atkinson et al., 1990; He et al., 1993; Levine et al., 1996; Mittler et al., 1997b).

Phosphonate up-regulates ethylene pathway, while phosphate up-regulates jasmonic acid pathway

DEGs involved in aminocyclopropane carboxylate (ACC) oxidase pathway, which catalyzes the last step in ethylene synthesis, were up-regulated after phosphonate treatment (Table 3.23), while most of the DEGs coding for lipoxygenase were up-regulated after phosphate treatment (Table 3.26). This shows that phosphonate treatment exerted its effect through ethylene pathway, while phosphate treatment exerted its effect through jasmonate pathway. Both treatments led to down-regulation of genes involved in abscisic acid (ABA) metabolism. Ethylene and jasmonic acid are considered as major regulators of induced resistance (Thomma et al., 2001; Mur et al., 2006). Ethylene is produced upon wounding or infection by pathogens as well as by treatment with elicitors of defense responses (Boller, 1990; Grosskopf et al., 1991). Short chain oligogalacturonides induced the accumulation of ethylene synthesis in tomato (Simpson et al., 1998), and BTH induced the expression of aminocyclopropane carboxylate (ACC) oxidase in bean (Iriti and Faoro, 2003). Glucan sulfate was also reported to induce ethylene-related defense pathways in tobacco and Arabidopsis (Ménard et al., 2004). As for jasmonic acid, a product of the lipoxygenase (LOX) pathway, it has been suggested as a signal transducer of defense reactions (Hamiduzzaman et al., 2005). In grapevine, many resistance inducers were shown to induce genes encoding for lipoxygenases. These included laminarin (Aziz et al., 2003), glucan sulfate (Trouvelot et al., 2008) and chitosan (Trotel-Aziz et al., 2006). Phosphate treatment in cucumber and barley also led to increased lipoxygenase expression (Mitchell and Walters, 2004). SA and JA/ET defense pathways are mutually antagonistic (Ghassemian et al., 2000; Abe et al., 2003; Wilkinson and Davies, 2009), however, evidences of synergistic interactions have also been reported (Schenk et al., 2000; Beckers and Spoel, 2006; Mur et al., 2006 This may explain the down-regulation of DEGs involved in ABA pathway, while DEGs in ethylene and jasmonic acid were up-regulated (Table 3.26). Another antagonism is reported between ethylene and auxin pathways, where defense responses involve down-regulation of auxin responsive genes. Therefore, it is assumed that auxin is involved in the attenuation of defense responses (Bari and Jones, 2009). Indeed, our results showed that DEGs coding for nitrilase, a key enzyme in auxin biosynthesis, were strongly down-regulated after treatment with phosphonate (Table 3.24).

Treatment with phosphonate led to cell wall modification

Both treatments led to up-regulation of most of the genes involved in cell wall metabolism (Table 3.19). Interestingly, there were no DEGs specific for phosphate treatment, while phosphonate treatment specifically led to up-regulation of genes involved in cell wall modification such as xyloglucan endotransglycosylase, genes coding for syringolide-induced protein and alpha expansin (Table 3.24). Plant pathogens penetrate through cell walls; therefore, various forms of cell-wall strengthening and/or modification play a role in plant defenses (Nicholson and Hammerschmidt, 1992; Mendgen et al., 1996; Vorwerk et al., 2004). BTH has been reported to cause cell wall alteration by inducing the expression of xyloglucan endo-transglycosylase (Heidel and Baldwin, 2004), while BABA led to more lignification in leaves of susceptible grapevine (Chasselas) (Hamiduzzaman et al., 2005). Xyloglucan endotransglycosylase integrates newly synthesized xyloglucans into the wall (Thompson and Fry 2001), while expansins cause cell wall loosening (Li and Cosgrove, 2001), thereby reinforcing the cell wall by allowing it to expand without undermining its structure (Campbell and Braam, 1999; Bourquin et al., 2002). *P. viticola* is a biotrophic pathogen that penetrates its host through the stomata, avoiding the host preformed barriers on the surface such as the cuticle and the epidermal cell wall (Kassemeyer et al., 2009), where encysted zoospores develop a penetration peg that grows through the stomatal pore (Kiefer et al., 2002; Riemann et al., 2002).

Treatment with phosphonate and phosphate led to down-regulation of photosynthesis and carbohydrate metabolism

Both treatments led to down-regulation of most of the genes involved in photosynthesis, especially genes in photosystem II (PSII) and genes coding for rubisco (Table 3.20). Plants respond to stresses in complex ways that are reflected by various metabolic responses (Petit et al., 2009). This may include metabolic adaptations affording stress protection. Therefore, a decrease of photosynthesis may be a part of the plant’s strategy to overcome environmental stresses (Chapin et al., 1993). Indeed, alteration of photosynthesis was reported to be accompanied by the stimulation of plant defense responses (Garcia et al., 2003). Moreover, it was reported that reduction in the photosynthesis process is a cost of induced resistance (Zangerl, 2003). Indeed, microarray studies generally confirmed that genes involved

in photosynthesis and growth are down-regulated during the expression of induced resistance (Scheideler et al., 2002; Heidel et al., 2004). However, Kehlenbeck et al. (1994) found that rates of photosynthesis were increased in the leaves of induced barley plants compared to non-induced controls. This may indicate that the plant and the type of elicitor determine the outcome. Alteration of photosynthesis may disturb the whole carbon balance of the plant (Petit et al., 2006; Saladin et al., 2003a, b; Lebon et al., 2005; Schwachtje and Baldwin, 2008). In our results, phosphonate and phosphate treatment caused most of DEGs involved in carbohydrate metabolism to be down-regulated. However, β-amylase and trehalose-6-phosphate phosphatase were up-regulated (Table 3.20). β -Amylase is an enzyme that degrades the β-1,4-glycosidic bonds from the non-reducing end of the starch molecules, releasing maltose (Gamm et al., 2011). The biosynthesis of trehalose, which regulates starch biosynthesis (Lunn et al., 2006), requires trehalose-6-phosphate phosphatase (Avonce et al., 2006). Plant pathogens are known to interfere with the source-sink balance of the host (Biemelt and Sonnewald, 2006), where in successful interaction pathogens reprogram the plant’s metabolism to theirown benefit (Kocal et al., 2008). The change in carbohydrate turnover may be because of the so-called high-sugar resistance. This type of resistance is associated with elevated levels of soluble carbohydrates (Horsfall and Dimond, 1957). The concept of high-sugar resistance has been supported by various studies demonstrating that provoking the accumulation of sugar in transgenic plants, can lead to activation of various defense responses (Herbers et al., 1996a, b; Johnson and Ryan, 1990). Beside being a source of nutrients, soluble sugars may act as signals leading to the down-regulation of genes involved in photosynthesis (Koch, 1996) as well as to an induction of defense gene expression (Herbers et al., 1996b; Rolland et al., 2006).

4.3.3 Similar transcriptional changes after elicitation with phosphate and phosphonate and subsequent inoculation

Elicitation with phosphonate or phosphate and subsequent inoculation with *P. viticola* led to differential expression of more genes than elicitation with either elicitor did, whereas inoculation alone led to differential expression of more genes than elicitation and inoculation together (Table 3.5). It was reported that phosphate and phosphonate have antimicrobial activity against oomycetes (Fenn and Coffey 1984; Smillie et al., 1989). However, recently it was found that foliar sprays of phosphate-containing fertilizers can also induce systemic protection against grapevine infecting pathogens (Reuveni and Reuveni et al., 2002; Creasy and Creasy, 2009). Phosphate salts can induce local and systemic protection against powdery mildew in many plants (Orober et al., 2002; Ehret et al., 2002; Mitchell and Walter, 2004) including grapevines (Reuveni and Reuveni, 1995). Although many DEGs were common between both treatments (Figure 3.17 and Table 3.28), some differences were observed and they will be discussed hereafter.

Phosphate treatment and subsequent inoculation up-regulates more genes in stress pathways

Phosphate and phosphonate led to a similar differential expression of many genes in stress pathways including the up-regulation of genes coding for PR-proteins such as PR-1, PR-10 and class IV chitinases (Table 3.29). Under elicitation only phosphate led to up-regulation of PR-1, while phosphonate did not. However, treatment with phosphate and phosphonate and subsequent inoculation led to up-regulation of PR-1, the expression of which is considered to be a molecular marker for a successful induction of SAR (Durrant and Dong, 2004). PR-1 can be induced to very high levels upon infection (reaching up to 1 to 2 percent of the total leaf protein) (Jayaraj et al., 2004) (discussed in details under elicitation). Moreover, phosphate treatment and subsequent inoculation led to up-regulation of all DEGs involved in the synthesis of heat shock proteins (HSPs) (Table 3.29). Interestingly, no differential expression of genes involved in HSPs was observed during *P. viticola*/grapevine interaction, while most of DEGs involved in the synthesis of HSPs were down-regulated after treatment with phosphate. HSPs were up-regulated under powdery mildew infection in grapevine, where they may be involved in the grapevine defense response (Marsh et al., 2010). In other studies aiming at induction of resistance, heat shock proteins in

barley were systemically up-regulated in the leaves of plants infested with strains of *Piriforomspora indica* and *Sebacina vermifera* (Waller et al., 2008), and in maize and cucumber upon inoculation with *Trichoderma harzianum* and *Trichoderma asperellum* (Segarra et al., 2007; Shoresh and Harman, 2008). In our experiment, genes coding for germin-like proteins were up-regulated after treatment with phosphate and subsequent inoculation (Table 3.29), although these genes were down-regulated after inoculation (discussed earlier). It was reported that germin-like proteins were down-regulated after infection with *P. viticola* (Godfrey et al., 2007; Legay et al., 2011).

Elicitation and subsequent inoculation up-regulates different pathways in secondary metabolism

Treatment with phosphonate and phosphate followed by inoculation led to up-regulation of DEGs involved in flavonoid pathways (e.g. flavonols and isoflavonols) (Table 3.30), unlike after inoculation, where genes coding for stilbene synthase were up-regulated and genes involved in isoflavonol and terpenoids pathways were down-regulated. Induced flavonoids may play a role in protecting the young berries or leaves from various phytopathogens, such as *P. viticola* (Dai et al., 1995; Malacarne et al., 2011). Pre-treatment of the susceptible grapevine cultivar (Chasselas) with BABA prior to inoculation with *P. viticola* primed the accumulation of flavonoids that are undetectable in non-BABA-primed plants (Slaughter et al., 2008). Although treatment with phosphonate and phosphate led to similar outcomes, different pathways were induced (Table 3.30). For example, phosphate led to up-regulation of genes coding for terpenoid synthase, while phosphonate up-regulated genes coding for cycloartenol synthase both of which are involved in the terpenoid pathway, (Logan et al., 2000). Treatment with phosphonate and subsequent inoculation led to up-regulation of two genes coding for stilbene synthase (resveratrol synthase), while treatment with phosphate and subsequent inoculation led to down-regulation of three genes coding for PAL (Table 3.30). It was reported that synthesis of stilbenes only occurs if PAL and subsequent genes are induced (Jeandet, et al., 2002). This may explain why phosphonate and subsequent inoculation led to up-regulation of stilbene synthase and not phosphate.

Phosphate and phosphonate followed by inoculation up-regulate R-genes

Treatment with phosphonate and phosphate and subsequent inoculation led to up-regulation of R-genes (resistance genes) that code for R-proteins that work as receptors to detect specific pathogens (Table 3.31). One way for plants to defend themselves against pathogens is the activation of specific resistance by R-genes (Li et al., 1999). Di Gaspero and Cipriani (2003) reported that Nucleotide Binding Site/Leucine-Rich Repeat (NBS-LRR) and Serin/Threonine Kinase genes, two of the known classes of resistance R-genes, occur in grapevine in large multigene families. The research of other authors has proven the presence of other resistance genes in genome of grapevine that are associated the resistance to *P*. viticola (Di Gaspero et al., 2007; Velasco et al., 2007; Moroldo et al., 2008). Most of the DEGs up-regulated in the signalling pathway code for serine/threonine kinases. They play a role in signal transduction (Mithofer et al., 2001; Yamamizo et al., 2006), where they participate in the activation of plant defense responses (Zhang and Klessig, 2001). Moreover, they generate diverse signalling molecules that regulate many processes leading to an amplification of the defense response (Lecourieux-Ouaked et al., 2000).

Phosphate and phosphonate treatments followed by inoculation activate systemic resistance (SAR and ISR)

Elicitation with phosphate and phosphonate and subsequent inoculation led to up-regulation of DEGs involved in salicylic acid and jasmonic acid metabolism (Table 3.32). Interestingly, most of DEGs involved in jasmonic acid and salicylic acid were down-regulated after inoculation, while only elicited plants did not show any significant changes in these pathways. This indicates that elicitation activated a systemic resistance in elicited and subsequently inoculated plants. Salicylic acid plays a crucial role in plant defense against biotrophic pathogens by establishing systemic acquired resistance (SAR) (Grant and Lamb, 2006) through accumulation of salicylic acid (Vleesschauwer and Höfte, 2009), since it is a key component for its activation. Up-regulated DEGs in jasmonate pathway code for lipoxygenases (LOXs). Transcripts involved in auxin signalling were up-regulated after phosphate treatment and subsequent inoculation (Table 3.45). These transcripts code for auxin induced SAUR like protein. SAUR-like genes are considered to be one of several classes of early auxin responsive genes (Abel and Theologis, 1996; Guilfoyle et al., 1998).

However, the function of the SAUR-like genes is still unknown. It has been suggested that they may encode short-lived nuclear proteins involved in auxin signalling by interacting with calmodulin (Yang and Poovaiah, 2000; Knauss et al., 2003). It is assumed that increased auxin levels could suppress (Chen et al., 2007) or attenuate (Bari and Jones, 2009) plant defenses and alter the host physiology in the favor of pathogen (Lopez et al., 2008). Moreover, most of ABA-responsive genes were up-regulated after phosphate treatment and inoculation (Table 3.45). Surprisingly, inoculation with *P. viticola* led to down-regulation of DEGs involved in auxin and ABA pathways. This may indicate that inoculated plants behave differently under elicitation. Indeed, inoculated plants showed down-regulation of DEGs involved in brassinosteroid pathway, while inoculated plants under elicitation with phosphate and phosphonate exhibited up-regulation of genes involved in brassinosteroid metabolism.

Elicitation and subsequent inoculation down-regulate photosynthesis but up-regulate carbohydrate metabolism

DEGs involved in photosynthesis were common between both treatments. There were no specific genes for each treatment (Table 3.33). These genes were down-regulated. Most of these genes are involved in photosystem II (PSII). Other genes that code for Rubisco were also down-regulated. Plants infected with biotrophic pathogens often show reduced rates of photosynthesis (Walters and Mc Roberts, 2006) such as in grapevine plants infected with *P. viticola* (Polesani et. al., 2010; Legay et al., 2011). However, this reduction in the photosynthesis might be a cost of induced resistance (Zangerl, 2003). Indeed, microarray studies generally confirmed that genes involved in photosynthesis and growth are down-regulated during the expression of induced resistance (Scheideler et al., 2002; Heidel et al., 2004), where hormonal and chemical defense responses can be costly in terms of plant growth and fitness (Tian et al., 2003; Zavala and Baldwin, 2004). Our results showed that elicitation with phosphonate and phosphate induced hormonal defense pathways in inoculated plants such as jasmonic acid and salicylic acid (Table 3.32). It was suggested that up-regulation of genes involved in jasmonic acid and salicylic acid pathways and the down-regulation of photosynthesis-related genes is part of a defense response (Bilgin et al., 2010). Moreover, silencing of a gene coding for Rubisco, which affected the resistance in *Nicotiana attenuata*, illustrated the linkage

between photosynthesis and defense responses (Giri et al., 2006; Mitra and Baldwin 2008). Therefore, it is suggested that the reduction of photosynthesis represents a "hidden cost" of defense (Bilgin et al., 2008; Zavala and DeLucia, 2009).

Interestingly, DEGs involved in carbohydrate metabolism were up-regulated in elicited and subsequently inoculated plants (Table 3.34), while DEGs involved in carbohydrate metabolism were down-regulated in inoculated plants (but not elicited) and in elicited plants (but not inoculated). Among the up-regulated DEGs were genes coding for sucrose synthase that were strongly up-regulated. Genes involved in starch biosynthesis were also up-regulated. It has been suggested that the reduced photosynthesis could be a negative feedback response to the accumulated levels of soluble sugars in the infected tissue (Kumar and Purohit, 2012), where sugars function as gene regulation signals (Lee and Daie, 1997) and, therefore, their presence could reduce the need for photosynthesis (Ludewig et al., 1998; Chou et al., 2000). Sucrose is the main form of sugar transported in plants (Gamm et al., 2011). Moreover, sugars such as glucose or sucrose repress the expression of genes encoding the small subunit of Rubisco among others (Gibson, 2005; Krapp et al., 1993), which is observed in our results as well. High levels of sucrose and glucose in the infected tissues have been observed in other biotrophic pathosystems (Jongebloed et al., 2004).

DEGs involved in trehalose metabolism such as trehalose-6-phosphate phosphatase were up-regulated in elicited and inoculated plants, although, they were down-regulated in inoculated plants (but not elicited). Previous studies have reported a trehalose accumulation in plant organs infected by pathogens (Fernandez et al., 2010) and in *P. viticola*-infected leaves (Gamm et al., 2011). Trehalose biosynthesis requires trehalose-6-phosphate phosphatase (Avonce et al., 2006) which was up-regulated after elicitation and inoculation. Trehaloses were found to activate the expression of genes encoding a defense-related transcription factor and PR-proteins (Schluepmann et al., 2004).

Elicitation and subsequent inoculation up-regulate cell wall metabolism

Elicitation with phosphonate and phosphate and subsequent inoculation led to up-regulation of DEGs involved in cell wall metabolism (Table 3.34). Most DEGs were involved in cellulose synthesis and cell wall modification. However, treatment with phosphate and subsequent inoculation led to up-regulation of more genes than phosphonate treatment did. Genes coding for syringolide-induced protein and expansins were also up-regulated. These proteins strengthen and/or modify the cell wall, therefore, they play a role in plant defense (Mendgen et al., 1996; Vorwerk et al., 2004). Expansins cause cell wall loosening (Li and Cosgrove, 2001), hereby allowing the cell wall to expand without undermining its structure. Genes coding for cellulose synthase were also up-regulated. It was reported that cellulose is a target polysaccharide for pathogens such as *B. cinerea* (El Ghaouth et al., 1998). When Ca^{2+} was applied to grapevine in the field, resistance to *B. cinerea* was increased and correlated with increased levels of cellulose (Miceli et al., 1999). Moreover, several genes coding for polygalacturonases were found up-regulated after elicitation with phosphonate and phosphate and subsequent inoculation. Polygalacturonases are known to cleave the galacturonide bonds in the pectin molecules (McLeod et al., 2003; Yan and Liou, 2005). Therefore, it is believed that polygalacturonases help releasing elicitor-active oligogalacturonides from plant cell walls (Gottstein & Kuc, 1989; Walters and Murray, 1992).

Change in some transcription factors after elicitation and subsequent inoculation

Elicitation with both elicitors and subsequent inoculation led to down-regulation of genes coding for WRKY transcription family (Table 3.35). WRKY factors are regarded as important regulators of resistance (Pandey and Somssich, 2009), since they play important roles in plant defense responses (Wang et al., 2006; Eulgem and Somssich 2007). Genes coding for WRKYs were up-regulated in inoculated (but not elicited) plants. Therefore, we suggest that elicitation suppressed some of the members of the WRKY family because they may interfere with the defense responses activated by phosphonate and phosphate. Indeed, some WRKYs were identified as negative regulators. They function as a direct negative regulator of SAR, while others can activate repressors of PR-1 (Van Verk et al., 2009). Moreover, some WRKYs interfere with SA and JA signalling by repressing downstream JA targets

such as lipoxygenases (Kim et al., 2008; Mao et al., 2007). Treatment with phosphate and subsequent inoculation led to up-regulation of MYB family and AP2 family (ethylene responsive element) (Table 3.47). AP2 and MYBs, among other transcription factors, are involved in plant defense (Chen et al., 2002; McGrath et al., 2005). AP2 proteins are involved in JA-inducible gene expression, while MYBs are involved in the regulation of the biosynthesis of secondary metabolites that play a role as defense compounds against pathogens (Van Verk et al., 2009).

Elicitation and subsequent inoculation led to up-regulation of other pathways

Elicitation with phosphonate and phosphate and subsequent inoculation led to up-regulation of DEGs involved in lipid metabolism and genes coding for glutathione-S-transferase (GST) (Table 3.35 and 3.36). Some reports have implicated the involvement of a lipid derived signal component in HR and SAR signalling (Brodersen et al., 2002; Watanabe and Lam, 2006). Lipid metabolism is activated consistently as reported for *V. riparia* and for the resistant grapevine cv. Regent upon downy mildew inoculation (Polesani et al., 2010; Figueiredo et al., 2012). However, it seems that lipid metabolism is affected in different manners under different pathosystems. In grapevine/powdery mildew and grapevine/*Eutypa lata* (ascomycete fungus) pathosystems, lipid metabolism was strongly down-regulated (Fung et al., 2008; Camps et al., 2010). Our results showed that genes involved in fatty acid synthesis, elongation and desaturation, e.g. stearoyl-ACP desaturase were up-regulated. Induction of desaturases (Gadea et al., 1996; Kirsch et al., 1997) was suggested to be an early component of the complex of responses associated with defense against pathogens (Kirsch et al., 1997). GSTs are known to be induced by infection, by elicitor treatments and by abiotic stress such as osmotic stress or extreme temperatures (Marrs et al., 1996). Indeed, genes encoding GSTs were induced during grapevine/downy mildew and grapevine/powdery mildew interactions (Legay et al., 2011; Fung et al., 2008). They play a major role as antioxidants in the maintenance of the redox balance (Camps et al., 2010), where they control the levels of reactive oxygen species through detoxification (Wojtaszek, 1997; Smirnoff, 2000) produced during defense responses.

4.3.4 Elicitation with Frutogard® did not induce many transcriptional changes

Although Frutogard® contains phosphonate and phosphate as ingredients; treatment with Frutogard® led to differential expression of fewer genes than the treatment with phosphate or phosphonate did (Table 3.5 and figure 3.15). This is maybe due to the other ingredients of Frutogard® such as brown algae extracts (*Ascophyllum nodosum*, Laminaria spp.) and plant amino acids that mitigated the effect of phosphonate and phosphate on the host genome. Treatment with Frutogard® led to down-regulation of most of DEGs, especially in hormone and cell wall metabolism. Most of DEGs involved in stress pathways and secondary metabolism were up-regulated. Interestingly, there were no DEGs in some pathways such as photosynthesis, glycolysis and polyamine metabolism, while other pathways had very few DEGs such as carbohydrate metabolism and lipid metabolism.

Treatment with Frutogard® led to up-regulation of genes involved in stress pathways and secondary metabolism

Treatment with Frutogard® led to up-regulation of DEGs coding for proteins that function as biotic stress receptors, mostly with nucleotide binding sites and leucine rich repeats domains (NBS-LRR) (Table 3.49). NBS-LRR genes are the most largely represented R-genes in plant genomes, as exemplified in the Arabidopsis (Meyers et al., 2003) and rice genomes (Zhou et al., 2004). These R-genes play a role in the plant innate immunity against bacterial, fungal or oomycete-induced diseases (Di Gaspero et al., 2007, Velasco et al., 2007). Two genes coding for germin-like proteins, the last classes of PR-proteins (PR-16) described in grapevine, were induced. Germins are expressed in response to a number of biotic and abiotic stresses (Bernier and Berna, 2001) and they often exhibit oxalate oxidase or superoxide dismutase activities, however, their exact role in plant defense is not yet elucidated (Gomès and Coutos-Thévenot, 2009). Treatment with BTH led to induction of genes coding for germin in *Nicotiana attenuata* (Heidel and Baldwin, 2004). A gene coding for chalcone synthase, an enzyme involved in phytoalexin production, was up-regulated (Table 3.49). In cowpea, BTH induced chalcone isomerase, when plants were subsequently challenged with bacteria, as compared to untreated controls (Latunde Ada and Lucas, 2001), while in bean, a rapid, strong, and lasting increased expression of transcripts for genes encoding chalcone

synthase was stimulated after spraying with Nutri-Phite®, a product formulated to produce inorganic phosphonates (Kim et al., unpublished data).

Treatment with Frutogard® led to up-regulation cytokinin and gibberellin

Genes coding for cytokinin dehydrogenase (cytokinin oxidase) were down-regulated (Table 3.50). These enzamyes catalyze the degradation of cytokinins; therefore, their down-regulation leads to up-regulation of cytokinin level. Cytokinins influence almost all stages of plant growth, most notably cell division, and seem to play a role in plant defense, although evidence is very scarce (Heil and Walters, 2009; Spaepen et al., 2009). It is believed that plants modulate their cytokinin levels to reduce plant susceptibility to pathogens that require living cells such as biotrophic pathogens (Brugiere et al., 2003). However, in other pathosystem it seems that elevated levels of cytokinins suppress the expression of stress related genes (Trillas and Segarra, 2009). It is believed that cytokinin enhances the expression of genes involved in carbohydrate transport such as hexose transporter, which is known to supply carbohydrates to the infected area, where the pathogen is located (Roitsch and Ehness, 2000; Walters and McRoberts, 2006; Walters et al., 2008). Cytokinins have been shown to induce programmed cell death and expression of PR-1, contributing to resistance against biotrophic pathogens (Carimi et al., 2003; Mlejnek and Prochazka, 2002; Sano et al., 1994).

As for gibberellins, they play important roles in diverse aspects of plant life (Hedden et al., 2000; Schwechheimer et al., 2008; Sun, 2011); however, there are no evidences in the literature that they have a direct effect on resistance. In a recent study, it was shown that jasmonate prioritizes defense over growth by interfering with gibberellin signalling cascade (Yang et al., 2012). Treatment with Frutogard® did not affect genes involved in the jasmonate pathways.

Treatment with Frutogard® led to down-regulation of genes involved in carbohydrate, cell wall and nitrilases metabolism

Genes involved in carbohydrate metabolism such as genes coding for hexokinase and inositol oxygenase were down-regulated (Table 3.51). However, β-amylase and trehalose-6-phosphate phosphatase were up-regulated. β-amylase and trehalose-6-phosphate phosphatase were also up-regulated after phosphonate and phosphate treatments. Hexokinases phosphorylate hexoses, six-carbon sugars (Cho et al., 2009), thereby making them more difficult to transport out of a cell. Inositol is involved in the biosynthesis of nucleotide sugar precursors for cell-wall matrix polysaccharides. Inositol is involved in many processes among which signal transduction pathways, hormone regulation and stress tolerance (Loewus and Murthy, 2000; Gillaspy, 2011). Treatment with Frutogard® also led to the down-regulation of DEGs involved in cell wall metabolism such as genes coding for cellulose synthase and pectinacetylesterase (Table 3.51). Moreover, several transcripts coding for nitrilase 4B (NIT4), a key enzyme in auxin biosynthesis, were strongly down-regulated after treatment (Table 3.51). Nitrilase activity is involved in defense pathways through the metabolism of cyanides (Howden and Gail, 2009), which provide protection to plants against pathogen attack (Vetter, 2000; Fahey et al., 2001; Halkier and Gershenzon, 2006).

4.3.5 Elicitation with Frutogard® and subsequent inoculation led to differential expression of very few genes

Elicitation with Frutogard® led to differential expression of 462 genes (Table 3.5). However, elicitation with Frutogard® and subsequent inoculation led to differential expression of 47 genes only (1529 after elicitation with phosphate, 1422 after elicitation with phosphonate and inoculated plants showed differential expression for 3466 genes) (Table 3.5). The reason for this is not known. It could be a technical problem during sample preparation or hybridization that led to the detection of very few signals. Among the DEGs that were up-regulated were gibberellin 2-oxidase (hormone signalling), phosphoenolpyruvate carboxylase (glycolysis), lipid transfer protein (lipid synthesis) and multidrug resistance-associated protein-like protein (transport), while disease resistance protein (biotic stress receptors) and expressed proteins that belong to signalling pathways were down-regulated (Table 3.52).

5. REFERENCES:

Abe H, Urao T, Ito T, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. The Plant Cell 15: 63–78.

Abood JK, Losel DM (2003) Changes in carbohydrate composition of cucumber leaves during the development of powdery mildew infection. Plant Pathology 52: 256-265.

Adie B A, Perez-Perez J, Perez-Perez MM, Godoy M, Sanchez-Serrano JJ, Schmelz EA, Solano R (2007) ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in Arabidopsis. The Plant Cell 19: 1665–1681.

Adrian M, Trouvelot S, Gamm M, Poinssot B, Héloir MC, Daire X (2012) Activation of Grapevine Defense Mechanisms: Theoretical and applied approaches in Mérillon and Ramawat (ed) plant defense: biological control 13: 313 – 332.

Aggarwal B, Shishodia S (2006) Resveratrol in Health and Disease. CRC Taylor and Francis, Boca Raton. Pp. 712.

Agrios GN (ed) (2005): Plant Pathology. Academic Press. San Diego, USA

Ahmad I, Farrar JF, Whitbread R (1983) Photosynthesis and chloroplast functioning in leaves of barley infected with brown rust. Physiology and Molecular Plant Pathology 23: 411-419.

Alexander D, Goodman RM, Gut-Rella M, Glascock C, Weyman K, Friedrich L, Maddox D, Ahl Goy P, Luntz T, Ward E, Ryals JA (1993) Increased tolerance to two oomycete pathogens in transgenic tobacco expressing pathogenesis-related protein 1a. Proceedings of the National Academy of Science 90: 7327-7331.

Alfano JR, Collmer A (2004) Type III secretion system effector proteins: double agents in bacterial disease and plant defense. Annual Review of Phytopathology 42: 385–414.

Allègre M, Daire X, Héloir MC, Trouvelot S, Mercier L, Adrian M, Pugin A (2006) Stomatal deregulation in *Plasmopara viticola*-infected grapevine leaves. New Phytologist 173: 832-840.

Allègre M, Héloir MC, Trouvelot S, Daire X, Pugin A, Wendehenne D, Adrian M (2009) Are grapevine stomata involved in the elicitor induced protection against downy mildew? Molecular Plant-Microbe Interactions 22: 977–986.

Allen PJ (1959) Physiology and biochemistry of defense. In: Horsfall JG, Dimond AE, eds. Plant Pathology, an Advanced Treatise. New York: Academic Press 1: 435–467.

Amâncio S, Tavares S, Fernandes JC, Sousa C (2009) Grapevine and sulfur: old partners, new achievements interactions. In Roubelakis-Angelakis, KA (ed) Grapevine Molecular Physiology and Biotechnology 2: 31-52. Springer Netherlands, Dordrecht.

Ammermann E, Lorenz G, Schelberger K, Mueller B, Kirstgen R, Sauter H (2000) BAS 500F: The new broad-spectrum strobilurin fungicide, Brighton Crop Protection Conference 541-548.

Andersen CL, Jensen JL, Orntoft TF (2004) Normalization of real-time quantitative reverse transcription-PCR data. A model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer Research 64:5245–5250.

Andrieu N, Jaworska G, Genet JL, Bompeix G (2001) Biological mode of action of famoxadone on *Plasmopara viticola* and *Phytophthora infestans*. Crop Protection 20: 253–260.

Apostol I, Heinstein PF, Low PS (1989) Rapid induction of an oxidative burst during elicitation of cultured plant cells. Plant Physiology 90: 109–116.

Arimura G, Kost C, Boland W (2005) Herbivore-induced, indirect plant defenses. Biochemistry and Biophysics Acta 1734: 91-111

Asselbergh B, De Vleesschauwer D, Höfte M (2008a) Global switches and fine-tuning-ABA modulates plant pathogen defense. Molecular Plant- Microbe Interactions 21: 709–719.

Atkinson MM,Keppler LD, Orlandi EW, Baker CJ, Mischke CF (1990) Involvement of plasma membrane calcium influx in bacterial induction of the K+/H+ exchange and hypersensitive responses in tobacco. Plant Physiology 92: 1241–1247.

Ausubel FM (2005) Are innate immune signaling pathways in plants and animals conserved? Nature Immunology 6: 973–979.

Avonce N, Mendoza-Vargas A, Morett E, Iturriaga G (2006) Insights on the evolution of trehalose biosynthesis. BMC Evolution Biology 6: 109.

Aziz A, Poinssot B, Daire X, Adrian M, Bezier A, Lambert B, Joubert JM, Pugin A (2003) Laminarin elicits defense responses in grapevine and induces protection against *Botrytis cinerea* and *Plasmopara viticola*. Molecular Plant–Microbe Interactions 16: 1118–1128.

Aziz A, Heyraud A, Lambert, B (2004) Oligogalacturonide signal transduction, induction of defense–related responses and protection of grapevine against *Botrytis cinerea*. Planta 218: 767–774.

Aziz A, Trotel-Aziz P, Dhuicq L, Jeandet P, Couderchet M, Vernet G (2006) Chitosan oligomers and copper sulfate induce grapevine defense reactions and resistance to gray mold and downy mildew. Phytopathology 96: 1188-1194.

Aziz A, Gauthier A, Bezier A, Poinssot B, Joubert JM, Pugin A, Heyraud A Baillieul F (2007) Elicitor and resistance-inducing activities of β-1,4 cellodextrins in grapevine, comparison with β-1,3 glucans and β-1,4 oligogalacturonides. Journal of Experimental Botany 58: 1463–1472.

Baider A, Cohen Y (2003) Synergistic interaction between BABA and mancozeb in controlling *Phytophthora infestans* in potato and tomato and Pse*udoperonospora cubensis* in cucumber. Phytoparasitica 31: 399–409.

Baldwin IT, Callahan P (1993) Autotoxicity and chemical defense: nicotine accumulation and carbon gain in solanaceous plants. Oecologia 94: 534–541.

Barlass M, Miller RM, Douglas TJ (1987) Development of methods for screening grapevines for resistance to downy mildew by resveratrol production. American Journal of Enology and Viticulture 38: 65–68.

Bari R, Jones JD (2009) Role of plant hormones in plant defense responses. Plant Molecular Biology 69: 473-488.

Bate NJ, Rothstein SJ (1998) C6-volatiles derived from the lipoxygenase pathway induce a subset of defense-related genes. Plant Journal 16: 561-569.

Bavaresco L, Petegolli D, Cantù E, Fregoni M, Chiusa G, Trevisan M (1997) Elicitation and accumulation of stilbene phytoalexins in grapevine berries infected by *Botrytis cinerea*. Vitis 36: 77-83.

Bavaresco L, Fregoni C, Van Zeller MB, Vezzulli S (2009) Physiology and molecular biology of grapevine stilbenes: an update interactions. In Roubelakis-Angelakis, KA (ed) Grapevine Molecular Physiology and Biotechnology 12: 341-364 Springer Netherlands, Dordrecht.

Bavaresco L, Fregoni C (2001) Physiological role and molecular aspects of grapevine stilbenic compounds. In: Roubelakis-Angelakis KA (ed) Molecular Biology and Biotechnology of the Grapevine. 153-182 Springer Netherlands, Dordrecht.

Beckers GJM, Spoel SH (2006) Fine-tuning plant defense signalling: Salicylate versus jasmonate. Plant Biology 8: 1–10.

Beckers GJM, Conrath U (2007) Priming for stress resistance: from the lab to the field. Current Opinion in Plant Biology 10: 425–431.

Bécot S, Pajot E, Le Core D, Monot C, Silué D (2000) Phytogard® (K2HPO3) induces localized resistance in cauliflower to downy mildew of crucifers. Crop Protection 19: 417-425.

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of Royal Stat Society 57: 289-300.

Berkelmann-Löhnertz B, Heibertshausen D, Baus-Reichel O, Hofmann, U, Kauer R (2008) Ohne Kupfer geht es nicht – Status quo im ökologischen Weinbau nach vier Jahren BÖL-Verbundprojekt. In: Reports from the Julius Kühn-Institut (BBA) – Plant protection in organic farming – 10th Workshop in Berlin, 142: 17-20.

Berkelmann-Löhnertz B, Baus O Hassemer-Schwarz H, Frühauf C (2011) Elaboration and validation of a downy mildew forecast model regarding soil borne infections. Journal of Plant Pathology 93: 45-46.

Berkelmann-Löhnertz B, Klärner S, Baus O, Herrmann G, Flemming B, Keicher R, Schwarz HP, Pfliehinger M, Löhnertz O (2012) Kupferreduzierung und Kupfersubstitution im Weinbau – zum Stand der Dinge aus Sicht der Forschung. In: Reports from the Julius Kühn-Institut – Plant protection in organic farming – 11th Workshop in Berlin 164: 65-70.

Bernier F, Berna A (2001) Germins and germin-like proteins: plant do-all proteins. But what do they do exactly? Plant Physiology and Biochemistry 39: 545–554.

Bertsch C, Joly D, Walter B (2003) Sequence of a putative *Vitis vinifera* PR-1. Vitis 42:103-104.

Biemelt S, Sonnewald U (2006) Plant-microbe interactions to probe regulation of plant carbon metabolism. Journal Plant Physiology 163: 307–318.

Bilgin D, Zavala J, Zhu J, Clough S, Ort D, DeLucia E (2010) Biotic stress globally downregulates photosynthesis genes. Plant Cell Environment 10: 1597–1613.

Blaise P, Gessler C (1990) Development of a forecast model of grape downy mildew on a microcomputer. Acta Horticulturae 276: 63–70.

Bleyer G, Kassemeyer HH, Krause R, Viret O, Siegfried W (2008) “Vitimeteo Plasmopara” – Prognosemodell zur Bekampfung von *Plasmopara viticola* (Rebenperonospora) im Weinbau Gesunde Pflanzen 60: 91–100.

Block A, Li G, Fu ZQ, Alfano JR (2008) Phytopathogen type III eVector weaponry and their plant targets. Current Opinion in Plant Biology 11: 396–403.

Blum M, Waldner M, Gisi U (2010) A single point mutation in the novel PvCesA3 gene confers resistance to the carboxylic acid amide fungicide mandipropamid in *Plasmopara viticola*. Fungal Genetics and Biology 47: 499-510.

Blume B, Nürnberger T, Nass N, Scheel D (2000) Receptor-mediated increase in cytoplasmic free calcium required for activation of pathogen defense in parsley. The Plant Cell 12: 1425-1440.

Boller T (1990) Ethylene and plantpathogen interactions. Current Topics in Plant Physiology 5: 138–45.

Bolstad BM, Irizarry RA, Astrand M, Speed TP (2003) A Comparison of normalization methods for high density oligonucleotide array data based on bias and variance. Bioinformatics 19: 185-193.

Bolton MD (2009) Primary metabolism and plant defense: Fuel for the fire. Molecular Plant Microbe Interaction 22: 487-497.

Bosshard E, Schuepp H (1983) Variability of selected strains of *Plasmopara viticola* with respect to their metalaxyl sensitivity under field conditions. Zeitschrift fuer Pflanzenkrankheiten und Pflanzenschutz 90: 449–459.

Bostock RM (2005) Signal crosstalk and induced resistance: straddling the line between cost and benefit. Annual Review of Phytopathology 43: 545–580.

Bourquin V, Nishikubo N, Abe H (2002) Xyloglucan endotransglycosylases have a function during the formation of secondary cell walls of vascular tissues. Plant Cell 14: 3073–3088.

Brem S, Rast DM, Ruffner HP (1986) Partitioning of photosynthate in leaves of *Vitis vinifera* infected with *Uncinula necator* or *Plasmopara viticola*. Physiological and Molecular Plant Pathology 29: 285–291.

Brendel G (1984) Einfluss von Kupferspritzungen auf die Rebsorte Riesling. Rebe und Wein 37: 272–274.

Brodersen P, Petersen M, Pike HM, Olszak B, Ødum N, Jørgensen LB, Brown RE, Mundy J (2002) Knockout of Arabidopsis ACCELERATED-CELL-DEATH encoding a shingosine transfer protein causes activation of programmed cell death and defense. Genes Development 16: 490–502.

Brook PJ (1979) Effect of light on sporulation of *Plasmopara viticola*. New Zealand Journal of Botany 17: 135–138.

Bru R, Selles S, Casado-Vela J, Belchi-Navarro S, Pedreno MA (2006) Modified cyclodextrins are chemically defined glucan inducers of defense responses in grapevine cell cultures. Journal of Agriculture Food Chemistry 54: 65-71.

Brugiere, N, Jiao SP, Hantke S, Zinselmeier C, Roessler JA, Niu XM, Jones RJ, Habben JE (2003) Cytokinin oxidase gene expression in maize is localized to the vasculature, and is induced by cytokinins, abscisic acid, and abiotic stress. Plant Physiology 132: 1228–1240.

Busam G, Kassemeyer H H, Matern U (1997a) Differential expression of chitinases in *Vitis vinifera* L. responding to systemic acquired resistance activators or fungal challenge. Plant Physiology 115: 1029-1038.

Calderon AA, Zapata JM, Munoz R, Pedreno MA, Barcelo AR. 1993. Resveratrol production as a part of the hypersensitive-like response of grapevine cells to an elicitor from *Trichoderma viride*. New Phytologist 124: 455–463.

Campbell P, Braam J (1999) Xyloglucan endotransglycosylases: diversity of genes, enzymes and potential wall-modifying functions. Trends in Plant Science 4: 361–366.

Campbell C (2004) Phylloxera: how Wine was Saved for the World. Harper Perennial, London.

Chapin FS, Autumn K, Pugnaire F (1993) Evolution of suites of traits in response to environmental stress. Amrican Nature 142: 78–92

Camps C, Kappel C, Lecomte P, Leon C, Gome s E, Coutos-Thevenot P, Delrot S, (2010) A transcriptomic study of grapevine *Vitis vinifera* cv. Cabernet-Sauvignon interaction with the vascular ascomycete fungus *Eutypa lata*. Journal of Experimental Botany 61: 1719–1737.

Carimi F, Zottini M, Formentin E, Terzi, M, Lo Schiavo F (2003) Cytokinins: new apoptotic inducers in plants. Planta 216: 413–421.

Cavalier-Smith T (1986) The Kingdom Chromista: origin and systematics, in Round EF, Chapman DJ (eds): Progress in Phycological Research, Biopress, Bristol, K, pp. 309-347.

Cesari A, Fiaccadori R, Velicogna E (1981) Verifica tecnico-economica su fungicidi endoterapici applicati nella difesa antiperonosporica della vite. Vignevini 8: 43–49.

Chang S, Pur Year J, Carney J (1993) A simple and efficient method for isolating RNA from pine trees. Plant Molecular Biology Reports 11: 113-116.

Chen C, Chen Z (2002) Potentiation of developmentally regulated plant defense response by AtWRKY18, a pathogen-induced Arabidopsis transcription factor. Plant Physiology 129: 706–716.

Chen WJ, Delmotte F, Richard-Cervera S, Douence L, Greif C, Corio-Costet MF (2007) At least two origins of fungicide resistance in grapevine downy mildew populations. Applied and Environmental Microbiology 73: 5162–5172.

Chen Z, Agnew JL, Cohen JD, He P, Shan L, Sheen J, Kunkel BN (2007) *Pseudomonas syringae* type III effector AvrRpt2 alters *Arabidopsis thaliana* auxin physiology. Proceedings of the National Academy of Sciences 104: 20131–20136.

Chester KS (1933) The problem of acquired physiological immunity in plants (continued). The Quarterly Review of Biology 8: 275–324.

Chisholm ST, Coaker G, Day B, Staskawicz B J (2006). Host–microbe interactions: Shaping the evolution of the plant immune response. Cell 124: 803–814.

Cho YS, Challa S, Moquin D (2009) Phosphorylation driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. Cell 137: 1112–1123.

Chong J, Henanff GL, Bertsch C, Walter B (2008) Identification, expression analysis and characterization of defense and signaling genes in *Vitis vinifera*. Plant Physiology and Biochemistry 46: 469–481.

Chou HM, Bundock N, Rolfe SA, Scholes JD (2000) Infection of *Arabidopsis thaliana* leaves with *Albugo candida* (white blister rust) causes a reprogramming of host metabolism. Molecular Plant Pathology 1: 99-113.

Claus P (1979): 90 Jahre Kupferanwendung im Weinbau und immer noch Erkenntnislücken? Weinberg and Keller 26: 142–172, 192–218.

Clerjeau M, Simone H (1982) Apparition en France de souches de mildiou (*Plasmopara viticola*) resistantes aux fongicides de la famille des anilides (metalaxyl, milfurame). Progrès Agricole et Viticole 99: 59-61.

Clerjeau M, Moreau C, Piganeau B, Malato G (1984) Resistance of Plasmopara viticola to anilide-fungicides: evaluation of the problem in France. Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 49: 179–184.

Cohen Y, Niderman T, Mösinger E, Fluhr R (1994) β-aminobutyric acid induces the accumulation of pathogenesis-related proteins in tomato (*Lycopersicon esculentum* L.) plant and resistance to late blight infection caused by *Phytophthora infestans*. Plant Physiology 104: 59-66.

Cohen Y, Reuveni M, Baider A (1999) Local and systemic activity of BABA (DL-3-aminobutyric acid) against *Plasmopara viticola* in grapevines. European Journal of Plant Pathology 105: 351-361.

Cohen Y (2002) b-Aminobutyric acid-induced resistance against plant pathogens. Plant Disease 86: 448-457.

Conrath U, Thulke O, Katz V, Schwindling S, Kohler A (2000) Priming as a mechanism in induced systemic resistance of plants. European Journal of Plant Pathology 107: 113–119.

Conrath U, Pieterse CMJ, Mauch-Mani B (2002) Priming in plant–pathogen interactions. Trends in Plant Science 7: 210–216.

Conrath, U, Beckers, GJM, Flors V, Garcıa-Agustın P, Jakab G, Mauch F, Newman MA, Pieterse CMJ, Poinssot B, Pozo M J, Pugin A, Schaffrath U et al. (2006) Priming: Getting ready for battle. Molecular Plant-Microbe Interactions 19: 1062–1071.

Copping LG, Duke SO (2007) Natural products that have been used commercially as crop protection agents. Pest Management Science 63: 524–554.

Cosgrove DJ (2001) Wall structure and wall loosening. A look backwards and forwards. Plant Physiology 125: 131–134.

Costa JM, Grant OM, Chaves MM (2010) Use of thermal imaging in viticulture: current application and future prospects. In Delrot S et al. (eds.) methodologies and results in grapevine research pp. 135–150. Springer: Dordrecht, the Netherlands.

Côté F, Ham K, Hahn M, Bergmann C (1998) Oligosaccharide elicitors in host pathogen interactions. Generation, perception, and signal transduction. Sub cellular Biochemistry 29: 385–432.

Creasy GL, Creasy (2009) Grapevine pests, diseases and disorders in Grapes (crop production science in horticulture series) 9: 185-222.

Custers JHHV, Harrison SJ, Sela–Buurlage MB, van Deventer E, Lageweg W, Howe PW, van der Meijs PJ, Ponstein AS, Simons BH, Melchers LS, Stuiver MH (2004) Isolation and characterisation of a class of carbohydrate oxidases from higher plants, with a role in active defense. Plant Journal 39: 147–160.

Da Cunha L, Sreerekha MV, Mackey D (2007) Defense suppression by virulence eVectors of bacterial phytopathogens. Current Opinion in Plant Biology 10, 349–357.

Dai GH, Andary C, Mondolot-Cosson L, Boubals D (1995) Histochemical studies on the interaction between three species of grapevine, *Vitis vinifera*, *V. rupestris* and *V. rotundifolia* and the downy mildew fungus, *Plasmopara viticola*. Physiological and Molecular Plant Pathology 46: 177–188.

Dangl JL, Jones JD (2001). Plant pathogens and integrated defense responses to infection. Nature 411: 826–833.

Davies C, Böttcher C (2009) Hormonal control of grape berry ripening interactions. In Roubelakis-Angelakis, KA (ed) Grapevine Molecular Physiology and Biotechnology 9: 229-262 Springer Netherlands, Dordrecht.

Davin LB, Lewis NG (2000) Dirigent proteins and dirigent sites explain the mystery of specificity of radical precursor coupling in lignan and lignin biosynthesis. Plant Physiology 123: 453–461

Davis EM, Croteau R (2000) Cyclization enzymes in the biosynthesis of monoterpenes sesquiterpenes and diterpenes. In Leeper FJ, Vederas JC (eds) Biosynthesis: Aromatic Polyketides Isoprenoids Alkaloids, Springer Berlin.

De Torres-Zabala M, Truman W, Bennett M H, Lafforgue G, Mansfield JW, Rodriguez Egea P, Bogre L, Grant M (2007) *Pseudomonas syringae* pv. tomato hijacks the Arabidopsis abscisic acid signalling pathway to cause disease. The EMBO Journal 26: 1434–1443.

Delledonne M, Polverari A, Murgia I (2003) The functions of nitric oxide-mediated signalling and changes in gene expression during the hypersensitive response. Antioxidants and Redox Signaling, 5: 33–41.

Delrot S, Medrano H, Or E, Bavaresco L, Grando S (2010) (eds) introduction in: methodologies and results in grapevine research, Springer.

Dempsey DA, Klessig DF (2012) SOS–too many signals for systemic acquired resistance? Trends Plant Science 17: 538–45

Desikan R, Cheung MK, Bright J, Henson D, Hancock JT, Neill SJ (2004) Hydrogen peroxide and nitric oxide signalling in stomatal guard cells. Journal of Experimental Botany 55: 205–212.

Deslandes L, Olivier J, Peeters N, Feng DX, Khounlotham M, Boucher C, Somssich I, Genin S, Marco Y (2003) Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. Proceedings of the National Academy of Sciences 100: 8024–8029.

Deverall BJ (1995) Plant protection using natural defense systems of plants. Advances in Plant Pathology 11: 211–228.

Dick MW (2001) The Peronosporomycetes. In McLaughlin DJ, McLaughlin EG, Lemke PA (eds.): The Mycota, Vol VII A (pp. 39–72). Berlin: Springer.

Dick MW (2002) Towards an understanding of the evolution of the downy mildews. In Spencer-Phillips PTN, Gisi U, Lebeda A (eds) Advances in downy mildew research, Vol 1 (pp. 1–57). Dordrecht: Kluwer.

Di Gaspero G, Cipriani G (2003) Nucleotide binding site/leucine-rich repeats, Pto-like and receptor-like kinases related to disease resistance in grapevine. Molecular genetics and genomics 269: 612–623.

Di Gaspero G, Cipriani G, Adam-Blondon AF, Testolin R (2007) Linkage maps of grapevine displaying the chromosomal locations of 420 microsatellite markers and 82 markers for R-gene candidates. Theoretical and Applied Genetics 114: 1249–1263.

Dodds PN, Lawrence GJ, Catanzariti AM, Teh T, Wang CI, AyliVe MA, Kobe B, Ellis JG (2006) Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. Proceedings of the National Academy of Sciences 103: 8888–8893.

D'Ovidio R, Raiola A, Capodicasa C, Devoto A, Pontiggia D, Roberti S, Galletti R, Conti E, O'Sullivan D, De Lorenzo G (2004) Characterization of the complex locus of bean encoding polygalacturonaseinhibiting proteins reveals subfunctionalization for defense against fungi and insects. Plant Physiology 135: 2424–2435.

Drennan PM, Smith MT, Golsworthy D, van Staden J, (1993) The occurrence of trehalose in the leaves of the desiccation-tolerant angiosperm *Myrrothammus flabellifolius*. Journal of Plant Physiology 142: 493–496.

Dubreuil-Maurizi C, Trouvelot S, Frettinger P, Pugin A, Wendehenne D, Poinssot B (2010) β-aminobutyric acid primes an NADPH oxidase-dependent reactive oxygen species production during grapevine-triggered immunity. Molecular Plant-Microbe Interactions 23:1012–1021.

Dunlevy JD, Kalua CM, Keyzers RA, Boss PK (2009) The production of flavour and aroma compounds in grape berries in Roubelakis-Angelakis, KA (ed) Grapevine Molecular Physiology and Biotechnology 11: 293-240 Springer Netherlands, Dordrecht.

Durrant WE, Dong X (2004) Systemic acquired resistance. Annual Review of Phytopathology 42: 185-209.

Dudareva N, Negre F, Nagegowda DA, Orlova I (2006) Plant volatiles: Recent advances and future perspectives. Critical Review of Plant Science 25: 417-440.

Egger E, (2008) La revisione non penalizza la lotta a peronospora e oidio. Informatore Agrario 13: 29–41.

Ehret DL, Menzies JG, Bogdanoff C, Ukthede RS, Frey B (2002) Foliar applications of fertilizer salts inhibit powdery mildew on tomato. Canadian Journal of Plant Pathology 24: 437-444.

Eulgem, T. and Somssich, I. E. (2007). Networks of WRKY transcription factors in defense signaling. Current Opinion in Plant Biology 10: 366–371.

Fahey J W, Zalcmann A T, Talalay P (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56: 5-51

Farmer EE, Almeras E, Krishnamurthy V (2003) Jasmonates and related oxylipins in plant responses to pathogenesis and herbivory. Current Opinion in Plant Biology 6: 372–378.

Fenn ME, Coffey MD (1984) Studies on the in vitro and in vivo antifungal activity of Fosetyl-Al and phosphorous acid. Phytopathology 74: 606-611.

Fernandez O, Béthencourt L, Quero A, Sangwan RS, Clément C (2010) Trehalose and plant stress responses: Friend or foe? Trends in Plant Science 15: 409-417.

Ferrari S, Galletti R, Vairo D, Cervone F, De Lorenzo G (2006) Antisense expression of the *Arabidopsis thaliana* AtPGIP1 gene reduces polygalacturonaseinhibiting protein accumulation and enhances susceptibility to *Botrytis cinerea*. Molecular Plant Microbe Interaction 19: 931–936.

Feussner I, Wasternack C (2002) The lipoxygenase pathway. Annual Review of Plant Biology 53: 275-297.

Figueiredo A, Fortes AM, Ferreira S, Sebastiana M, Choi YH, Sousa, Acioli-Santos B, Pessoa F, Verpoorte R, Pais MS (2008) Transcriptional and metabolic profiling of grape (*Vitis vinifera* L.) leaves unravel possible innate resistance against pathogenic fungi. Journal of Experimental Botany 59: 3371–3381.

Figueiredo A, Monteiro F, Fortes AM, Bonow-Rex M, Zyprian, E, Sousa L, Pais MS (2012) Cultivar-specific kinetics of gene induction during downy mildew early infection in grapevine. Functional Integrated Genomics 12: 379-386.

Fischer BM, Salakhutdinov I, Akkurt M, Eibach R, Edwards KJ, Töpfer R, Zyprian EM (2004) Quantitative trait locus analysis of fungal disease resistance factors on a molecular map of grapevine. Theoretical and Applied Genetics 108: 501–515.

Flor HH (1971) Current status of the gene–for–gene concept. Annual Review of Phytopathology 9: 275–296.

Flores-Vélez LM, Ducaroir J, Jaunet AM, Robert M (1996) Study of the distribution of copper in an acid sandy vineyard soil by three different methods. European Journal of Soil Science 47: 523–32.

Fu XQ, Dong X (2013) Systemic Acquired Resistance: Turning Local Infection into Global Defense. Annual Review of Plant Biolpgy. 64: 1–25.

Fung RW, Gonzalo M, Fekete C, Kovacs LG, He Y, Marsh E, McIntyre LM, Schachtman DP, Qiu W (2008) Powdery mildew induces defense-oriented reprogramming of the transcriptome in a susceptible but not in a resistant grapevine. Plant Physiology 146: 236-249.

Gabriel DW, Rolfe BG (1990) Working models of specific recognition in plant-microbe interactions, Annual Review of Phytopathology 28: 365-391.

Gadea J, Mayda ME, Conejer V, Vera P (1996) Characterization of defenserelated genes ectopically expressed in viroid-infected tomato plants. Molecular Plant Microbe Interaction 9: 409-15.

Gaffney T, Friedrich L, Vernooij B, Negrotto D, Nye G, Uknes S, Ward E, Kessmann H, Ryals J (1993) Requirement of salicylic acid for the induction of systemic acquired resistance. Science 261: 754–756.

Gamm M, Héloir MC, Bligny R, Vaillant-Gaveau N, Trouvelot S, Gérard Alcaraz G, Frettinger P, Clément C, Pugin A, Wendehenne D, Adrian M (2011) Changes in Carbohydrate Metabolism in *Plasmopara viticola*-Infected Grapevine Leaves. Molecular plant microbe interactions. 24: 1061–1073.

Gams W, Hoekstra ES, Aptroot A (1998) CBS course of mycology. CBS Baarn, The Netherlands.

Gang DR, Costa MA, Fujita M, Dinkova-Kostova AT, Wang H-B, Burlat V, Martin W, Sarkanen S, Davin LB, Lewis NG (1999) Regiochemical control of monolignol radical coupling: a new paradigm for lignin and lignin biosynthesis. Chemical Biology 6: 143–151.

Garcia PC, Rivero RM, Ruiz JM, Romero L (2003) The role of fungicides in the physiology of higher plants: implications for defense responses. Botanical Review 69: 162–172

Garcia-Brugger A, Lamotte O, Vandelle E, Bourque S, Lecourieux D, Poinssot B, Wendehenne D, Pugin A (2006) Early signalling events induced by elicitors of plant defenses. Molecular Plant Microbe Interaction 19: 714-724.

Gärtel W (1961) Einfluss der im Weinbau gebräuchlichen Pflanzenschutzmittel auf Keimung und Schlauchwachstum bei Rebpollen. Biologische Bundesanstalt Land- und Forstwirtschaft 104: 108– 112.

Gaumann E (1946) Pflanzliche Infektionslehre (Basel: Birkhauser Verlag).

Gay-Bellile F, Lacouture J, Sarrazin JF, Courlit Y, Menard E (1983) Le point sur le mildiou de la vigne. Les fongicides systémiques. Les souches résistantes. Progres Agricole et Viticole 100: 83–89.

Gessler C, Pertot L, Perazzolli M (2011) *Plasmopara viticola*: a review of knowledge on downy mildew of grapevine and effective disease management. Phytopatholgia Mediterranea 50: 3–44.

Ghassemian M, Nambara E, Cutler S, Kawaide H, Kamiya Y, McCourt P (2000) Regulation of abscisic acid signaling by the ethylene response pathway in Arabidopsis. Plant Cell 12: 1117–1126.

Gibson SI (2005) Control of plant development and gene expression by sugar signaling. Current Opinion of Plant Biology 8: 93-102.

Gindro K, Pezet R, Viret O (2003) Histological study of the response of two *Vitis vinifera* cultivars (resistant and susceptible) to Plasmopara viticola infections. Plant Physiology and Biochemistry 41: 846–853.

Gindro K, Spring JL, Pezet R, Richter H, Viret O (2006) Histological and biochemical criteria for pbjective and early selection of grapevine cultivars resistent to Plas*mopara viticola*. Vitis 45: 191-196.

Gindro K, Godard S, de Groote I, Viret O (2007) Peut-on stimuler les mécanismes de défense de la vigne? Une nouvelle méthode pour évaluer le potentiel des éliciteurs. Revue Suisse De Viticulture Arboriculture, Horticulture 126: 553–558.

Gindro K, Alonso-Villaverde V, Voinesco F, Spring JL, Viret O, Dubuis PH (2012) Susceptibility to downy mildew in grape clusters: New microscopical and biochemical insights. Plant Physiology and Biochemistry 52: 140-146.

Giri AP, Wunsche H, Mitra S, Zavala JA, Muck A, Svatos A, Baldwin IT (2006) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. VII. Changes in the plant's proteome. Plant Physiol142: 1621–1641.

Gisi U, Harr J, Sandmeijer S, Wiedmer H (1983) A new systemic oxazolidinone fungicide (SAN 371 F) against diseases caused by Peronosporales. Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 48: 541–549.

Gisi U,Sierotzki H, Cook A, McCaffery A (2002) Mechanisms influencing the evolution of resistance to Qo inhibitor fungicides. Pest Management Science 58: 859–867.

Gisi U, Waldner M, Kraus N, Dubuis PH, Sierotzki H (2007) Inheritance of resistance to carboxylic acid amide fungicides in *Plasmopara viticola*. Plant Pathology 56: 199-208.

Gisi U, Sierotzki H (2008) Fungicide modes of action and resistance in downy mildews, Springer: 157-167.

Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annual Review of Phytopathology 43: 205–227.

Gobbin D, Jermini M, Loskill B, Pertot I, Raynal M, Gessler C (2005) Importance of secondary inoculum of *Plasmopara viticola* to epidemics of grapevine downy mildew. Plant Pathology 54: 522–534.

Gobel C, Feussner I, Hamberg M, Rosahl S (2002) Oxylipin profiling in pathogen-infected potato leaves. Biochimica et Biophysica Acta 1548: 55–64.

Godard S, Slacanin I, Viret O, Gindro K (2009) Induction of defense mechanisms in grapevine leaves by emodin- and anthraquinone-rich plant extracts and their conferred resistance to downy mildew. Plant Physiology and Biochemistry 47: 827–837.

Goddijn OJ, Verwoerd TC, Voogd E, Krutwagen RW, De Graaf PT, van Dun K, Poels J, Ponstein AS, Damm B, Pen J (1997) Inhibition of trehalase activity enhances trehalose accumulation in transgenic plants. Plant Physiology 113: 181-190.

Goddijn OJ, Smeekens S (1998) Sensing trehalose biosynthesis in plants. Plant Journal 14:143-146.

Godet F, Roques JF, Compagnon JM (1997) L'azoxystrobine: fongicide polyvalent cereales et vigne. Phytoma 50: 52–54.

Godfrey D, Able AJ, Dry IB (2007) Induction of a grapevine germin-like protein (VvGLP3) gene is closely linked to the site of *Erysiphe necator* infection: a possible role in defense? Molecular Plant Microbe Interaction 20: 1112-1115

Gomès E, Coutos-Thévenot P (2009). Molecular aspects of grapevine-pathogenic fungi interactions. In Roubelakis-Angelakis, KA (ed) Grapevine Molecular Physiology and Biotechnology 15: 407-428. Springer Netherlands, Dordrecht.

Gonzalez-Alvarez M, Gonzalez-Barreiro C, Cancho-Grande B, Simal-Gandara J (2012) Impact of phytosanitary treatments with fungicides (cyazofamid, famoxadone, mandipropamid and valifenalate) on aroma compounds of Godello white wines. Food Chemistry 131: 826–836.

Gottstein HD, Kuc J (1989) Induction of systemic resistance to anthracnose in cucumber by phosphates. Phytopathology 79: 176–179.

Gouot JM (2006) Field efficacy of Profiler, a fluopicolide and fosetyl-Al fungicide combination for the control of grape downy mildew (*Plasmopara viticola*). Pflanzenschutz- Nachrichten Bayer 59: 293–302.

Gozzo F (2003) Systemic acquired resistance in crop protection: from nature to a chemical approach. Journal of Agriculture and Food Chemistry 51: 4487–4503.

Grant M, Lamb C (2006) Systemic immunity. Current Opinion in Plant Biology 9: 414–420.

Grosskopf DG, Felix G, Boller T (1991) A yeast-derived glycopeptide elicitor and chitosan or digitonin differentially induce ethylene biosynthesis, phenylalanine ammonia-lyase and callose formation in suspension-cultured tomato cells. Journal of Plant Physiology 138: 741–46.

Gullino ML, Mescalchin E, Mezzalama M (1997) Sensitivity to cymoxanil in populations of *Plasmopara viticola* in northern Italy. Plant Pathology 46: 729–736.

Hahlbrock K, Scheel D (1989) Physiology and molecular biology of phenylpropanoid metabolism. Annual Review of Plant Physiology and Plant Molecular Biology 40: 347-369.

Hahn M, Mendgen K (2001) Signal and nutrient exchange at biotrophic plant fungus interfaces. Current Opinion in Plant Biology 4: 322–327.

Halitschke R, Baldwin IT (2003) Antisense LOX expression increases herbivore performance by decreasing defense responses and inhibiting growth-related transcriptional reorganization in *Nicotiana attenuata*. Plant Journal 36: 794–807

Halkier B A, Gershenzon J (2006) Biology and biochemistry of glucosinolates. Annual Review of Plant Biology 57: 303-333.

Hall JL, Williams LE (2000) Assimilate transport and partitioning in fungal biotrophic interactions. Australian Journal of Plant Physiology 27: 549–560

Hamiduzzaman MM, Jakab G, Barnavon L, Neuhaus JM, Mauch-Mani B (2005) ß-Aminobutyric acidinduced resistance against downy mildew in grapevine acts through the potentiation of callose formation and jasmonic acid signaling. Molecular Plant Microbe Interactions 18: 819–829.

Hammerschmidt R, Kuc J (1995) Induced resistance to disease in plants, Kluwer Dordrecht.

Hammerschmidt R, Smith-Becker JA (1999) The role of salicylic acid in disease resistance. In Agrawal AA et al. (eds) induced plant defenses against pathogens and herbivores: biochemistry, ecology and agriculture. American Phytopathological Society Press pp. 37–7153.

Hammerschmidt R, Nicholson RL (1999) A survey of plant defense responses to pathogens. In Agrawal AA et al. (eds) induced plant defenses against pathogens and herbivores: biochemistry, ecology and agriculture. American Phytopathological Society Press 55–71.

Hammerschmidt R (1999) Induced disease resistance: how do induced plants stop pathogens? Physiology Molecular Plant Pathology 55: 77–84.

Hammerschmidt R. (2007) Introduction: definitions and some history. In Walters D et al. (eds) Induced resistance for plant disease control: a sustainable approach to crop protection. Oxford: Blackwell Publishing; 1–8.

Hammerschmidt R (2009) Systemic acquired resistance. Advances in Botanical Research 51: 173–222.

Hardham AR (2007) Cell biology of plant–oomycete interactions. Cellular Microbiology 9: 31–39.

Harm A, Kassemeyer HH, Seibicke T, Regner F (2011) Evaluation of chemical and natural resistance inducers against downy mildew (*Plasmopara viticola*) in grapevine. American Journal of Enology and Viticulture 62: 184-192.

Haubrick LL, Assmann SM (2006) Brassinosteroids and plant function:some clues, more puzzles. Plant Cell Environment 29: 446-457

Hayes MA, Feechan A, Dry IB (2010) Involvement of abscisic acid in the coordinated regulation of a stressinducible hexose transporter (VvHT5) and a cell wall invertase in grapevine in response to biotrophic fungal infection. Plant Physiology 153: 211–221.

He SY, Huang HC, Collmer A (1993) *Pseudomonas syringae* pv. Syringae Harpin PSS: a protein that is secreted by the Hrp pathway and elicits the hypersensitive response in plants. Cell 73: 1255–1266.

Heaney S P, Hall A A, Davies S A, Olaya G (2000) Resistance to fungicides in the QoI STAR cross resistance group: current perspectives. Proceedings of the BCPC Conference – Pests and Diseases 755–762.

Heath MC (2000) Nonhost resistance and nonspecific plant defenses. Current Opinion in Plant Biology, 3: 315–319.

Hedden P, Phillips AL (2000) Gibberellin metabolism: new insights revealed by the genes. Trends in Plant Science 5: 523–530.

Heibertshausen DS (2009) PhD thesis: Befallsreduzierung von Plasmopara viticola (Berk. & Curt. ex de Bary) Berl. & de Toni im ökologischen Weinbau auf der Basis von Maßnahmen zur Kupferminimierung und Verfahren der Induzierten Resistenz. Justus-Liebig-Universität Giessen and Forschungsanstalt Geisenheim.

Heidel AJ, Baldwin IT (2004) Microarray analysis of salicylic acid- and jasmonic acid-signalling in responses of *Nicotiana attenuata* to attack by insects from multiple feeding guilds. Plant, Cell and Environment 27: 1362–1373.

Heil M (1999) Systemic acquired resistance: available information and open ecological questions. Journal of Ecology 87: 341–346.

Heil M, Walters D (2009) Ecological consequences of plant defense signalling. In van Loon LC (ed.) Plant Innate Immunity. 15: 667-716 Elsevier, Amsterdam.

Heil M, Bostock RM (2002) Induced systemic resistance (ISR) against pathogens in the context of induced plant defenses. Annals of Botany 89: 503–51.

Heitefuss R (ed) (2000) Pflanzenschutz: Grundlagen der praktischen Phytomedizin. Thieme, Stuttgart.

Herbers K, Meuwly P, Frommer WB, Metraux JP, Sonnewald U (1996a) Systemic acquired resistance mediated by the ectopic expression of invertase: Possible hexose sensing in the secretory pathway. The Plant Cell 8: 793–803.

Herbers K, Meuwly P, Metraux JP, Sonnewald U (1996b) Salicylic acid independent induction of pathogenesis-related protein transcripts by sugars is dependent on leaf developmental stage. Federation of European Biochemical Societies Letters 397: 239–244.

Herbers K, Meuwly P, Metraux JP, Sonnewald U (1996b) Salicylic acidindependent induction of pathogenesis-related protein transcripts by sugars is dependent on leaf developmental stage. FEBS Lett 397: 239–244.

Herms S, Seehaus K, Koehle H, Conrath U (2002) A strobilurin fungicide enhances the resistance of tobacco against TMV and *Pseudomonas syringae* pv. tabaci. Plant Physiology 130: 120-127.

Hewitt WB, Pearson RC (1988) Downy mildew. In: Pearson RC, Goheen AC (eds) Compendium of Grape Diseases. St Paul, MN, USA: APS Press, 11±3.

Hildebrandt A, Guillamón M, Lacorte S, Tauler R, Barceló D (2008) Impact of pesticides used in agriculture and vineyards to surface and groundwater quality (North Spain). Water Research 42: 3315–3326.

Hijwegen T (1963) Lignification, a possible mechanisms of active response against pathogens. Netherlands Journal of Plant Pathology 69: 314-317.

Hofmann U (2009) Optimization of downy mildew (Plasmopara viticola) control in organic viticulture with low copper doses, new copper formulations and plant strengtheners, results of 20 years of on farm research. ECO-CONSULT, Geisenheim, Germany.

Howe GA (2004) Jasmonates as signals in the wound response. Journal of Plant Growth Regulation 23: 223–237.

Howe GA, Jander G (2008) Plant immunity to insect herbivores. Annual Review of Plant Biology 59: 41–66.

Hofmann U (2003) Kupferreduzierung und Kupferersatz im ökologischen Weinbau Ergebnisse aus dem BÖW Ringversuch. In: Berichte aus der BBA – Pflanzenschutz im Ökologischen Landbau, Heft 118: 27-37.

Horsfall JG, Dimond AE (1957) Interactions of tissue sugar, growth substances, and disease susceptibility. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 27: 415–421.

Huang HT (2000) Fermentations and food science. In: Needham J (ed) Science and Civilisation in China. Volume 6: Biology and Biological Technology. Cambridge University Press, Cambridge, UK.

Hückelhoven R (2005) Powdery mildew susceptibility and biotrophic infection strategies. Microbiology Letters 245: 9–17.

Hückelhoven R (2007) Cell wallassociated mechanisms of disease resistance and susceptibility. Annual Review of Plant Phytopathology 45: 127.

Hunt M, Ryals J (1996) Systemic acquired resistance signal transduction, Critical Reviews in Plant Science 15: 583-606.

Iriti M, Faoro F (2003) Does benzothiadiazole-induced resistance increase fitness cost in bean? Journal of Plant Pathololology 85: 265–70.

Iriti M, Faoro F (2004) Plant defense and human nutrition: the phenylpropanoids on the menu. Current Topics in Nutraceutical Research 2: 47–65.

Iriti M, Castorina G, Vitalini G, Mignani I, Soave C, Fico G, Faoro F (2010) Chitosan-induced ethylene-independent resistance does not reduce crop yield in bean plants. Biological Control 54: 241–247.

Iriti M, Vitalini S, Di Tommaso G, D'Amico S, Borgo M, Faoro F (2011) New chitosan formulation prevents grapevine powdery mildew infection and improves polyphenol content and free radial scavenging activity of grape and wine. Australian Society of Viticulture and Oenology pp. 263-269.

Irizarry, RA, Hobbs, B, Collin, F, Beazer-Barclay, YD, Antonellis, KJ, Scherf, U, Speed, TP (2003) Exploration, Normalization, and Summaries of High Density Oligonucleotide Array Probe Level Data. Biostatistics 4: 249-264.

Jabs T, Tschöpe M, Colling C, Hahlbrock K, Scheel D (1997) Elicitor stimulated ion fluxes and O2 from the oxidative burst are essential components in triggering defense gene activation and phytoalexin synthesis in parsley. Proceedings of the National Academy of Sciences 94: 4800 4805.

Jacobson AR, Dousset S, Guichard N, Baveye PC, Andreux F (2005) Diuron mobility through vineyard soils contaminated with copper. Environment Pollution 138: 250–259.

Jager CE, Symons GM, Ross JJ, Reid JB (2008) Do brassinosteroids mediate the water stress response? Physiology Plant 133: 417–425.

Jalloul A, Montillet JL, Assigbetse K, Agnel JP, Delannoy E, Triantaphylides C, Daniel JF, Marmey P, Geiger JP, Nicole M (2002) Lipid peroxidation in cotton: Xanthomonas interactions and the role of lipoxygenases during the hypersensitive reaction. The Plant Journal 32: 1–12.

Janick J (2002) Ancient Egyptian agriculture and the origins of horticulture. Acta Horticulturae 582: 23-39.

Jayaraj J, Yi H, Liang GH, Muthukrishnan S, Velazhahan R (2004) Foliar application of *Bacillus subtilis* AUBS1 reduces sheath blight and triggers defense mechanisms in rice. Journal of Plant Diseases and Protection 111: 115–125.

Jeandet P, Douillet-Breuil AC, Bressis R, Debord S, Spaghi M, Adrian M (2002) Phytoalexins from the Vitaceae: Biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity and metabolism. Journal of Agriculture and food chemistry 50: 2731–2741.

Jeong ST, Goto-Yamamoto N, Hashizume K, Esaka M (2006) Expression of the flavonoid 30- hydroxylase and flavonoid 3′,5′-hydroxylase genes and flavonoid composition in grape (Vitis vinifera). Plant Science 170: 61–69.

Jia Y, McAdams SA, Bryan GT, Hershey HP, Valent B (2000) Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. The EMBO Journal 19: 4004–4014.

Jiang CJ, Shimono M, Sugano S, Kojima M, Yazawa K, Yoshida R, Inoue H, Hayashi N, Sakakibara H, Takatsuji H (2010) Abscisic acid interacts antagonistically with salicylic acid signaling pathway in rice-*Magnaporthe grisea* interaction. Molecular Plant Microbe Interaction 23: 791–798.

Jones JD, Dangl JL (2006) The plant immune system. Nature 444: 323–329.

Johnson R, Ryan CA (1990) Wound-inducible potato inhibitor II genes: Enhancement of expression by sucrose. Plant Molecular Biology 14: 527–536.

Jongebloed U, Szederkenyi J, Hartig K, Schobert C, Komor E (2004) Sequence of morphological and physiological events during natural ageing and senescence of a castor bean leaf: sieve tube occlusion and carbohydrate back-up precede chlorophyll degradation. Physiology of Plant 120: 338–346.

Kamoun S, Hraber P, Sobral S, Nuss D, Govers F (1999) Initial assessment of gene diversity for the Oomycete pathogen *Phytophthora infestans* based on expressed sequences, Fungal Genetics and Biology 28: 94–106.

Kant MR, Bleeker PM, Van wijk M, Schuurink RC, Haring MA (2009) Plant volatiles in defense in Van Loon LC (ed) plant innate immunity (advances in botanical research) Vol. 51, 614-667 Elsevier.

Karita, E., Yamakawa, H., Mitsuhara, I., Kuchitsu, K, Ohashi, Y (2004) Three types of tobacco calmodulins characteristically activate plant NAD kinase at different $Ca2^{+}$ concentrations and pHs. Plant Cell Physiology 45: 1371–1379.

Kassemeyer HH (1996) Integrated management of grapevine diseases and pests, Brighton Crop Protection Conference, 119-124.

Kassemeyer HH (2008) So wächst die Rebenperonospora. Der Badische Winzer 5: 28-30.

Kast WK (1996) Untersuchungen zur Wirksamkeit sehr geringer Kupfermengen gegen *Plasmopara viticola* im Weinbau. Nachrichtenblatter der Deutsche Pflanzenschutzverein 48: 63-65

Kauer R (2003) Sicherheit durch phosphorige Säure. Das Deutsche Weinmagazin 12: 24 – 27.

Kehlenbeck H, Krone C, Oerke EC, Schonbeck F (1994) The effectiveness of induced resistance on yield of mildewed barley. Journal of Plant Disease and Protection 101: 11–21.

Kennelly MM, Gadoury DM, Wilcox WF, Magarey PA, Seem RC (2007) Primary infection, lesion productivity and survival of sporangia in the grapevine downy mildew pathogen *Plasmopara viticola*. Phytopathology 97: 512–522.

Kessmann H, Staub T, Hofmann C, Maetzke T, Herzog J, Ward E, Uknes S, Ryals J (1994) Induction of systemic acquired disease resistance in plants by chemicals. Annual Review in Phytopathology 32: 439-459.

Khilare VC, Deokate AS, Ganawane LV (2003) Occurrence of aluminium phosethyle (allitte) resistance in *Plasmopara viticola* causing downy mildew of grapevine in Maharashtra. Journal of Phytological Research 16: 239 – 241.

Kiefer B, Riemann M, Büche C, Kassemeyer H H, Nick P (2002) The host guides morphogenesis and stomatal targeting in the grapevine pathogen *Plasmopara viticola*. Planta 215: 387-393.

Kim YC, Blee KA, Robins J, Anderson AJ (2001) Oxycom™ under field and laboratory conditions increases resistance responses in plants. European Journal of Plant Pathology 107: 129–136.

Kim KC, Lai Z, Fan B, Chen Z (2008) Arabidopsis WRKY38 and WRKY62 transcription factors interact with histone deacetylase 19 in basal defense. The Plant Cell 20: 2357–2371.

Kirsch C, Hahlbrock K, Somssich IE (1997) Rapid and transient induction of parsley microsomal delta 12 fatty acid desaturase mRNA by fungal elicitor. Plant Physiology 115:283–9.

Kirk PM, Cannon PF, David JC, Stalpers, J A (2001) Ainsworth and Bisby's dictionary of the fungi (9th ed). Wallingford: CAB International.

Klarzynski O, Plesse B, Joubert JM, Yvin JC, Kopp M, Kloareg B, Fritig B (2000) Linear b-1,3 glucans are elicitors of defense responses in tobacco. Plant Physiology 124: 1027–1038.

Kliebenstein DJ, Rowe HC (2008) Ecological costs of biotrophic versus necrotrophic pathogen resistance, the hypersensitive response and signal transduction. Plant Science 174: 551–556.

Kliewe H (Ed) (1981) Wein und Gesundheit. Verlag und Druckerei D. Meininger GmbH, Neustadt a. d. Weinstraße.

Kocal N, Sonnewald U, Sonnewald S (2008) Cell Wall-Bound Invertase Limits Sucrose Export and Is Involved in Symptom Development and Inhibition of Photosynthesis during Compatible Interaction between Tomato and *Xanthomonas campestris* pv *vesicatoria*. Plant physiology 148: 1523–1536.

Koch KE (1996) Carbohydrate-modulated gene expression in plants. Annual Review of Plant Physiology and Plant Molecular Biology 47: 509–540.

Komárek M, Cadková E, Chrastný V, Bordas F, Bollinger J (2010) Contamination of vineyard soils with fungicides: a review of environmental and toxicological aspects. Environmental Interaction 36: 138–151.

Kortekamp A, Wind R, Zyprian E (1998) Investigation of the interaction of *Plasmopara viticola* with susceptible and resistant grapevine cultivars. Journal of Plant Disease Protection 105: 475-488.

Koornneef A, Pieterse CMJ (2008) Cross talk in defense signaling. Plant Physiology 146: 839–844.

Kortekamp A (2005) *Growth, occurrence and development of septa in Plasmopara viticola* and other members of the Peronosporaceae using light- and epifluorescence-microscopy. Mycology Research 109: 640–648.

Kortekamp A (2006) Expression analysis of defense-related genes in grapevine leaves after inoculation with a host and a non-host pathogen. Plant Physiology and Biochemistry 44: 58–67.

Kortekamp A, Welter L, Vogt S, Knoll A, Schwander F, Topfer R, Zyprian E (2008) Identification, isolation and characterization of a CC-NBS-LRR candidate disease resistance gene family in grapevine. Molecular Breeding 22: 421-432.

Krapp A, Hofmann B, Schafer C, Stitt M (1993) Regulation of the expression of rbcS and other photosynthetic genes by carbohydrates: A mechanism for the sink regulation of photosynthesis. Plant Journal 3: 817- 828.

Krisa S, Teguo PW, Decendit A, Deffieux G, Vercauteren J, MerillonJM (1991) Production of 13C-labelled anthocyanins by *Vitis vinifera* cell suspension cultures. Phytochemistry 51: 651–656.

Krishna P, (2003) Brassinosteroid-mediated stress responses. Journal of Plant Growth Regulation 22: 289–297.

Kuc J (1957) A biochemical study of the resistance of potato tuber tissue to attack by various fungi. Phytopathology 47: 676–680.

Kuc J, Shockley G, Kearney K (1975) Protection of cucumber against *Colletotrichum lagenarium* by *Colletotrichum lagenarium*. Physiological Plant Pathology 7: 195–199.

Kuc J (1982) Induced immunity to plant disease. Bioscience 32: 854–856.

Kuc J (2001) Concepts and direction of induced systemic resistance in plants and its application. European Journal of Plant Pathology 107: 7–12.

Kühne S, Friedrich B, Röhrig R (2011) Berichte aus dem Julius Kühn-Institut (164). Fachgespräch: Kupfer als Pflanzenschutzmittel Berlin-Dahlem.

Kundert J (1956) Die Peronospora der Rebe und ihre Bekämpfung im Jahre 1955. Schweizerische Zeitschrift für Obst- und Weinbau 65, 135–139.

Kunkel BN, Brooks DM (2002) Cross talk between signaling pathways in pathogen defense. Current Opinion in Plant Biology 5: 325-331.

Lamb C, Dixon RA (1997) The oxidative burst in plant disease resistance. Annual Review of Plant Physiology and Plant Molecular Biology 48: 251-275.

Langcake P, Pryce RJ (1976) The production of trans-resveratrol by *Vitis vinifera* and other members of the Vitaceae as a response to infection or injury, Physiological Plant Pathology 9: 77-86.

Langcake P and Pryce RJ (1977a) Oxidative dimerisation of 4-hydroxystilbenes in vitro: production of a grapevine phytoalexin mimic. Journal of the Chemical Society, Chemical Communications 7: 208-210.

Langcake P, Pryce RJ (1977b) A new class of phytoalexins from grapevines. Experientia 33: 151-152.

Langcake P, Cornford CA and Pryce RJ (1979) Identification of pterostilbene as a phytoalexin from *Vitis vinifera* leaves. Phytochemistry 18: 1025-1027.

Latorse MP, Gouot JM, Pepin R (1998) Curative activity of RPA407213 against downy mildew on grapevine: biological and microscopical study. In: Proceedings of an International Conference, Brighton Crop Protection Conference: Pests and Diseases. Brighton, UK 3: 863–868.

Latorse MP, Holah D, Bardsley R (2006) Fungicidal properties of fluopicolide-based products. Pflanzenschutz- Nachrichten Bayer 59: 185–200.

Latunde-Dada AO, Lucas JA (2001) The plant defense activator acibenzolar-Smethyl primes cowpea (*Vigna unguiculata* (L.) Walp) seedlings for rapid induction of resistance. Physiology Molecular Plant Pathology 58: 199–208.

Lebon G, Duchêne E, Brun O, Clément C (2005) Phenology of Xowering and starch accumulation in grape (*Vitis vinifera* L.) cuttings and vines. Annual Botany 95:943–948.

Lecourieux F, Pugin A, Lebrun A (2000) Phosphoproteins involved in the signal transduction of cryptogein, an elicitor of defense reactions in tobacco. Molecular Plant Microbe Interaction 13: 821–829.

Lecourieux D, Mazars C, Pauly N, Ranjeva R, Pugin A (2002) Analysis and effects of cytosolic free calcium increases in response to elicitors in *Nicotiana plumbaginifolia* cells. The Plant Cell 14: 2627-2641.

Lecourieux D, Raneva R, Pugin A (2006) Calcium in plant defense-signalling pathways. New Phytologist 171: 249–269.

Legay G, Marouf E, Berger D, Neuhaus JM, Mauch-Mani B, Slaughter A (2011) Identification of genes expressed during the compatible interaction of grapevine with *Plasmopara viticola* through suppression subtractive hybridization (SSH). European journal of plant pathology 129: 281–301.

Legendre L, Heinstein PF, Low PS (1992) Evidence for the participation of GTP–binding proteins in the elicitation of rapid oxidative burst in cultured soybean cells. Journal of Biological Chemistry 267: 20140–20147.

Leon-Reyes A, Du Y, Koornneef A, Proietti S, Ko¨ rbes AP, Memelink J, Pieterse CMJ, Ritsema T (2010) Ethylene signaling renders the jasmonate response of Arabidopsis insensitive to future suppression by salicylic acid. Molecular Plant Microbe Interact 23: 187–197.

Leroux P, Clerjeau M (1985) Resistance of *Botrytis cinerea* and *Plasmopara viticola* to fungicides in French vineyards. Crop Protection 4: 137–160.

Levine A, Pennell RI, Alvarez ME, Palmer R, Lamb C (1996) Calcium-mediated apoptosis in a plant hypersensitive disease resistance response. Current Biology 6: 427–437.

Li X, Zhang Y, Clarke J D, Li Y, Dong X (1999) Identification and cloning of a negative regulator of systemic acquired resistance, SNI1, through a screen for suppressors of npr1-1. Cell 98: 329–339.

Li LC, Cosgrove DJ (2001) Grass group I pollen allergens (beta-expansins) lack proteinase activity and do not cause wall loosening via proteolysis. European Journal of Biochemistry 268: 4217–4226.

Lindenthal M, Steiner U, Dehne HW, Oerke EC (2005) Effect of downy mildew development on transpiration of cucumber leaves visualized by digital infrared thermography. Phytopathology 95: 233–240.

Liswidowati F, Melchior F, Hohmann F, Schwer B, Kindl H (1991) Induction of stilbene synthase by *Botrytis cinerea* in cultured grapevine cells. Planta, 183: 307–314.

Llorente F, Muskett P, Sa´nchez-Vallet A, Lo´pez G, Ramos B, Sa´nchez-Rodrı´guez C, Jorda´ L, Parker J, Molina A (2008) Repression of the auxin response pathway increases Arabidopsis susceptibility to necrotrophic fungi. Molecular Plant pathology 1: 496–509.

Logan BA, Monson RK, Potosnak MJ (2000). Biochemistry and physiology of foliar isoprene production. Trends in Plant Science 5: 477–481.

Lopez MA, Bannenberg G, Castresana C (2008) Controlling hormone signaling is a plant and pathogen challenge for growth and survival. Current Opinion in Plant Biology 11: 420–427.

Loskill B, Gobbin D, Berkelmann-Lochnertz B, Gessler C (2006) Disease dynamics and genetic variability of *Plasmopara viticola* in three untreated vineyards. Bulletin OILB/SROP 29, 11: 33–36.

Lunn JE, Feil RJ, Hendriks J, Gibon Y, Morcuende R, Osuna D, Scheible WR, Carillo R, Hajirezaei MR, Stitt M (2006) Sugar-induced increases in trehalose 6-phosphate are correlated with redox activation of ADPglucose pyrophosphorylase and higher rates of starch synthesis in *Arabidopsis thaliana*. Biochemistry Journal 397:139-148.

Lyon GD, Newton AC, 1999. Implementation of elicitor mediated induced resistance in agriculture. In Agrawal AA, Tuzun S, Bent E (eds): Induced plant defenses against pathogens and herbivores. APS Press, 299–318.

Lyon G (2007) Agents that can elicit induced resistance. In Walters D, Newton A, Lyons G (eds): induced resistance for plant defense, A Sustainable approach to crop protection. Blackwell Publishing, Oxford, UK.pp. 9–29.

Ma W, Berkowitz, GA (2007) The grateful dead: calcium and cell death in plant innate immunity. Cellular Microbiology 9: 2571–2585.

Madamanchi NR, Kuc J (1991) Induced systemic resistance in plants, in G. T. Cole, Hoch, HC (ed.) The fungal spore and disease initiation in plants and animals, Plenum Press New York, pp. 347-362.

Magarey PA, Wicks TJ, MF Wachtel (1990) Phosphonic (phosphorous) acid controls Plasmopara viticola the cause of downy mildew of grapevines. Australasian Plant Pathology 19: 126–127.

Magarey PA, Wachtel MF, Newton MR (1991a) Evaluation of phosphonate, fosetyl-Al and several phenylamide fungicides for post-infection control of grapevine downy mildew caused by Plasmopara viticola. Australasian Plant Pathology 20: 34–40.

Malacarne G, Vrhovsek U, Zulini L, Cestaro A, Stefanini M, Mattivi F, Delledonne M, Velasco R, Moser C (2011) Resistance to *Plasmopara viticola* in a grapevine segregating population is associated with stilbenoid accumulation and with specific host transcriptional responses. BMC Plant Biology 11: 114.

Mao P, Duan M, Wei C, Li Y (2007) WRKY62 transcription factor acts downstream of cytosolicNPR1 and negatively regulates jasmonate-responsive gene expression. Plant and Cell Physiology 48: 833–842.

Marais PG, Tromp A (1981) Metaxanine, a systemic fungicide against *Plasmopara viticola* on wine grapes: disease control, residues and effect on fermentation and wine quality. South African Journal for Enology and Viticulture 2: 67–70.

Marrs KA (1996) The functions and regulation of glutathione S-transferases in plants. Annual Review of Plant Physiology and Plant Molecular Biology 47: 127-158.

Martz F, Maury S, Pinon G, Legrand M (1998) cDNA cloning, substrate specificity and expression study of tobacco caffeoylCoA 3Omethyltransferase, a lignin biosynthetic enzyme. Plant Molecular Biology 36: 427437.

Mathieu, Y, Kurkdjian A, Xia H, Guern J, Koller A, Spiro MD, O'Neill M, Albersheim P, Darvill A (1991) Membrane responses induced by oligogalacturonides in suspension–cultured tobacco cells. Plant Journal 1: 333–343.

Matthysse AG, Marry M, Krall L, Kaye M, Ramey BE, Fuqua C, White AR (2005) The effect of cellulose overproduction on binding and biofilm formation on roots by *Agrobacterium tumefaciens*. Molecular Plant Microbe Interactions 18: 1002–1010.

Mattivi F, Reniero F, Korhammer S (1995) Isolation, characterization, and evolution in red wine vinification of resveratrol monomers. Journal of Agriculture and Food Chemistry 43: 1820-1823.

Mauch F, Mauch-Mani B, Boller T (1988) Antifungal hydrolases in pea tissue: Inhibition of fungal growth by combinations of chitinase and -1,3- glucanase. Plant Physiology 88: 936-942.

Mauch-Mani B (2002) Host resistance to downy mildew diseases. In PTN Spencer-Phillips, Gisi U, Lebeda A (eds) Advances in downy mildew research (pp. 59–83). Dordrecht: Kluwer.

Mauch-Mani B, Mauch F (2005) The role of abscisic acid in plant-pathogen interactions. Current Opinions in Plant Biology 8: 409– 414.

McGrath KC, Dombrecht B, Manners JM, Schenk PM, Edgar CI, Maclean DJ, Scheible WR, Udvardi MK, Kazan K (2005) Repressor- and activator-type ethylene response factors functioning in jasmonate signaling and disease resistance identified via a genome-wide screen of Arabidopsis transcription factor gene expression. Plant Physiology 139: 949–959.

McLeod A, Smart CD, Fry WE (2003) Characterization of 1,3-β-glucanase and 1,3;1,4-β-glucanase genes from *Phytophthora infestans*. Fungal Genetics and Biology 38:250–263.

Melan MA, Dong X, Endara ME, Davis KR, Ausubel FM, Peterman TK (1993) An *Arabidopsis thaliana* lipoxygenase gene can be induced by pathogens, abscisic acid and methyl jasmonate. Plant Physiology 99S:220.

Melchior F, Kindl H (1991) Coordinate- and elicitor-dependent expression of stilbene synthase and phenylalanine ammonia-lyase genes in Vitis cv. Optima. Achieves of Biochemistry and Biophysics 288: 552-557.

Melotto M, Underwood W, Koczan J, Nomura K, He SY (2006) Plant stomata function in innate immunity against bacterial invasion. Cell 126: 969–980.

Mendgen K, Hahn M, Deising H (1996) Morphogenesis and mechanisms of penetration by plant pathogenic fungi. Annual Review of Phytopathology 34: 367–386.

Menard R, Alban S, de Ruffray P, Jamois F, Franz G, Fritig B, Yvin JC, Kauffmann S (2004) β-1,3 glucan sulphate, but not β-1,3 glucan, induces the salicylic acid signaling pathway in tobacco and Arabidopsis. The Plant Cell 16: 3020–3032.

Mercer RT, Lacroix G, Gouot JM, Latorse MP (1998) RPA 407213: a novel fungicide for the control of downy mildews, late blight and other diseases on a range of crops. In: Proceedings of an International Conference, Brighton Crop Protection Conference: Pests and Diseases. Brighton, UK 2: 319–326.

Meyers BC, Kozik A, Griego A, Kuang H, Michelmore RW (2003) Genome-wide analysis of NBS-LRR-encoding genes in Arabidopsis. Plant Cell 15: 809–834.

Mitchell AF, Walters DR (2004) Potassium phosphate induces systemic protection in barley to powdery mildew infection. Pest Management Science 60: 126–134.

Mithofer A, Fliegmann J, Daxberger A, Ebel C, Neuhaus- Url G, Bhagwat AA (2001) Induction of H(2)O (2) synthesis by beta-glucan elicitors in soybean is independent of cytosolic calcium transients. Federation of European Biochemical Societies Letters 508: 191–195.

Mitra S, Baldwin IT (2008) Independently silencing two photosynthetic proteins in N*icotiana attenuata* has different effects on herbivore resistance. Plant Physiology 148: 1128–1138.

Mittler R, Simon L, Lam E (1997b) Pathogen-induced programmed cell death in tobacco. Journal of Cell Science 110: 333–1344.

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science 9: 405–410.

Mlejnek P, Prochazka S (2002) Activation of caspase-like proteases and induction of apoptosis by isopentenyladenosine in tobacco BY-2 cells. Planta 215: 158–166.

Mohr HD (ed) (2005): Farbatlas Krankheiten, Schädlinge und Nützlinge an der Weinrebe. E. Ulmer, Stuttgart.

Mohr PG, Cahill DM (2007) Suppression by ABA of salicylic acid and lignin accumulation and the expression of multiple genes, in Arabidopsis infected with *Pseudomonas syringae* pv. tomato. Functional Integral Genomics 7: 181–191.

Moiseyev GP, Fedoreyeva LI, Zhuravlev YN, Yasnetskaya E, Jekel PA, Beintema JJ (1997) Primary structures of two ribonucleases from ginseng calluses. New members of the PR-10 family of intracellular pathogenesis- related plant proteins. Federation of European Biochemical Societies Letters 407: 207–210.

Moreau C, Clerjeau M, Morzieres, JP (1987) Bilan des essais detection de souches de *Plasmopara viticola* resistantes aux anilides anti-oomycetes (metalaxyl, ofurace, oxadixyl et benalaxyl) dans le vignoble francais en. Pont de la Maye: Rapport G.R.I.S.P. de Bordeaux.

Morel G (1946) Essais de laboratoire sur le mildiou de la Vigne. Revue de Viticulture 92: 210–213.

Moroldo M, Paillard S, Marconi R, Fabrice L, Canaguier A, Cruaud C, De Berardinis V, Guichard C, Brunaud V, Le Clainche I, Scalabrin S, Testolin R, Di Gaspero G, Morgante M, Adam-Blondon AF (2008) A physical map of the heterozygous grapevine 'Cabernet Sauvignon' allows mapping candidate genes for disease resistance. BMC Plant Biology 8: 66.

Mucharromah E, Kuc J (1991) Oxalate and phosphates induce systemic resistance against diseases caused by fungi, bacteria and viruses in cucumber. Crop Protection 10: 265–270.

Muckensturm N (1995) Modelisation du mildiou de la vigne en Champagne: Bilan de trois ans de validation du modele MILVIT et d'un an d'utilisation au service des avertissements agricoles, Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 60: 477-481.

Mur LAJ, Kenton P, Atzorn R, Miersch O, Wasternack C (2006) The outcomes of concentration specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. Plant Physiology 140: 249–262.

Müller KO (1959) Hypersensitivity In: Horsfall JG, Dimond AE, eds. Plant Pathology: an Advanced Treatise. New York: Academic Press, 1: 459–519.

Müller K, H Sleumer (1934) Biologische Untersuchungen über die Peronosporakrankheit des Weinstockes. Landwirtschaftliche Jahrbucher 79: 509–576.

Mullins MG, Bouquet A, Williams LE (1992) Biology of the Grapevine. Cambridge University Press, Cambridge.

Murolo G, Stanich I (1980) Prove di lotta antiperonosporica in Campania con Ridomil. Vignevini 7: 31–32.

Nandi A, Krothapalli K, Buseman CM, Li M, Welti R, Enyedi A, Shah J (2003) Arabidopsis sfd mutants aVect plastidic lipid composition and suppress dwarfing, cell death, and the enhanced disease resistance phenotypes resulting from the deficiency of a fatty acid desaturase. The Plant Cell 15: 2383–2398.

Navarro L, Jay F, Nomura K, He SY, Voinnet O (2008) Suppression of the micro RNA pathway by bacterial eVector proteins. Science 321: 964–967.

Nicholson RL, Hammerschmidt R (1992) Phenolic compounds and their role in disease resistance. Annual Review of Phytopathology 30: 369-389.

Niderman T, Genetet I, Bruyere T, Gees R, Stintzi A, Legrand M, Fritig B, Mosinger E (1995) Pathogenesis-related PR-1 proteins are antifungal. Plant Physiology 108: 17–27.

O'Connell RJ, Panstruga R (2006) tête-à-tête inside a plant cell: establishing compatibility between plants and biotrophic fungi and oomycetes. New Phytologist 171: 699–718.

OIV (2006) State of the vitiviniculture world report. L'Organisation Internationale de la Vigne et du Vin, Paris.

Oliver RP, Ipcho SVS (2004) Arabidopsis pathology breathes new life into the necrotrophs-vs.-biotrophs classification of fungal pathogens. Molecular Plant Pathology 5: 347–352.

Olmo HP (1996) The origin and domestication of the vinifera grape. In the origins and ancient history of wine. McGovern PE et al. (Eds.), pp. 31-43. Gordon and Breach, Amsterdam.

Orlandini S, Gozzini B, Rosa M, Egger E, Storchi P, Maracchi G, Miglietta F (1993) PLASMO: a simulation model for control of *Plasmopara viticola* on grapevine. Bulletin OEPP 23, 4: 619–626.

Orober M, Siegrist J, Buchenauer H (2002) Mechanisms of phosphate-induced disease resistance in cucumber. European Journal of Plant Pathology 108, 345–353.

Pajor E, Le Corre D, Silue D (2001) Phytogard® and DL-β-amino butyric (BABA) induce resistance to downy mildew (*Bremia lactucae*) in lettuce (*Lactuca sativa* L). European Journal of Plant Pathology 107: 861–869.

Pandey SP, Somssich IE (2009) The role of WRKY transcription factors in plant immunity. Plant Physiology 150: 1648-1655.

Parat C, Chaussod R, Lévêque J, Dousset S, Andreux F (2002) The relationship between copper accumulated in vineyard calcareous soils and soil organic matter and iron. European Journal Soil Science 53: 663–9.

Park SW, Kaimoyo E, Kumar D, Mosher, Klessig DF (2007) Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. Science 318: 113–116.

Parlevliet JE (1992) Selecting components of partial resistance, in H.T. Stalker and J.P. Murphy (eds), Plant Breeding in the 1990s, Proceedings of the Symposium on Plant Breeding in the 1990s, C.A.B. International, Oxon, pp. 281-302.

Pasquer F, Isidore E, Zarn J, Keller B (2005) Specific pattern of changes in wheat gene expression after treatment with three antifungal compounds. Plant Molecular Biology 57: 693–707.

Pearson RC, Goheen AC (1988) Compendium of Grape Diseases, APS Press, St. Paul, MN, USA.

Perazzolli M, Dagostin S, Ferrari A, Elad Y, Pertot I (2008) Induction of systemic resistance against *Plasmopara viticola* in grapevine by *Trichoderma harzianum* T39 and benzothiadiazole. Biological Control 47: 228–234.

Petit AN, Vaillant N, Boulay M, Clément C, Fontaine F (2006) Alteration of photosynthesis in grapevines affected by esca. Phytopathology 96: 1060–1066.

Pezet R, Pont V, Hoang–Van K (1991) Evidence for oxidative detoxification of pterostilbene and resveratrol by a laccase–like stilbene oxidase produced by Botrytis cinerea. Physiological and Molecular Plant Pathology 39: 441–450.

Pezet R., K. Gindro, O. Viret and H. Richter (2004) Effects of resveratrol, viniferins and pterostilbene on *Plasmopara viticola* zoospore mobility and disease development. Vitis 43: 145–148.

Pieterse CM, Van Loon LC (2004) NPR1: the spider in the web of induced resistance signaling pathways. Current Opinion in Plant Biology 7: 456–464.

Pieterse CMJ, Schaller A, Mauch-Mani B, Conrath U (2006) Signaling in plant resistance responses: divergence and cross-talk of defense pathways. In: Tuzun S, Bent E, eds. Multigenic and induced systemic resistance in plants. New York: Springer, pp. 166–196.

Pieterse CMJ, Dicke M (2007) Plant interactions with microbes and insects: from molecular mechanisms to ecology. Trends in Plant Science 12: 564–569.

Pietrzak U, McPhail DC (2004) Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia. Geoderma 122: 151–66.

Pimentel JC, Marques F (1969) “Vineyard sprayer's lung” a new occupational disease. Thorax 24 :678–88.

Poinssot B, Vandelle E, Bentejac M, Adrian M, Levis C, Brygoo Y, Garin J, Sicilia F, Coutos-Thevenot P, Pugin A (2003) The endopolygalacturonase 1 from *Botrytis cinerea* activates grapevine defense reactions unrelated to its enzymatic activity. Molecular Plant Microbe Interactions 16: 553–564.

Polesani M, Desario F, Ferrarini A, Zamboni A, Pezzotti M, Kortekamp A, Polverari A (2008) cDNAAFLP analysis of plant and pathogen genes expressed in grapevine infected with *Plasmopara viticola*. BMC Genomics 9: 142.

Polesani M, Bortesi L, Ferrarini A, Zamboni A, Fasoli M, Zadra C, Lovato A, Pezzotti M, Delledonne M, Polverari A (2010) General and species-specific transcriptional responses to downy mildew infection in a susceptible (*Vitis vinifera*) and a resistant (*V. riparia*) grapevine species. BMC Genomics 11: 117.

Prial FJ (1987) Wine; stealing from thieves. The New York Times March 15.

Pryce-Jones E, Carver T, Gurr SJ (1999) The roles of cellulase enzymes and mechanical force in host penetration by *Erysiphe graminis* f.sp. hordei. Physiological and Molecular Plant Pathology 55: 175–182.

Radonic A, Thulke S, Mackay IM, Landt O, Siegert W, Nitsche A (2004) Guideline for reference gene selection for quantitative real-time PCR. Biochemistry and Biophysics Research Communication 313: 856–862.

Rancé I, Fournier J, Esquerré-Tugaye MT (1998) The incompatible interaction between *Phytophthora parasitica* var. *nicotianae* race 0 and tobacco is suppressed in transgenic plants expressing antisense lipoxygenase sequences. Proceedings of the National Academy of Science 95: 6554-6559.

Rasmussen JB, Hammerschmidt R, Zook MN (1991) Systemic induction of salicylic acid accumulation in cucumber after inoculation with Pseudomonas syringae pv syringae. Plant Physiology 97: 1342–1347.

Raynal M, Debord C, Guittard S, Vergnes M (2007) EPIcure, a geographic information decision support system risk assessment of downy and powdery mildew epidemics in Bordeaux vineyards. Institut Français de la Vigne et du vin (IFV) 39, rue Michel Montaigne, 33290 Blanquefort, France.

Regnault-Roger C (2012) Trends for Commercialization of Biocontrol Agent (Biopesticide) Products in Approaches in Mérillon (ed) plant defense: biological control.

Reglinski T, Dann E, Deverall B (2007) Integration of induced resistance in crop production. In Walters D, Newton A, Lyons G (eds) Induced Resistance for Plant Defense, A Sustainable Approach to Crop Protection pp. 201–228.

Reid KE, Olsson N, Schlosser J, Peng F, Lund ST (2006) An optimized grapevine RNA isolation procedure and statistical determination of reference genes for real-time RT-PCR during berry development. BMC Plant Biology 6: 27-37.

Remor AP, Totti CC, Moreira DA, Dutra GP, Heuser VH, Boeira JM (2009) Occupational exposure of farm workers to pesticides: biochemical parameters and evaluation of genotoxicity. Environmental Interactions 35:273–8.

Repka V (2001b) Elicitor-stimulated induction of defense mechanisms, defense gene activation in grapevine cell suspension cultures. Biology of Plant 44: 555-565.

Repka V (2006) Early defense responses induced by two distinct elicitors derived from a *Botrytis cinerea* in grapevine leaves, cell suspensions. Biol Plant 50: 94-106.

Restrepo S, Myers KL, del Pozo O, Martin GB, Hart AL, Buell CR (2005) Gene profiling of a compatible interaction between *Phytophthora infestans* and *Solanum tuberosum* suggests a role for carbonic anhydrase. Molecular Plant Microbe Interactions 18: 913–922.

Reuveni M, Reuveni R (1995) Efficacy of foliar sprays of phosphates in controlling powdery mildews in field-grown nectarine, mango trees and grapevines. Crop Protection 4: 311–314.

Reuveni R, Reuveni M, Agapov V (1996) Foliar sprays of NPK fertilizers induce systemic protection against *Puccinia sorghi* and *Exserohilum turcicum* and growth response in maize. European Journal of Plant Pathology 102: 339348.

Reuveni R, Reuveni M (1998) Foliar-fertilizer therapy: a concept in integrated pest management. Crop Protection 17: 111–118.

Reuveni M (2001) Activity of trifloxystrobin against powdery and downy mildew diseases of grapevines. Canadian Journal of Plant Pathology 23: 52–59.

Reuveni M, Reuveni R (2002) Mono-potassium phosphate fertilizer (PeaK) – a component in integrated control of powdery mildews in fruit trees and grapevines. Acta Horticulturae 594: 619–625.

Ribolzi O, Valles V, Gomez L, Voltz M (2002) Speciation and origin of particulate copper in runoff water from a Mediterranean vineyard catchment. Environment Pollution 117: 261–71.

Rickard DA (2000) Review of phosphorus acid and its salts as fertilizer materials. Journal of Plant Nutrition 2: 161–180.

Ridley BL, O'Neill MA, Mohnen D (2001) Pectins: structure, biosynthesis, and oligogalacturonide-related signalling. Phytochemistry 57: 929–967.

Riemann M, Büche C, Kassemeyer H H, Nick P (2002) Cytoskeletal response during early development of the downy mildew of grapevine (*Plasmopara viticola*). Protoplasma 219: 13-22.

Robert N, Ferran J, Breda C, Coutos-Thevenot P, Boulay M, Buffard D, Esnault R, (2001) Molecular characterization of the incompatible interaction of *Vitis vinifera* leaves with *Pseudomonas syringae* pv. pisi: expression of genes coding for stilbene synthase, class 10 PR protein. European Journal of Plant Pathology 107:249-261.

Robert N, Roche K, Lebeau Y, Breda C, Boulay M, Esnault R, Buffard D (2002) Expression of grapevine chitinase genes in berries, leaves infected by fungal, bacterial pathogens. Plant Science 162:389-400.

Robert-Seilaniantz A, Navarro L, Bari R, Jones JD (2007) Pathological hormone imbalances. Current Opinion in Plant Biology 10: 372–379.

Roitsch T (1999) Source-sink regulation by sugar and stress. Current Opinion in Plant Biology 2:198-206.

Roitsch T, Ehness R (2000) Regulation of source/sink relations by cytokinins. Plant Growth Regulation 32: 359–367.

Rolland F, Baena-Gonzalez E, Sheen J (2006) Sugar sensing and signaling in plants: conserved and novel mechanisms. Annual Review of Plant Biology 57: 675–709.

Romero-Pérez AI, Ibern-Gómez M, Lamuela Raventos RM, De la Torre-Boronat MC (1999) Piceid, the major resveratrol derivative in grape juices. Journal of Agriculture and Food Chemistry 47: 1533-1536.

Roßberg D (2009) NEPTUN – Weinbau. Berichte aus dem Julius Kühn-Institut. Julius Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen Institut für Strategien und Folgenabschätzung im Pfl anzenschutz.

Ross AF (1961) Systemic acquired resistance induced by localized virus infections in plants. Virology 14: 340–358.

Ross AF (1966) Systemic effects of local lesion formation, in A. B. R. Beemster, Dijkstra J (ed) Viruses of Plants, North-Holland Publishing Amsterdam, pp. 127-150.

Rumbolz J, Wirtz S, Kassemeyer HH, Guggenheim R, Schafer E, Buche C (2002) Sporulation of *Plasmopara viticola*: differentiation and light regulation. Plant Biology 4: 413–422.

Rusterucci C, Montillet JL, Agnel JP, Battesti C, Alonso B, Knoll A (1999) Involvement of lipoxygenase-dependent production of fatty acid hydroperoxides in the development of the hypersensitive cell death induced by cryptoge in on tobacco leaves. Journal of Biological Chemistry 274: 36446–36455.

Ryals J, Uknes S, Ward E (1994) Systemic acquired resistance. Plant Physiology 104: 1109–1112.

Saladin G, Magné C, Clément C (2003a) Physiological stress responses of *Vitis vinifera* L. to the fungicides Xudioxonil and pyrimethanil. Pesticide Biochemistry and Physiology 77: 125–137.

Saladin G, Magné C, Clément C (2003b) Effects of Xudioxonil and pyrimethanil, two fungicides used against *Botrytis cinerea*, on carbohydrate physiology in *Vitis vinifera* L. Pest Management Science 59: 1083–1092.

Samoucha Y, Gisi U (1987a) Use of two- and threeway mixtures to prevent buildup of resistance to phenylamide fungicides in Phytophthora and Plasmopara. Phytopathology 77: 1405–1409.

Sanders D, Pelloux J, Brownlee C, Harper JF (2002) Calcium at the crossroads of signaling. Plant Cell 14: 401–417.

Sano H, Seo S, Orudgev E, Youssefian S, Ishizuka K, Ohashi Y (1994) Expression of the gene for a small GTP binding protein in transgenic tobacco elevates endogenous cytokinin levels, abnormally induces salicylic acid in response to wounding, and increases resistance to tobacco mosaic virus infection. Proceedings of the National Academy of Sciences 91: 10556–10560.

Santić Z, Puvacić Z, Radović S, Puvacić S (2005) Higher mortality risk of lung carcinoma in vineyard sprayers. Bosnian Journal of Basic Medicinal Science 5: 65–69.

Sanz A, Moreno JI, Castresana C (1998) PIOX, a new pathogen-induced oxygenase with homology to animal cyclooxygenase. Plant Cell 10: 1523–1537.

Schäfer P, Pfiffi S, Voll LM, Zajic D, Chandler PM, Waller F, Scholz U, Pons-Kühnemann J, Sonnewald S, Sonnewald U, Kogel, K (2009) Manipulation of plant innate immunity and gibberellin as factor of compatibility in the mutualistic association of barley roots with Piriformospora indica. Plant Journal 59: 461–474.

Scheible W, Pauly M (2004) Glycosyltransferase and cell wall biosynthesis: novel players and insights. Current Opinion in Plant Biology 7: 285–295.

Scheideler M, Schlaich NL, Fellenberg K, Beissbarth T, Hauser NC, Vingron M, Slusarenko AJ, Hoheisel JD (2002) Monitoring the switch from housekeeping to pathogen defense metabolism in Arabidopsis thaliana using cDNA arrays. Journal of Biological Chemistry 277: 10555–10561.

Schenk PM, Kazan K, Wilson I, Anderson JP, Richmond T, Somerville SC, Manners JM (2000) Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. Proceedings of National Academy of Science 97: 11655–11660.

Scherer E, Gisi U (2006) Characterization of genotype and mating type in European isolates of *Plasmopara viticola*. Journal of Phytopathology 154: 489–495.

Schilder A, Gillet J, Sysak R, Wise J (2002) Evaluation of environmentally friendly products for control of fungal diseases of grapes. In: 10th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing and Viticulture, Weinsberg, Germany, 163–167.

Schlauch KA, Grimplet J, Cushman J, Cramer GR (2010) Transcriptomics analysis methods: microarray data processing, analysis and visualization using the Affymetrix Genechip® *Vitis Vinifera* Genome Array. In Delrot S (eds.) Methodologies and results in grapevine research pp. 318–334. (Springer: Dordrecht, the Netherlands.

Schlösser E (1997) Allgemeine Phytopathologie, Thieme-Verlag, Stuttgart, New York.

Schluepmann H, Pellny TI, van Dijken A, Smeekens S, Paul M (2003) Trehalose 6-phosphate is indispensable for carbohydrate utilization and growth in Arabidopsis thaliana. Proceedings of the National Academy of Sciences 100: 6849-6854.

Schluepmann H, van Dijken A, Aghdasi M, Wobbes B, Paul M, Smeekens S (2004) Trehalose mediated growth inhibition of Arabidopsis seedlings is due to trehalose-6-phosphate accumulation. Plant Physiology 135: 879-890.

Schmitt A, Kunz S, Nandi S, Seddon B, Ernst A (2002) Use of Reynoutria sachalinensis plant extracts, clay preparations and *Brevibacillus brevis* against fungal diseases of grape berries. In: 10th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit- Growing and Viticulture, Weinsberg, Germany, 146–151.

Schmitt CA, Heiss EH, Dirsch VM (2010) Effect of resveratrol on endothelial cell function: molecular mechanisms. Biofactors 36(5):342–349.

Schnee S, Viret O, Gindro K (2008) Role of stilbenes in the resistance of grapevine to powdery mildew. Physiological and Molecular Plant Pathology 72: 128–133.

Scholes JD, Lee PJ, Horton P, Lewis DH (1994) Invertase: understanding changes in the photosynthetic and carbohydrate metabolism of barley leaves infected with powdery mildew. New Phytologist 126: 213–22.

Schwachtje J, Baldwin IT (2008) Why does herbivore attack reconfigure primary metabolism? Plant Physiology 146: 845–851.

Segarra G, Casanova E, Bellido D, Odena MA, Oliveira E, Trillas I (2007) Proteome, salicylic acid, and jasmonic acid changes in cucumber plants inoculated with *Trichoderma asperellum* strain T34. Proteomics 7: 3943–3952.

Serres JM, Carraro GA (1976) DPX-3217, a new fungicide for the control of grape downy mildew, potato late blight and other Peronosporales. Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent 41: 645–650.

Seskar M, Shulaev V, Raskin I (1998) Endogenous methyl salicylate in pathogen-inoculated tobacco plants. Plant Physiology 116: 387–392.

Shen QH, Saijo Y, Mauch S, Biskup C, Bieri S, Keller B, Seki H,Ulker B, Somssich IE, Schulze-Lefert P (2007) Nuclear activity of MLA immune receptors links isolate-specific and basal disease-resistance responses. Science 315: 1098–1103.

Shoresh M, Harman GE (2008) The molecular basis of shoot responses of maize seedlings to *Trichoderma harzianum* T22 inoculation of the root: A proteomic approach. Plant Physiology 147: 2147–2163.

Shulaev V, Silverman P, Raskin I (1997) Airborne signalling by methyl salicylate in plant pathogen resistance. Nature 385: 718–721.

Shulaev V, Leon J, Raskin I (1995) Is salicylic acid a transported signal of systemic acquired resistance in tobacco? The Plant Cell 7: 1691–1701.

Schultz H (1992) An empirical model for the simulation of leaf appearance and leaf area development of primary shoots of several grapevine (*vitis vinifera* L.) canopy-systems. Scientia Horticulturae 52: 179-200.

Siegfried W, Bosshard E, Schuepp H (1992) Erste Erfahrungen mit Plasmopara-Warngeraten im Rebbau, Schweizerische Zeitschrift für Obst- und Weinbau 128: 143-150.

Siegrist J, Orober M, Buchenauer H (2000) β-aminobutyric acid-mediated enhancement of resistance in tobacco to tobacco mosaic virus depends on the accumulation of salicylic acid. Physiological and Molecular Plant Pathology 56: 95-106.

Siemann EH, Creasy LL (1992) Concentration of the phytoalexin resveratrol in wine. American Journal of Enology and Viticultre 43: 49–52.

Sierotzki H, Kraus N, Assemat P, Stanger C, Cleere S, Windass J, Gisi U (2005) Evolution of resistance to QoI fungicides in *Plasmopara viticola* populations in Europe. In: Modern Fungicides and Antifungal Compounds IV. Dehne HW, Gisi U, Kuck KH, Russell PE, Lyr H (eds). BCPC, Alton, UK pp. 73-80.

Silue D, Pajot E, Cohen Y (2002) Induction of resistance to downy mildew (Peronospora parasitica) in cauliflower by DL-β-amino-n-butanoic acid (BABA). Plant Pathology 51: 97-102.

Simpson SD, Ashford AD, Harvey DJ, Bowles DJ (1998) Short chain oligogalacturonides induce ethylene production and expression of the gene encoding aminocyclopropane 1-carboxylic acid oxidase in tomato plants. Glycobiology 8: 579–583.

Slaughter AR, Hamiduzzaman MM, Gindro K, Neuhaus JM, Mauch-Mani B (2008) Beta-aminobutyric acid-induced resistance in grapevine against downy mildew: Involvement of pterostilbene. European Journal of Plant Pathology 122: 185-195.

Smillie R, Grant BR, Guest D (1989) The mode of action of phosphite: evidence for both direct and indirect modes of action on three *Phytophthora* spp. in plants. Phytopathology 79, 921-926.

Smirnoff N (2000) Ascorbic acid: Metabolism and functions of a multi-facetted molecule. Current Opinion in Plant Biology 3: 229-235.

Smyth GK (2005) Limma: linear models for microarray data. In Gentleman R et al. (eds.) Bioinformatics and computational biology solutions using R and Bioconductor. New York: Springer 397-420.

Spaepen S, Vanderleyden J, Okon Y (2009) Plant growth-promoting actions of rhizobacteria defense in Van Loon LC (ed) plant innate immunity (advances in botanical research), Vol. 51, Elsevier.

Sparla F, Rotino L, Valgimigli MC, Pupillo P, Trost P (2004) Systemic resistance induced by benzothiadiazole in pear inoculated with the agent of fire blight (*Erwinia amylovora*). Scientia Horticulturae 101: 269–279.

Spoel SH, Dong X (2008) Making sense of hormone crosstalk during plant immune responses. Cell Host and Microbe 3: 348–351.

Staub T, Sozzi D (1981) Résistance au métalaxyl en pratique et les conséquences pour son utilisation. Phytiatrie – Phytopharmacie 30: 283–291.

Stenzel K, Pontzen R, Seitz T, Tiemann R, Witzenberger A (1998) SZX 722: a novel systemic oomycete fungicide. Brighton Crop Protection Conference: Pests and Diseases 2: 367–374.

Sticher L, Mauch-Mani B, Metraux JP (1997) Systemic acquired resistance. Annual Review of Phytopathology 35: 235–270.

Symons GM, Davies C, Shavrukov Y, Dry IB, Reid JB, Thomas MR (2006) Grapes on steroids. Brassinosteroids are involved in grape berry ripening. Plant Physiology 140:150-158.

Tamm L; Amsler T; Schaerer H, Lévite D, Häseli A (2006) Current downy mildew control strategies in Swiss organic vineyards. Beitrag präsentiert bei der Konferenz: Joint Organic Congress, Odense, Denmark.

Tamm L, Fuchs JG, Böger N, Mühletaler L, Amsler A, Levite D, Häseli A (2004) Eigenschaften von Tonerdepräparaten: Erfahrungen aus der Schweiz [Properties of acidified clay preparations:the Swiss experience]. Internationales Symposium für ökologischen Weinbau 27-36.

Tang X, Frederick RD, Zhou J, Halterman DA, Jia Y, Martin GB (1996) Initiation of plant disease resistance by physical interaction of AvrPto and Pto kinase. Science 274: 2060–2063.

Tassoni A, Fornale S, Franceschetti M, Musiani F, Michael AJ, Perry B, Bagni N, Franceschetti M, Ferri M, Tassoni A (2005) Jasmonates, Na-orthovanadate promote resveratrol production in *Vitis vinifera* cv. Barbera cell cultures. New Phytologist 166: 895-905.

Tassoni A, Fornalé S, Franceschetti M, Musini F, Michael AJ, Perry B, Bagni N (2005) Jasmonates and Na-orthovanadate promote resveratrol production in *Vitis vinifera* cv Barbera cell cultures. New Phytologist 166: 895-905

Thomma BP, Penninckx IA, Broekaert WF, Cammue BP (2001) The complexity of disease signalling in Arabidopsis. Current Opinion in Immuology 1: 63–68.

Thompson JE, Fry SC (2001) Restructuring of wall-bound xyloglucan by transglycosylation in living plant cells. Plant Journal 26: 23–34.

Thordal–Christensen H, Zhang Z, Wei Y, Collinge DB (1997) Subcellular localization of H_2O_2 in plants. H_2O_2 accumulation in papillae and hypersensitive response during the barley–powdery mildew interaction. The Plant Journal 11: 1187–1194.

Thuleau P, Ward JM, Ranjeva R, Schroeder JI (1994) Voltage-dependent calciumpermeable channels in the plasma membrane of a higher plant cell. The EMBO Journal 13: 2970–2975.

Tian D, Traw MB, Chen JQ, Kreitman M, Bergelson J (2003) Fitness costs of R-gene-mediated resistance in Arabidopsis thaliana. Nature 423: 74–77.

Trillas MI, Segarra G, (2009) Interactions between nonpathogenic fungi and plants in Van Loon LC (ed) plant innate immunity (advances in botanical research), Vol. 51, Elsevier.

Trotel-Aziz P, Couderchet M, Vernet G, Aziz A (2006) Chitosan stimulates defense reactions in grapevine leaves and inhibits development of *Botrytis cinerea*. European Journal Plant Pathology 114: 405-413.

Trouvelot S, Varnier AL, Allègre M, Mercier L, Baillieul F, Arnould C, Gianinazzi-Pearson V, Klarzynski O, Joubert JM, Pugin A, Daire X (2008) A b-1,3 glucan sulfate induces resistance in grapevine against *Plasmopara viticola* through priming of defense responses, including HR-like cell death. Molecular Plant Microbe Interactions 21: 232–243.

Turner JG, Ellis C, Devoto A (2002) The jasmonate signal pathway. Plant Cell 14: S153–S164.

Ueda H, Yamaguchi Y, Sano H (2006) Direct interaction between the tobacco mosaic virus helicase domain and the ATP-bound resistance protein, N factor during the hypersensitive response in tobacco plants. Plant Molecular Biology 61: 31–45.

Vallad GE, Goodman RM (2004) Systemic acquired resistance and induced systemic resistance in conventional agriculture. Crop Science 44: 1920–1934.

Van der Biezen EA, Jones JDG (1998) Plant disease-resistance proteins and the gene-for-gene concept. Trends in Biochemical Sciences 23: 454–456.

Van der Auwera GDBR, Van de Peer Y, De Rijk P, Van den Broeck I, De Wachter R (1995) The phylogeny of the Hyphochytriomycota as deduced from ribosomal RNA sequences of Hyphochytrium catenoides. Molecular Biology and Evolution 12: 671–678.

Van der Hoorn RAL, Kamoun S (2008) From guard to decoy: A new model for perception of plant pathogen effectors. The Plant Cell 20: 2009–2017.

Van Hulten M, Pelser M, Van Loon LC, Pieterse CMJ, Ton J (2006) Costs and benefits of priming for defense in Arabidopsis. Proceedings of the National Academy of Sciences 103: 5602–5607.

Van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Annual Review of Phytopathology 36: 453–483.

Van Loon LC and Van Strien EA (1999) The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. Physiological and Molecular Plant Pathology 55: 85 97.

Van Loon LC, Rep M, Pieterse CMJ (2006) Significance of inducible defense-related proteins in infected plants. Annual Review of Phytopathology 44: 135–162.

Van verk MC, Gatz C, Linthorst HJM (2009) Transcriptional regulation of plant defense responses in Van Loon LC (ed) plant innate immunity (advances in botanical research), Vol. 51, Elsevier.

Vandelle E, Poinssot B, Wendehenne D, Bentejac M, Pugin A (2006) Integrated signaling network involving calcium, nitric oxide, and active oxygen species but not mitogen-activated protein kinases in BcPG1-elicited grapevine defenses. Molecular Plant–Microbe Interactions 19: 429–440.

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biology 3: 1-11.

Varadarajan DK, Karthikeyan AS, Matilda PD, Raghothama KG (2002) Phosphite, an analog of phosphate, suppresses the coordinated expression of genes under phosphate starvation. Plant Physiology 129: 12321240.

Velasco R, et al. (2007). A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. Public Library of Science (PLOS One) 2: 1326.

Vellosillo T, Martinez M, Lopez MA, Vicente J, Tomas Cascon T, Dolan L, Hamberg M, Castresanaa C (2007) oxylipins produced by the 9-lipoxygenase pathway in Arabidopsis regulate lateral root development and defense responses through a specific signaling cascade. The Plant Cell 19: 831–846.

Vercesi A, Zerbetto F, Rho G (1999) Impiego dei modelli EPI e PRO nella difesa antiperonosporica del vigneto. Frustula Entomologica 22: 92–97.

Vernooij B, Friedrich L, Morse A, Reist R, Kolditz-Jawhar R, Ward E, Uknes S, Kessmann H, Ryals J (1994) Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. The Plant Cell 6: 959–965.

Verhage A, van Wees S, Pieterse C, (2010) Plant Immunity: It's the hormones talking, but what do they say?. Plant Physiology 154: 536–540.

Viennot-Bourgin G (1981) History and importance of downy mildews. In: Spencer, DM (ed) The Downy Mildews. Academic Press, London, UK, pp. 1–14.

Vivier MA, Pretorius IS (2000) Genetic improvement of grapevine: tailoring grape varieties for the third millennium. South African Journal of Enology and Viticulture 21: 5–26.

Vleesschauwer D, Hofte M (2009) Rhizobacteria-induced systemic resistance. Advances in Botanical Research 51: 223–281.

Vlot AC, Klessig DF, Park SW (2008a) Systemic acquired resistance: the elusive signal(s). Current Opinion in Plant Biology 11: 436–442.

Vlot AC, Liu PP, Cameron RK, Park SW, Yang Y, Kumar D, Zhou F, Padukkavidana T, Gustafsson C, Pichersky E, Klessig DF (2008b) Identification of likely orthologs of tobacco salicylic acid-binding protein 2 and their role in systemic acquired resistance in Arabidopsis thaliana. The Plant Journal 56: 445–456.

Vögele RT, Mendgen K (2003) Rust haustoria: Nutrient uptake and beyond. New Phytologist 159: 93–100.

Vorwerk S, Somerville S, Somerville C (2004) The role of plant cell wall polysaccharide composition in disease resistance. Trends in Plant Science 9: 203–209.

Walker AR, Lee E, Bogs J, McDavid DAJ, Thomas MR, Robinson SP (2007) White grapes arose through the mutation of two similar and adjacent regulatory genes. Plant Journal 49: 772-785.

Waller F, Mukherjee K, Deshmukh SD, Achatz B, Sharma M, Schaefer P. Kogel KH (2008) Systemic and local modulation of plant responses by *Piriformospora indica* and related Sebacinales species. Journal of Plant Physiology 165: 60–70.

Walker AR, Lee E, Bogs J, McDavid DAJ, Thomas MR, Robinson SP (2007) White grapes arose through the mutation of two similar and adjacent regulatory genes. Plant Journal 49: 772-785.

Walling LL (2001) Induced resistance: from the basic to the applied. Trends in Plant Science 6: 445–447.

Walters DR, Murray DC (1992) Induction of systemic resistance to rust in *Vicia faba* by phosphate and EDTA: effects of calcium. Plant Pathology 41: 444–448.

Walters DR (2003) Polyamines and plant disease. Phytochemistry 64: 97–107.

Walters D, Walsh D, Newton A, Lyon G (2005) Induced resistance for plant disease control: maximizing the efficacy of resistance elicitors. Phytopathology 95: 1368–1373.

Walters DR, McRoberts N (2006) Plants and biotrophs: A pivotal role for cytokinins? Trends Plant Science 11: 581–586.

Walters DR, McRoberts N (2006) Plants and biotrophs: a pivotal role for cytokinins? Trends in Plant Science 11: 581–586.

Walters DR, Heil M (2007) Costs and trade-offs associated with induced resistance. Physiological and Molecular Plant Pathology 71: 3–17.

Walters DR, McRoberts N, Fitt BD (2008) Are green islands red herrings? Significance of green islands in plant interactions with pathogens and pests. Biological Reviews 83: 79–102.

Wang D, Amornsiripanitch N, Dong X (2006) A genomic approach to identify regulatory nodes in the transcriptional network of systemic acquired resistance in plants. Public Library of Science (PLoS) Pathogens 2: e123.

Wang D, Pajerowska-Mukhtar K, Culler AH, Dong X (2007) Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. Current Biology 17: 1784–1790.

Wang L, Mitra RM, Hasselmann KD, Sato M, Lenarz-Wyatt L, Cohen JD, Katagiri F, Glazebrook J (2008a) The genetic network controlling the Arabidopsis transcriptional response to *Pseudomonas syringae* pv. *maculicola*: Roles of major regulators and the phytotoxin coronatine. Molecular Plant-Microbe Interactions 21: 1408–1420.

Waterhouse AL, Lamuela-Raventos RM (1994) The occurrence of piceid, a stilbene glucoside, in grape berries. Phytochemistry 37: 571-573.

Wen PF, Chen JY, Kong WF, Pan QH, Wan SB, Huang WD (2005) Salicylic acid induced the expression of phenylalanine ammonia-lyase gene in grape berry. Plant Science 169: 928-934.

Weichert H, Stenzel I, Berndt E, Wasternack C, Feussner I (1999) Metabolic profiling of oxylipins upon salicylate treatment in barley leaves: Preferential induction of the reductase pathway by salicylate. Federation of European Biochemical Societies Letters 464: 133–137.

Wicks T (1980) The control of *Plasmopara viticola* by fungicides applied after infection. Australasian Plant Pathology 9: 2–3.

Wicks T, Hall B (1990) Efficacy of dimethomorph (CME 151) against downy mildew of grapevines. Plant Disease 74: 114–116.

Wielgoss A, Kortekamp A (2006) Comparison of PR1 expression in grapevine cultures after inoculation with a host- and a non-host pathogen. Vitis 45: 9-13

Wilkinson S, Davies WJ (2009) Ozone suppresses soil drying- and abscisic acid (ABA)-induced stomatal closure via an ethylene-dependent mechanism. Plant Cell and Environment 8: 949–959.

Winkler E (1980) Kupferbehandlung – Einfluss auf Menge und Güte des Ertrages sowie auf die Winterfrostfestigkeit der Reben. Rebe und Wein 33: 260–263.

White RF (1979) Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. Virology 99: 410–412.

White PJ, Broadley MR (2003) Calcium in plants. Annual Botany 92: 487–511.

Wojtaszek P (1997) Oxidative burst: An early plant response to pathogen infection. Biochemical Journal 322: 681-692.

Wong FP, Wilcox WF (2000) Distribution of baseline sensitivities to azoxystrobin among isolates of *Plasmopara viticola*. Plant Disease 84: 275–281.

Wong FP, Burr HN, Wilcox WF (2001): Heterothallism in *Plasmopara viticola*. 50: 427-432.

Wright DP, Baldwin BC, Shephard MC, Scholes JD (1995) Source-sink relationships in wheat leaves infected with powdery mildew: Alterations in carbohydrate metabolism. Physiology and Molecular Plant Pathology 47: 237-253.

Wu J, Zhang Y, Zhang H, Huang H, Folta KM, Lu J (2010) Whole genome wide expression profiles of *Vitis amurensis* grape responding to downy mildew by using Solexa sequencing technology. BMC Plant Biology 10: 234.

Yamamizo C, Kuchimura K, Kobayashi A, Katou S, Kawakita K, Jones JD, (2006) Rewiring mitogen-activated protein kinase cascade by positive feedback confers potato blight resistance. Plant Physiology 140: 681–692.

Yan HZ, Liou RF (2005) Cloning and analysis of pppg1, an inducible endopolygalacturonase gene from the oomycete plant pathogen *Phytophthora parasitica*. Genetics and Biology 42: 339–350

Zangerl AR (2003) Evolution of induced responses to herbivores. Basic and Applied Ecology 4: 91–103.

Zavala JA, Baldwin IT (2004) Fitness benefits of trypsin proteinase inhibitor expression in *Nicotiana attenuata* are greater than their costs when plants are attacked. BMC Ecology 4: 11.

Zhang S, Klessig DF (2001) MAPK cascades in plant defense signaling. Trends in Plant Science 6: 520-527.

Zhang L, Lu YT (2003) Calmodulin-binding protein kinases in plants. Trends in Plant Science 8: 123–127.

Zhou F, Menke FLH, Yoshioka K, Moder W, Shirano Y, Klessig DF (2004) High humidity suppresses ssi4-mediated cell death and disease resistance upstream of MAP kinase activation, H2O2 production and defense gene expression. The Plant Journal 39: 920–932.

Zhou JM, Chai J (2008) Plant pathogenic bacterial type III effectors subdue host responses. Current Opinion in Microbiology 11: 179–185.

Zimmerli L, Jakab G, Metraux JP and Mauch-Mani B (2000) Potentiation of pathogen specific defense mechanisms in Arabidopsis by beta-aminobutyric acid. Proceedings of the National Academy of Science 97: 12920-12925.

Zipfel C, Felix G (2005) Plants and animals: A different taste formicrobes. Current Opinion in Plant Biology 8: 353–360.

Zobrist L (1954) L'efficacité des fongicides organiques en viticulture. Proceedings III int. Congress on Phyto-pharmacologie, 2: 682–688.

Zuskin E, Mustajbegovic J, Schachter EN, Kern J, Pavicic (19979) Respiratory function in vineyard and orchard workers. American Journal of Industrial Medicine 31: 250–255.

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