Expression of Defense Signaling Genes

in the Potato-*Verticillium dahliae* Interaction

BY

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ABSTRACT

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The interaction between plants and pathogens involves an initial defense reaction by the plant followed by a counter-defense reaction by the pathogen. This back-and-forth relationship between plants and pathogens is constantly evolving leading to complex and unique interactions. It is known that plant hormones are involved in defense against a wide range of pathogens. *Verticillium dahliae* Kleb. is the causal agent of Verticillium wilt in potato and is part of the complex that causes potato early dying. The potato early dying complex can cause yield losses up to 50%. This research investigates the role of hormone defense signaling in the potato-*V. dahliae* interaction. A gene expression analysis was performed on a susceptible and a moderately resistant (MR) cultivar of potato after inoculation with either a highly aggressive (HA) isolate or a weakly aggressive (WA) isolate of *V. dahliae*. This pathogen infects potato plants via the roots and travels through the vascular system to the aboveground portions of the plant. Genes related to the different hormone signaling pathways were analyzed for expression in the roots and the leaves of infected and wounded control plants. Genes related to the salicylic acid (SA), jasmonic acid/ethylene (JA/ET), and abscisic acid (ABA) pathways showed higher expression in the MR cultivar than the susceptible cultivar, indicating that they may contribute to resistance. Other genes related to the ABA pathway and an SA-related gene showed the opposite trend, indicating that they potentially contribute to susceptibility. In addition, a

number of genes showed a delayed reaction in the susceptible cultivar and only in plants inoculated with the HA isolate. This trend was observed in genes related to the SA and JA/ET pathways and may indicate a delayed defense response by the susceptible cultivar. Overall, this research indicates that more than one hormone signaling pathway may contribute to potato defenses against *V. dahliae*. Future work in this area could include overexpression and gene silencing studies, exogenous application of hormones, or direct measurements of hormone levels.

1.0 INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the world's most important food crops. It is cultivated in over 150 countries with China being the top world producer of potatoes (Anonymous 2007b). Canada is the 13th largest world potato producer (Anonymous 2007b) with Prince Edward Island and Manitoba being the top producing provinces (Anonymous 2009). When potato was first cultivated as a field crop, it served as famine relief in areas where the grain harvest was poor (Zuckerman 1998, McNeill 1999). It is believed that in Ireland in the early 1800s, one acre of potato land could feed a six-person family for one year (Zuckerman 1998). This dependence on potato in Ireland led to the most notable historical famine, the Great Irish Famine when the late blight disease hit the crop from 1845 to 1852.

Beside late blight, Verticillium wilt became a concerning challenge to producers in Canada in recent years. Verticillium wilt is caused by *Verticillium dahliae* Kleb.. This fungus infects a wide-range of species and the overwintering structures, microsclerotia, remain viable in the soil for up to 15 years (Pataky 1997). These two characteristics alone make this pathogen very difficult to control. *Verticillium dahliae* is a major contributor to the potato early dying complex, which has become a limiting factor for potato growth in many regions of Canada (Barker 2008). Promising control methods include the cultivation of resistant cultivars (Corsini et al. 1988, Mohan et al. 1990) and the use of green manures (Davis et al. 1996). Control strategies require improvement and further testing before they become widely accepted. In addition, further understanding of

the plant-pathogen interaction is important in any attempt to develop widespread and efficient means to control *V. dahliae* in potato.

It is known that plant hormone signaling pathways play an important role in plant defense against a wide range of pathogens. It is generally accepted that two main pathways are involved in plant's defense against pathogens, the salicylic acid (SA) pathway and the jasmonic acid/ethylene (JA/ET) pathway (Glazebrook 2005, Bari & Jones 2009). Recent research has indicated a role also for abscisic acid (ABA) in defense in some plant-pathogen interactions (Adie et al. 2007). In many plant-pathogen systems the different hormone pathways interact during the defense response. There can be positive interactions (cooperation) in that two or more pathways can work together to fight the pathogen. On the other hand, negative interactions (antagonism) may also occur where one pathway can inhibit another pathway during the defense response. The ability of the plant to activate or inhibit these pathways allows it to fine-tune its defense against a wide range of pathogens as well as abiotic stresses. Studying the hormone defense signaling pathways gives insight into the methods of defense that plants employ and will provide important information for future development of control methods against pathogens such as *V. dahliae*.

2.0 LITERATURE REVIEW

2.1 Potato (*Solanum tuberosum*)

2.1.1 History

Potato (*Solanum tuberosum*) is an economically important crop in Canada and worldwide. It is considered the fourth most important food crop in the world behind rice, wheat, and maize (Anonymous 2007b). The potato originated in southern Peru (Spooner et al. 2005), but the potato cultivars grown today are native to the Chiloé Archipelago on the coast of Chile (Ames & Spooner 2008). Potatoes were first cultivated as a field crop in northern Europe in the late 18th century (McNeill 1999) and quickly became a staple food crop for many countries. The dependence on potatoes in Ireland led to the Great Irish Famine which resulted in the death of over one million people in Ireland and the emigration of over one million people to North America (McNeill 1999).

2.1.2 Potato Production

Potatoes grow best under conditions of greater than 65% available soil water capacity and average daily temperatures of 21°C (Anonymous 2003). Potatoes have five growth stages and the fourth stage, tuber bulking, is most critical in regards to yield potential (Gudmestad 2008). Physiological days (P-days) are measured to determine the area best suited to potato production (Sands et al. 1979). P-days are a measurement of temperature, day length, and moisture levels useful for growth and development of potatoes (Sands et al. 1979). The area from Lake Manitoba to the US border has the highest number of

P-days on the Canadian prairies and therefore has the biggest potential for potato production (Anonymous 2003).

Canada is the 13th largest producer of potatoes in the world, producing nearly 5 million metric tonnes in 2007 (Anonymous 2007b). From 1989 to 2003, the annual potato production in Canada doubled (Anonymous 2007a). This increase coincided with the North American Free Trade Agreement in the late 1980s and was also related to the fluctuation of the Canadian dollar versus the American dollar (Anonymous 2007a). From 2003-2010 potato production has remained steady with minor increases and decreases. Manitoba contributes 19% of the potato production in Canada, second only to Prince Edward Island (Anonymous 2009). The potato growing regions of Manitoba are mostly restricted to the Red River Valley and the area surrounding Portage la Prairie (Shaykewich & Blatta 2009).

2.1.3 Uses of Potato

The nutritional value of potato contributes to its popularity worldwide with fewer calories than the common alternatives rice and pasta (Anonymous 2003). Many areas of the world have different ways of preparing potatoes ranging from samosas in India to latkes in Jewish communities to French fries in North America. In Canada, more than 50% of the potatoes grown are processed into French fries and potato chips (Anonymous 2008). Canada is the second largest exporter of French fries in the world. The remainder of the potatoes grown in Canada is used as table or seed potatoes (Anonymous 2008).

Potatoes also have non-culinary uses including utilization of the starch by textile, soap, and paste companies (Anonymous 2008). The sugars from potatoes are used in pharmaceutical preparations. Potatoes can also be used to produce vinegar, alcohol, and wine (Anonymous 2008).

2.2 Verticillium Wilt

2.2.1 Pathogen

Verticillium dahliae is a widespread soil-borne pathogen that affects more than 300 plant species (Pataky 1997). Examples of plants affected by this pathogen include tomato, potato, strawberries, maple, elm, and alfalfa (Pataky 1997). *Verticillium dahliae* is a member of the Deuteromycetes (Agrios 2005) and is considered a hemibiotroph (Glazebrook 2005). The fungus survives in the soil as microsclerotia for up to 15 years (Pataky 1997). Germination of the microsclerotia is stimulated by exudates naturally released from plant roots (Mol 1995). The fungus infects the outer cortex of the roots and travels to the vascular tissue where it proliferates in the xylem and infects systemic tissue (Pataky 1995). As systemic tissue begins to die the fungus colonizes the dying tissue and is returned to the soil to re-infect in later years (Rowe & Powelson 2002).

2.2.2 Symptoms

Verticillium dahliae is the causal agent of Verticillium wilt, but can also interact with other organisms in the soil to contribute to the early dying complex (EDC) in potato (Powelson & Rowe 1993). Specifically, *V. dahliae* and the nematode *Pratylenchus penetrans* are the major contributors to potato EDC (Powelson & Rowe 1993). The specific role of *P. penetrans* has not yet been

determined, but it is thought to alter the host's physiology making it more susceptible to infection by *V. dahliae* (Bowers et al. 1996).

The symptoms of Verticillium wilt and EDC in potato include chlorosis and wilting of the lower leaves of the plant (Pataky 1997). These symptoms move up the plant, but the stem typically remains erect (Powelson et al. 1993). Occasionally, the wilting and chlorosis is seen on one side of the petiole, leaves, or stems (Powelson et al. 1993). This particular symptom does not occur in all cases, but if present is indicative of infection by *V. dahliae*. Internal symptoms included necrosis of the vascular system which can be seen by taking a cross section of the lower stem (Powelson et al. 1993, Powelson & Rowe 1993, Pataky 1997, Rowe & Powelson 2002). Together, these symptoms contribute to early senescence of the plant and decreased tuber yield and quality. Potato EDC can cause yield losses up to 50% and is considered to be a limiting factor of potato growth in affected areas (Barker 2008).

2.2.3 Control Strategies

Currently, there are no widely accepted control methods for *V. dahliae* in potato. Soil fumigants have been used in many areas to control the levels\ of *V. dahliae* propagules in the soil (Powelson et al. 1993, Powelson & Rowe 1993, Rowe & Powelson 2002). This control method is expensive and has adverse environmental effects (Powelson & Rowe 1993). Tolerant to moderately resistant cultivars have been identified although many commercial cultivars do not fall into these categories (Corsini et al. 1988, Mohan et al. 1990, Powelson et al. 1993, Powelson & Rowe 1993, Johnson & Dung 2010). Transgenic potatoes are

currently being tested for their suitability (Gao et al. 2000). Solarization has proven effective, but can only be utilized in certain climates (Johnson & Dung 2010). Crop rotation is a common control strategy, but is not very effective against *V. dahliae* because this pathogen has a wide range of hosts and the overwintering structures can survive in the soil for many years (Powelson et al. 1993, Powelson & Rowe 1993, Rowe & Powelson 2002, Johnson & Dung 2010). The use of green manures to control potato EDC has been met with some success (Davis et al. 1996). Sudan grass (*Sorghum vulgar* var. *sudanense*), amongst other green manure crops, has shown large reductions in disease incidence (Davis et al. 1996). At this point, the use of green manures is not commercially accepted and multi-year studies are required to determine the viability of this form of control (Rowe & Powelson 2002). Research is ongoing in this area and the use of green manures could prove to be a very valuable source of control. In order to develop more effective control strategies researchers need to fully understand the interaction between the plant and the pathogen and how potato plants naturally defend themselves against *V. dahliae*.

2.3 Plant Defense Signaling Pathways

The interaction between plants and pathogens is complex and involves defense by the plant followed by counter-defense by the pathogen. The defense strategies by the plant and pathogen are constantly evolving as each entity struggles to overcome its counterpart. The understanding of these interactions is important in order for more sustainable control strategies to be developed. Hormone signaling in the plant is known to play a major role in defense by plants

against most pathogens. Salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) are the main hormones involved in plant defense signaling. These hormones can act alone during plant defense or may interact with one another through crosstalk between the signaling pathways.

The crosstalk between the pathways can be negative or positive. Negative crosstalk will be referred to as antagonism and the positive crosstalk will be referred to as cooperation. The antagonism between pathways can act in one direction or can be mutual. To add to the complexity of the hormone signaling systems, there may be shifts in the pathways depending on the stage of the interaction.

General trends have been observed regarding hormone defense signaling, but there are exceptions to every trend. Overall, each plant-pathogen system is unique and needs to be studied individually to fully understand the role of hormone defense signaling within each interaction. Plants that have mutations affecting a signaling pathway are commonly used to elucidate which pathways are involved in a specific interaction and the crosstalk between the pathways. In addition, it is known that certain genes, such as Pathogenesis-Related (PR) genes, are related to specific signaling pathways. This information allows for the use of gene expression analyses in the research of defense signaling pathways.

Overall, a number of studies have been conducted towards identifying hormone signaling pathways in plant-pathogen interactions. Some interactions have been studied more than others and a lot of work has been done on Arabidopsis because of the availability of mutant plants. It is impossible to

determine a specific role for each hormone pathway from these studies as it varies from interaction to interaction, but these studies are important to understand the role of hormone defense signaling in different plant-pathogen systems.

2.3.1 Salicylic Acid (SA)

Salicylic acid plays an important role in many plant-pathogen interactions and is typically the pathway involved in defense against biotrophic pathogens (Glazebrook 2005, Spoel et al. 2007). Furthermore, in the case of hemibiotrophs, SA is shown to play a role early in the infection during the biotrophic stage of the pathogen (Halim et al. 2007). An example of an early defense response in which SA plays a role is the hypersensitive response (HR). The HR is defined as the localized death of cells to contain the invading pathogen. It has been shown that in some interactions low levels of SA are required for the HR (Feys et al. 2001).

SA also plays a role in other defense responses such as systemic acquired resistance (SAR) (Gaffney et al. 1993, Lawton et al. 1994, Lawton et al. 1995). SAR leads to whole organism resistance to subsequent infections by a pathogen after initial infection. Through grafting experiments, it was determined that methyl salicylate (MeSA), a derivative of SA, is the mobile signal in SAR. MeSA travels via the phloem to secondary tissues where SA-binding-protein 2 (SABP2) binds to it and converts it to SA (Park et al. 2007). SA activates defense responses and leads to resistance to subsequent infections.

There are a number of marker genes that are used to indicate a SA-mediated response by the plant. The pathogenesis-related genes *PR-1*, *PR-2*,

and *PR-5* are often used as marker genes (Linthorst & Van Loon 1991). These genes are partially controlled through the action of *NPR1*, an important regulatory gene in the SA pathway (Cao et al. 1994, Delaney et al. 1994, Shah et al 1997). *NPR1* acts downstream of SA and is especially important for SAR. *NPR1* interacts with the TGA family of transcription factors and loss of this interaction leads to loss of SAR (Deprés et al. 2000). It is important to note that *NPR1* also plays a role in induced systemic resistance (ISR), but not through the expression of PR-genes as it does in SAR (Pietrese et al. 1998).

2.3.2 Jasmonic Acid and Ethylene (JA and ET)

The JA/ET pathway also plays a major role in many plant-pathogen interactions. These hormones are typically thought to work together to play a larger role in defense against necrotrophic pathogens (Glazebrook 2005, Spoel et al. 2007). Overall, the JA pathway appears to play a role against a broad range of pathogens and differential regulation of this pathway within the plant allows the plant to defend itself (De Vos et al. 2005). It is important to note that these two hormones (JA & ET) do not always work in concert with one another.

The expression of ISR requires both JA- and ET-response pathways as well as functional *NPR1* (Pietrese et al. 1998, Johansson et al. 2006). ISR differs from SAR in that the systemic resistance is achieved via colonization by the non-pathogenic rhizobacteria (Schippers et al. 1988). The JA/ET signaling pathway reacts after initial recognition between the beneficial bacteria and the host plant.

Like the SA pathway, the JA/ET pathway has certain genes that are studied as target genes. These genes included *PDF1.2*, a defensin, (Penninckx

et al. 1996, Thomma et al. 1998), *PR-4*, a hevein-like protein that binds chitin, *PR-3*, a chitinase, and *THI2.1* (Epple et al. 1995, Norman-Setterbald et al. 2000). *THI2.1* is a thionin induced by pathogens that has antifungal activity. *THI2.1* is induced by methyl jasmonate (a derivate of JA), but not by ET (Epple et al. 1995). Therefore, the expression of this gene is an example of when ET and JA are not active in the same pathway.

2.3.3 Abscisic Acid (ABA)

Abscisic acid (ABA) is known to play a role in response to abiotic stresses, but more recently is being explored as a potential player in response to biotic stresses. In some plant-pathogen interactions ABA has been shown to increase susceptibility to the pathogen (Anderson et al. 2004, de Torres-Zabala et al. 2007), but this is not always the case and reiterates why it is important to study each interaction independently.

It has been discovered that ABA plays a role in callose-deposition which acts as a mode of defense against necrotrophic pathogens (Ton & Mauch-Mani 2004). The ABA signaling pathway is required for the priming of the deposition of callose by β-amino butyric acid (BABA). This production of callose is a key factor in resistance against *Plectrosphaeria cucumerina* and *Alternaria brassicola* in *Arabidopsis thaliana*. In addition, other studies have shown that ABA plays a role in plant defense beyond the deposition of callose (Adie et al. 2007).

2.3.4 Crosstalk between the SA and JA/ET Pathways

Interaction between the SA and JA/ET pathways has been well-documented. Overall, there are more cases of antagonism than cooperation,

specifically antagonism by SA on the JA/ET pathway. Research has also revealed antagonism that occurs in the opposite direction and in other cases, mutually between the pathways. Cooperation between SA and JA and/or ET is not as commonly observed, but some research has indicated that this type of interaction can occur (Adie et al. 2007).

2.3.4.1 Antagonism. The antagonism between SA and JA often occurs via the action of the regulatory protein NPR1 (Spoel et al. 2003). The gene encoding this protein was mentioned previously regarding its role in both SAR and ISR, so it is important to reinforce that the role of NPR1 is not always negative towards the JA pathway. In the case of the interaction between *Pseudomonas syringae* pv. *tomato* and *Arabidopsis thaliana*, *NPR1* is required for functional SA signaling. If the signaling of the SA pathway is impaired through a mutation in *NPR1* it results in enhanced expression of *LOX2* (Spoel et al. 2003) (Fig.2.1). *LOX2* encodes an enzyme which is a key player in the octadecanoid pathway leading to synthesis of JA. Other studies have indicated that SA can also have an effect on ET synthesis (Leslie & Romani 1988). In addition, *NPR1* is also known to play a role in antagonism by SA on the JA/ET pathway in the *A. thaliana*/*Alternaria brassicicola* interaction (Spoel et al. 2007).

As mentioned previously, NPR1 is involved in the expression of defense genes in the SA pathway. The activation of these defense genes by NPR1 requires the localization of this regulatory protein to the nucleus. In contrast, the antagonism of the JA pathway that occurs via NPR1 occurs without localization of this protein to the nucleus (Spoel et al. 2003). Therefore, it is proposed that

this protein plays different roles depending on its location in the cell (Spoel et al. 2003) (Fig.2.1). This may allow for regulation by the plant depending on the role of NPR1 that is required.

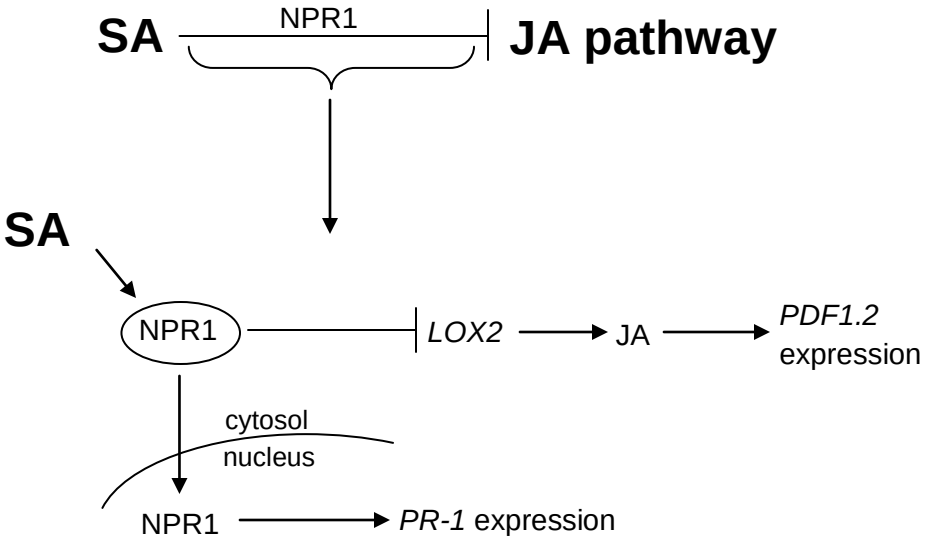


Figure 2.1. Cytosolic NPR1 acts to antagonize the jasmonic pathway via *LOX2* while localization of this protein to the nucleus induces expression of *PR-1*. Adapted from research by Spoel et al. (2003).

Many examples of antagonism are discovered through the use of mutants. Mutants that are affected in the SA pathway and show an increase in JA and/or ET levels indicate antagonism by SA on the JA/ET pathway. Studies looking at mutations in *A. thaliana* resulting in enhanced disease susceptibility to *Erysiphe orontii*, *A. brassicicola*, and *P. syringae* pv. *maculicola* corresponded to decreased levels of SA and increased expression levels of genes related to the JA pathway (Dewdney et al. 2000, Brodersen et al. 2006, Gupta et al. 2000). Brodersen et al. (2006) argue that the EDS1 protein acts as a SA pathway activator and ET/JA pathway repressor. In contrast, some research has shown that defense against *A. brassicicola* in *A. thaliana* can be induced after treatment

with methyl-JA and this defense is compromised when SA is also applied (Spoel et al. 2007). Therefore, in this case JA contributes to disease resistance and not to enhanced susceptibility.

The PR-genes that are related to the JA/ET pathway provide a target for analysis of antagonism by the SA pathway. Many studies have shown that SA-insensitive/deficient plants have higher levels of defensin (*PDF1.2*) compared to wild-type plants (Penninckx et al. 1996, Jirage et al. 2001). Plant defensins are known to be related the JA/ET pathway (Penninckx et al. 1998, Thomma et al. 1998). Proteinase inhibitors are also defense genes induced by the JA/ET pathway and the induction is inhibited by SA and SA derivates (Doherty et al. 1988, Doares et al. 1995). This indicates that the negative effect of SA on the JA/ET pathway can have a negative effect on resistance. Such is the case with *Nicotiana tabacum* and culture filtrates of *Erwinia caratovara* subsp. *caratovoara*. The resistance to this disease is known to act in a SA-independent fashion and causes expression of genes known to be related to the JA/ET pathway such as a basic glucanase and a basic chitinase (Vidal et al. 1997). The expression of these genes is repressed when SA is applied exogenously (Vidal et al. 1997).

In another example, the transcription factor WRKY70 is affected by both SA and JA, and in turn affects the genes that respond to these two pathways (Li et al. 2004). *WRKY70* expression is activated by SA and repressed by JA in the interaction between *A. thaliana* and *E*. *carotovara* and *P. syringae* pv. *tomato*. To a further extent, WRKY70 activates genes induced in the SA pathway, such as *PR-2* and *PR-5*, and represses genes that respond to JA, such as *VSP*

(VEGATATIVE STORAGE PROTEIN) and *PDF1.2*. But expression of *WRKY70* at low levels can induce the expression of the same JA-responsive genes (Li et al. 2004). Consequently, it is assumed that there is a threshold level of WRKY70 that is reached for this change in action to occur. This indicates the importance of this transcription factor as an integrator between the two pathways.

This antagonism can also work in the opposite direction in that JA can negatively affect the SA pathway, although examples of this type of interaction are not as well documented. In the interaction between *A. thaliana* and *Pythium irregulare*, plants that were mutated in their ability to detect JA showed increased levels of SA accumulation (Adie et al. 2007). Therefore, through some mechanism JA was inhibiting accumulation of SA in wildtype plants. Similarly, JA has been shown to inhibit the expression of PR-genes that are up-regulated by SA in tobacco plants (Niki et al. 1998). MPK4 is required for *PDF1.2* and *THI2.1* expression through the JA/ET pathway and acts as a negative regulator of SAR which is controlled by the SA pathway (Petersen et al. 2000). The role of MPK4 in hormone signaling can be compared to the role of EDS1, in that EDS1 acts as a SA pathway inducer and a repressor of the JA/ET pathway and MPK4 has the opposite effect (Brodersen et al. 2006).

In tomato and Arabidopsis, JA (Kloek et al. 2001, Traw et al. 2003, Cui et al 2005) and ET (Berrocal-Lobo et al. 2002) have been shown to negatively affect SA in regards to defense against *P. syringae*. In the studies looking at JA antagonism, *coi* plants which were insensitive to JA showed increased expression of *PR-1* through the SA pathway and the action of NPR1 (Kloek et al.

2001, Cui et al. 2005). This is especially interesting with *P. syringae* as this pathogen produces the phytotoxin coronatine which is an analogue of JA (Brooks et al. 2005, Cui et al. 2005) although the effects of coronatine on the SA pathway are much stronger than the effects of JA (Cui et al. 2005). It is thought that the virulence of *P. syringae* is dependent on its ability to modulate the SA signaling defense pathways via coronatine (Kloek et al. 2001, Brooks et al. 2005, Cui et al. 2005). In the *P. syringae*-Arabidopsis interaction it is also important to consider the role of DELLA proteins. These proteins are thought to antagonize the SA pathway and have a positive effect on the JA/ET pathway (Brooks et al. 2005).

In addition to one-way antagonism these two pathways are also involved in mutual antagonism. In *Arabidopsis* plants constitutively expressing JA and ET responses (*cev1* mutation) SA was found to suppress the expression of *PDF1.2* (Ellis et al. 2002). Conversely, JA signaling was found to antagonize the induction of *PR-1* by SA. This antagonism acted through JA, but not through ET (Ellis et al. 2002). This is comparable to the results of Fidanstef et al. (1999) where the induction of defense genes by a SA-derivate was diminished by the addition of JA and vice versa.

SA can also have an inhibitory effect on JA/ET pathways not-related to pathogen defense such as pathways responding to wounding or herbivores. *AtVSP* encodes a vegetative storage protein acid phosphatase and the expression of this gene is regulated by JA in response to wounding. In *A. thaliana* SA can inhibit the expression of this gene (Norman-Setterbald et al. 2000). Some studies have indicated that SA inhibits the JA-related wound

response by the plant by blocking JA biosynthesis (Peña-Cortés et al. 1993, Harms et al. 1998). In addition, SA can also inhibit the expression of *ACCS* which is involved in synthesis of ET in response to wounding (Li et al. 1992). JA-regulated herbivore defense can also be antagonized by the SA pathway (Nishiuchi et al. 1997, Niki et al. 1998, Felton et al. 1999, Preston et al. 1999, Stotz et al. 2002, Thaler et al. 2002, Cui et al. 2002). It is important to note that Nishiuchi et al. (1997) found differences in that this inhibition only occurred in the roots, but not in the stems or leaves.

An extreme of the SA-JA relationship in regards to herbivores is represented by Tobacco Mosaic Virus (TMV) and *Manduca sexta* attacking *Nicotiana tabacum* (Preston et al. 1999). In this study, the plants that had been inoculated with TMV did not mount any response to herbivory by *M. sexta*. The SA pathway mediated defense against TMV and the JA pathway regulated defense against the herbivore by inducing the production of nicotine (Preston et al. 1999). This could represent a shift in resources by the plant to allocate more resources towards pathogen defense via the SA pathway and less energy towards herbivory and general stress responses. On the other hand, one study showed that the inhibitory action of the SA pathway is induced by the herbivore (Egyptian cotton worm, *Spodoptera littoralis*) in order to lessen the effects of the plant defenses against said herbivore (Stotz et al. 2002). Therefore, each interaction needs to be studied individually and thoroughly before any conclusions can be drawn.

2.3.4.2 Cooperation. A positive interaction between the SA and JA pathways is less common. One example of when these two pathways cooperate in the defense reaction is in *Arabidopsis thaliana* against the pathogen *Pythium irregulare* (Adie et al. 2007). Meta-analysis of the transcriptome showed genes that were up-regulated in defense that required both sensitivity to JA and a functioning SA pathway. Other studies have shown that these two pathways can both induce a leaf thionin in barley known to play a role in plant defense against pathogens (Wasternack et al. 1994). Likewise, it has been proven that both JA and SA induce class I and class IV chitinases in the genus *Pinus* (Davis et al. 2002). EREBP1 (Ethylene Response Element Binding Protein 1) represents an integration point between the pathways as it is induced by JA, ET, as well as SA (Horvath et al. 1998). This protein is a transcription factor that binds to response elements in basic PR genes (Ohme-Takagi & Shinsi 1995).

An example of cooperation between JA and SA involving basic PR genes is with the gene *PR-1b*. The accumulation of this protein showed a synergistic effect when methyl jasmonate and SA were exogenously applied together (Xu et al. 1994). ET has also been indicated to play a role in the induction of *PR-1* in *Nicotiana glutinosa* (Kim et al. 1998). In the study by Kim et al. (1998), it was determined that SA can induce the expression of ACC oxidase, an enzyme involved in the synthesis of ET further signifying the role of cooperating pathways in this interaction.

This crosstalk between the JA and SA pathways can also occur further upstream. A study looking at the Arabidopsis CPR6 protein determined this

protein to be an important regulator of PR genes related to the SA pathway and defense genes related to the JA pathway (Clarke et al. 1998). This common regulator represents an integration point between the two pathways. A protein from the same family, CPR5, was also found to be a regulator of both pathways. Mutations in this gene led to resistance to different pathogens and activation of both the JA and SA pathways (Bowling et al. 1997).

Pep-13, a pathogen-associated molecular pattern (PAMP), from *Phytophthora infestans* acts as an elicitor for plant defense in potato (Halim et al. 2004, Halim et al. 2009). This elicitor results in a defense pathway in which both JA and SA play a role (Halim et al. 2004, Halim et al. 2009). SA accumulation results in the production of reactive oxygen species (ROS) and HR-like cell death. JA is required in the pathway from SA to ROS and HR, but not necessarily in a linear fashion (Halim et al. 2009). JA-deficient mutants showed reduced levels of ROS and HR cell death, but these responses were still present (Halim et al. 2009). It is possible that JA potentiates the effects of SA on the production of these defense responses.

The concept of one hormone pathway potentiating another hormone pathway is important when discussing cooperation between signaling molecules. There are a couple of documented cases where ET is hypothesized to have a potentiating effect on SA. For example, ET has been shown to enhance the sensitivity to SA in some tissues. Levels of *PR-1* mRNA show a dose-dependent relationship on SA in Arabidopsis (Lawton et al. 1994). The levels of *PR-1* mRNA at low dosage levels of SA were increased when the tissue was exposed to ET

immediately following SA treatment (Lawton et al. 1994, Johansson et al. 2006). This potentiating effect was only seen in early timings (3 days post-inoculation (dpi)); whereas later timings (7 dpi) showed that ET had a negative effect on expression of *PR-1* representing a possible shift in defense strategies (Johansson et al. 2006).

NPR1 has been mentioned previously in this review. It is known to play a role in resistance to many pathogens through its effect on the SA pathway. In addition, it also plays a key role in the antagonism between the SA and JA pathways. One study has suggested that SA mediates two types of resistance to pathogens, one that is dependent on *NPR1* and one that is independent from this gene (Clarke et al. 2000). This study also determined that the *NPR1*-independent resistance required JA/ET sensitivity along with SA (Clarke et al. 2000). This *NPR1*-independent pathway may involve the gene *SSI1* as explored by Shah et al. (1999).

In other cases the cooperation between the JA and SA pathways is not as direct. For example, it was determined that both the JA and SA pathways are involved in defense against *Pseudomonas syringae* pathogens in *Arabidopsis* spp. (Fidanstef et al. 1999, Ellis et al. 2002). Induction of ISR and SAR also showed increased resistance to *P. syringae* pv. *tomato*, but it was determined that these pathways acted parallel to one another without any direct crosstalk (van Wees et al. 2000). *Arabidopsis* plants mutated in their ability to detect ethylene or JA showed greater susceptibility to *Plectosphaerella cucumerina* (Berrocal-Lobo et al. 2002) and *Xanthomonas campestris* pv. *armoraciae* (Ton et

al. 2002), as did plants with reduced levels of SA. This indicates a potential role for all of these hormones in defense against these pathogens.

A unique interaction between JA and SA was elucidated in a study looking at *Arabidopsis* plants infected with an avirulent strain of *Pseudomonas syringae* (Truman et al. 2007). Infection by this strain of the pathogen resulted in systemic resistance that was correlated with an accumulation of JA (Truman et al. 2007). Systemic resistance is generally related to the SA pathway, this could be an example where both pathways are involved in this type of resistance.

2.3.5 Crosstalk between the JA and ET Pathways

For most purposes relating to plant defense the JA and ET pathways are grouped together. These two pathways commonly work together to help the plant defend against invading pathogens. Although the cooperative interaction between these two pathways is very common, there is also evidence to the contrary. The antagonism between these pathways has been shown in response to wounding as well as in response to pathogen infection.

2.3.5.1 Cooperation. As mentioned previously, the pathway leading to ISR requires both JA and ET. This is a key example of how these two hormones cooperate to contribute to plant defense. The roles of these two hormones act in succession with JA acting downstream of ET (Pietrese et al. 1998). *NPR1*, the gene that plays a regulatory role in SAR, is also required for ISR through a SA-independent pathway. *npr1* plants are blocked in their ability to respond to both methyl jasmonate (JA derivative) and 1-aminocyclopropane-1-carboxylate (ET precursor) therefore *NPR1* acts downstream from the hormonal effects (Pietrese

et al. 1998). *NPR1* also plays a role in the JA and ET cooperation involved in defense in *Arabidopsis* against *Verticillium longisporum* (Johansson et al. 2006). In this defense reaction, both JA and ET are required although JA again is believed to act downstream of ET (Johansson et al. 2006).

Another important way that JA and ET cooperate is through the activation of the transcription factor ERF1 (Ethylene Response Factor1) (Fig.2.2). The two pathways synergistically induce the expression of *ERF1* which plays a role in defense gene expression and plant resistance (Berrocal-Lobo et al. 2002, Lorenzo et al. 2003). It up-regulates the expression of defense genes such as *b-CHI* and *PDF1.2* (Lorenzo et al. 2003). In *A. thaliana*, ERF1 has been shown to play an important resistance role against necrotrophic pathogens such as *Botrytis cinerea* and *Plectosphaerella cucumerina* (Berrocal-Lobo et al. 2002).

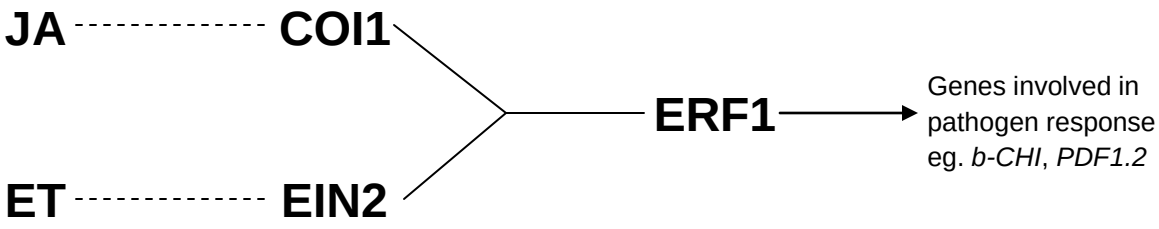


Figure 2.2. Cooperation between JA and ET in activating ERF1 leading to the expression of defense genes. COI1 and EIN2 represent proteins required for functional JA and ET pathways, respectively. Adapted from Berrocal-Lobo et al. 2002 and Lorenzo et al. 2003.

The expression of PR-genes plays a major role in all of the hormone defense signaling pathways. *PDF1.2* encodes a plant defensin and the expression of this gene requires the synthesis of ET and JA (Penninckx et al. 1996, Penninckx et al. 1998, Schenk et al. 2000). In pathogen-challenged

Arabidopsis plants, these hormones act in parallel pathways that converge to induce the expression of *PDF1.2* (Penninckx et al. 1998). The expression of this gene represents a synergistic interaction between these two pathways as either pathway acting alone can weakly induce this gene, but together there is a very strong induction of expression (Penninckx et al. 1998). A number of other defense genes are activated when the JA and ET pathways cooperate. In tobacco seedlings, the application of MeJA and ET together resulted in the activation of the osmotin promoter (Xu et al. 1994). Osmotin has been classified as homolog of family five PR proteins (Koiwa et al. 1994). Similarly, the expression of *PR-1b* was also induced by MeJA and ET in a synergistic fashion (Xu et al. 1994). SA also induced the expression of osmotin and *PR-1b*, but to a lesser extent than ET/MeJA applied together (Xu et al. 1994).

JA and ET are also known to cooperate in response to wounding. Together these two hormones regulate the increased expression of proteinase inhibitor (pin) genes in tomato plants in after wounding (O'Donnell et al. 1996). In addition, the two hormones directly affect one another as ET regulates the levels of endogenous JA upon wounding and exogenous application of JA induces ethylene biosynthesis (O'Donnell et al. 1996). This interaction is of importance as JA and ET do not always work together during wounding responses, as will be illustrated in the next section of this review.

2.3.5.2 Antagonism. The response to wounding in *Arabidopsis* is one of the few documented responses in which JA and ET have an antagonistic relationship (Lorenzo et al. 2004). JA acts via two different transcription factors, AtMYC2 and

ERF1, depending on the stress that is present. These two transcription factors are mutually antagonistic (Fig.2.3). AtMYC2 acts in response to wounding and requires both JA and ABA whereas ERF1 responds to pathogen attack and requires JA and ET (Lorenzo et al. 2004). The activation of AtMYC2 induces the expression of genes such as *VSP2* and *TAT* which are marker genes for the wound response. The activation of ERF1 leads to the expression of genes involved in pathogen defense such as *PDF1.2* and *PR4* (Lorenzo et al. 2004). This is important when studying plant-pathogen interaction as it portrays how a plant conserves energy by having separate wound response and pathogen response pathways that are mutually antagonistic.

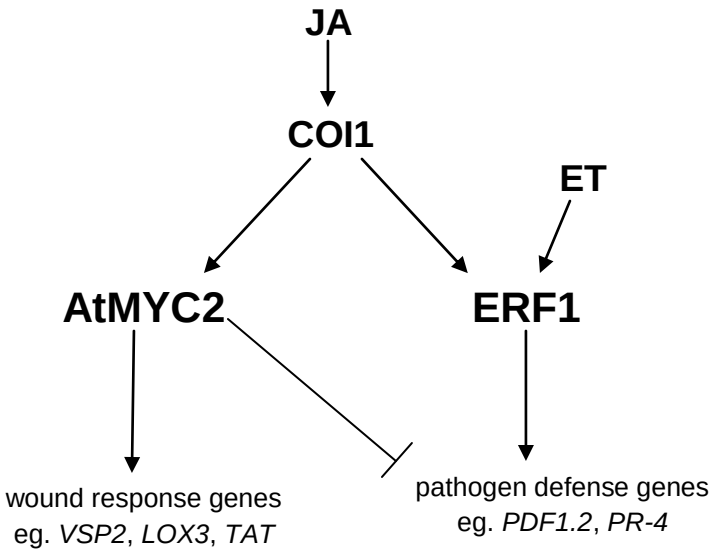


Figure 2.3. Schematic diagram illustrating wound and pathogen responses in Arabidopsis via the mutually antagonistic pathway involving the transcription factors AtMYC2 and ERF1. COI1 represents a protein required for a function JA signaling pathway. Adapted from Lorenzo et al. (2004)

2.3.6 Crosstalk Involving Abscisic Acid

2.3.6.1 ABA and JA/ET. Wound and stress responses are important examples of when JA and ABA cooperate in a signaling pathway. As stated in the last section, together these two hormones regulate the transcription factor AtMYC2 which leads to the expression of stress defense genes such as *VSP2* and *RD22* (Anderson et al. 2004). These two hormones also cooperate to induce the expression of *PIN2* after wounding in tomato and potato plants (Peña-Cortés et al. 1996). *PIN2* encodes the PR-protein proteinase inhibitor II. It is thought that JA acts downstream of ABA in this interaction and that the ABA plays a role in the synthesis of JA. This is comparable to *Arabidopsis*, where wounding was found to induce the expression of *LOX1*, an important gene in the JA biosynthesis pathway (Melan et al. 1993). The expression of this gene was also induced by exogenous application of ABA or application of MeJA (Melan et al. 1993).

ABA can also work with the JA/ET pathway to contribute to pathogen defense. Osmotin, a PR-5 protein was found to be co-regulated by ABA and ET (Nelson et al. 1992). These two hormones appeared to act independently of one another, but both play a role. In a study performed on *A. thaliana* and *Pythium irregular*, it was discovered that JA and ABA both play a role in plant defense signaling (Adie et al. 2007). JA was determined to be the most important signaling molecule with ABA being the second most important. Further investigation indicated that ABA was required for JA biosynthesis. Plants that

were unable to synthesize ABA also showed a significant decrease in the overall levels of JA (Adie et al. 2007).

It is important to note that ABA can also play an antagonistic role regarding plant defense. As stated previously, the activation of the AtMYC2 transcription factor antagonizes the transcription factor ERF1 which is involved in pathogen defense via the JA/ET pathway (Lorenzo et al. 2004). In a study looking at the interaction between *Arabidopsis thaliana* and *Fusarium oxysporum*, it was concluded the AtMYC2 leads to increased susceptibility to this pathogen (Anderson et al. 2004). ET was shown to repress the activity of AtMYC2 and restore resistance to *F. oxysporum*. The antagonism in this plant-pathogen interaction is complex and has not been completely elucidated.

2.3.6.2 ABA and SA. ABA is known to play a role in the formation of callose which is a common response in plants during fungal infections (Ton & Mauch-Mani 2004). An interesting study was done looking at the role of callose in the interaction between *Arabidopsis* and powdery mildew (*Erysiphe cichoracearum*) (Nishimura et al. 2003). Through the use of mutant plants it was found that mutants with increased resistance to powdery mildew had decreased levels of callose deposition. This is unexpected as callose is known to play a defensive role. However, with this decrease in callose deposition there was also an activation of the SA pathway (Nishimura et al. 2003). This induction of the SA pathway was also indicated in another study showing increased expression of *PR-1* in the same mutants (Vogel & Sommerville 2000). Further work needs to be done to understand the role of ABA in this interaction, but it is interesting to note

that a mutation repressing a process involved in the ABA signaling pathway also resulted in the activation of the SA pathway and increased resistance.

The objectives of this research were to: (i) analyze the gene expression levels of selected genes involved in hormone defense signaling and (ii) determine the hormone defense signaling pathways involved in the potato-*V. dahliae* interaction. The expression of the target genes identified for each of the pathways was measured to determine differences over time, between cultivars of varying resistance, and between reactions to pathogens of varying aggressiveness. This information was used to deduce potential hormone signaling pathways that are involved in the potato-*V. dahliae* interaction.

3.0 MATERIALS AND METHODS

3.1 Biological Material and Growth Conditions

Two potato cultivars were chosen for this study: 'Kennebec' is susceptible to *Verticillium dahliae* and 'Ranger Russet' is considered moderately resistant (Anonymous 2003). In addition, two isolates of the pathogen were used to inoculate each cultivar separately. Vs 06-14 is an isolate of *V. dahliae* that was determined to be weakly aggressive (WA) on potato and Vd 1396-9 is a highly aggressive (HA) isolate (Uppal et al. 2007, Alkher et al. 2009). Three biological replicates of each isolate/cultivar combination were used in this study.

Potato tuber pieces were planted in 10-cm diameter plastic pots filled with a pasteurized sand-soil mixture (1:1, by volume) containing granular NPK fertilizer (16:20:16). Plants were grown for 25 days under 20°/16°±2°C day/night with a 16 hour photoperiod at a light intensity of 350 µmol m^{-2} s^{-1}. After inoculation, plants were transplanted into new 10-cm diameter plastic pots containing the same soil components and returned to the same growing conditions.

3.2 Inoculation

25-day-old plants were used for inoculations. Inoculum of each *V. dahliae* isolate was prepared from single-spore cultures grown on potato dextrose agar (PDA) for two weeks at 20°C (Fisher Scientific Incubator, Model 146E). Conidial suspensions were prepared to a final concentration of 10^6 conidia mL^{-1}. The plants were inoculated via 'root dip' inoculation (Daayf et al. 1998). The plants were uprooted from the soil, gently washed with water, the root tips were cut with

scissors and the root systems were immersed in the conidial suspensions for one minute before being transplanted. The plants for the wounded control treatment had their root tips cut before immersion in sterile distilled water (SDW). The cultivars were grown separately, but under the same growing conditions.

3.3 Tissue Harvesting

Plant roots and leaves were harvested and stored separately. The tissue was harvested at 0 hours, 4 hours, 21 hours, 3 days, 7 days, and 14 days post-inoculation. For 0 hours post-inoculation, the tissue was harvested immediately after being placed in the conidial suspension (SDW in the case of the wounded control). All leaves and roots were harvested from each plant and pooled together. A random sampling from this pool of tissue was used for the next steps of RNA extraction and gene expression analysis.

3.4 RNA Extraction

RNA was extracted from the leaf tissue using TRIzol reagent (Invitrogen, Burlington). Leaf tissue was ground in liquid nitrogen and homogenized in 1000 µl of TRIzol reagent. The homogenized sample was centrifuged at 10 000 rpm for five minutes. After centrifiguation, 300 µl of chloroform was added to the upper phase of the sample and they were mixed using a vortex. The mixed sample was centrifuged at 13 000 rpm for 15 minutes to separate the phases. The aqueous phase was then mixed with 1000 µl of isopropyl alcohol and incubated at -20°C for 24 hours. After the RNA had precipitated, the sample was centrifuged for 15 minutes at 13 000 rpm. The supernatant was removed and the pellet was washed with 500 µl of 75% ethanol and centrifuged at 12 000 rpm for 10 minutes.

The RNA pellet was dried at room temperature and then dissolved in 55 µl of RNase-free water.

RNA was extracted from the root tissue following the manufacturer's protocol for the RNeasy kit (Qiagen, Toronto). A different kit was used because of difficulties with extracting stable RNA from the root tissue. After root tissue was ground in liquid nitrogen, 450 µl of Buffer RLT was added to the sample. The homogenate was mixed with a vortex and then incubated at 56°C for three minutes. The mixture was then transferred to a QIAshredder spin column and centrifuged for two minutes at 13 000 rpm. One half volume of 100% ethanol was added to the supernatant of the flow-through and mixed by pipetting then transferred to an RNeasy spin column. The column was centrifuged for 15 seconds at 10 000 rpm. The sample was then washed according to Qiagen's protocol; one wash with 700 µl of Buffer RW1 and two consecutive washes with 500 µl of Buffer RPE. The samples were eluted to a final volume of 40 µl.

3.5 Gene Expression Analysis

Reverse transcription-PCR was used to synthesize first-strand cDNA from the RNA extracted from the plant material. The first-strand cDNA was synthesized from 5 µg of RNA in a 50 µl solution containing 5 ng μl^{-1} of oligodT, 500 µM of each dNTP, 0.4X First-Strand buffer (20 mM Tris-HCl (pH 8.3), 30 mM KCl, 1.2 mM $MgCl_2$), 4 mM DTT, 40 U RNaseOUT, and 200 U M-MLV (Moloney Murine Leukemia Virus) reverse transcriptase (Invitrogen). The RNA was mixed with the OligodT, dNTPs, and DEPC-water and heated to 65°C for 5 minutes. The remaining components were added and the first-strand cDNA was

synthesized at 37°C for two hours; the reaction was inactivated by heating to 70°C for 15 minutes.

A number of primers were designed based on genes known to be related to the different signaling pathways. Forward and reverse primers were designed for each of these genes based on potato genome sequences (Table 3.1). *Elongation Factor1α* (*EF-1α*) is constitutively expressed in potato and was used as an internal control to compare to expression levels of the genes of interest.

For the second strand gene amplification a polymerase chain reaction (PCR) was performed using 1 µl of the first-strand cDNA. The first-strand cDNA was mixed with 1x amplification buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$), 1 mM $MgCl_2$, 0.1 mM of each dNTP, 0.1 µM of each primer for gene of interest, and 2 U of *Taq* DNA polymerase. Reactions were run on a C100 Thermal Cycler (Bio-Rad, Hercules, CA) under the following conditions: 5 minutes at 94°C followed by a pre-determined number of cycles (Table 3.1) of 30 seconds at 94°C, 30 seconds at the annealing temperature (T_m) for the specific primers (Table 3.1), and 60 seconds at 72°C. A final extension period of 10 minutes at 72°C completed the reaction.

After the genes were amplified using the PCR the product and a 1Kb Plus DNA ladder was run on a 1% agarose gel stained with ethidium bromide in TAE buffer (0.04 mM Tris-base, 65 µl/L glacial acetic acid, 1.0 mM EDTA). The gel images were captured from these gels using a UV transilluminator (Alpha Innotech, San Leandro, CA). The images captured on these gels were used for quantification using the Spot Denso feature on AlphaEase FC imaging software

Table 3.1. Primer sequences, T_m (°C), and number of cycles used for polymerase chain reaction (F-Forward, R-Reverse)

Gene	Primer Sequences (5' → 3')	T_m (°C)	# cycles (leaves, roots)
***1-Aminocyclopropane-1-Carboxylate Synthase* (*ACCS*)**	F: GGGTTCCCTGGATTTAGGGTTG R: CAACCTGGCTCTGAGCAATGAA	55	35,28
***ABA-responsive element binding protein* (*AREB*)**	F: CACAGCAGCAACCAATTTTCC R: GACCCCACTGGCTCCTAAACCT	55	35,25
***Ethylene Response Factor1* (*ERF1*)**	F: GTATTGCCGGAGTTCAAGTTTCA R: TCTCTGTCGGCGGAGAAACAGAAG	55	30,28
***Proteinase Inhibitor I* (*INH1*)**	F: CGATTCGTCACGTCATAATCTCA R: GGGGACAAACATATATTGGATAGG	54	30,30
***Neoxanthin Synthase* (*NXS*)**	F: GAAGAGAAATGTGTGATCCCTATGG R: AAAGGCCACAAACCATTCCA	55	-,35
***Phenylalanine Ammonia Lyase1* (*PAL1*)**	F: TTGCACAAGTTGCATCCATT R: AAGAGCACCACCATTTTTGG	55	35,30
***Phenylalanine Ammonia Lyase2* (*PAL2*)**	F: GCACCATCAATTGCACAAAA R: TGCAACTTGTGCAACAGTCA	55	35,30
***Lipoxygenase* (*POTLX3*)**	F: GTCAATTTTGGGCAATACCCTTA R: GTCCCGTTGTCCAAGGTAAA	55	35,32
PR-1	F: TCAGGTGCAGGAGAGAACCT R: AATGAACCACCATCCGTTGT	38.1	35,35
PR-2	F: ATTTGGTGCCACACAAGACA R: TTGGGGAAAACAATCCAAAA	50	30,25
PR-3	F: TCTGGATGACAGCACAGGAT R: TTCACCAGTGGGAACATTCA	50	30,30
PR-5	F: AGCGTTTTCAGCCAAAGTGT R: ATTGTCCCTTCACGGTATGG	60	35,32
PR-9	F: GCCAAACTTGGTAGCTGCTC R: TTCTGCTGTGTTTCCTGACG	53	35,28
***Wound-Induced Protein 2* (*WIN2*)**	F: GGTTCTTTGCATCAGCCTAACC R: TGATATGTTGCACGAACGTTTT	55	30,28
***Zeaxanthin Epoxidase* (*ZEP*)**	F: TTCACTCCAGCAGTGGAACGT R: ACCTTATGCCATCAGCACCA	64	35,30
***Elongation Factor1* (*EF-1α*)**	F: GATGGTCAGACCCGTGAACAT R: TGTTACAGCAGCAGATCATTTGC	55	

(Alpha Innotech, San Leandro, CA). Expression levels are expressed relative to *EF-1α*. All gene expression data was statistically analyzed using the General Linear Model (GLM) in SigmaPlot 11.0 (Systat Software Inc., Chicago, IL). Two-way analyses of variance (ANOVA) were performed to test differences between the treatments and the means were compared using Fisher's LSD Test ($P < 0.05$). The two-way ANOVA was performed to detect significant differences between the cultivars within a single treatment as well as detect differences over time within individual treatments of a single cultivar. A second two-way ANOVA was performed comparing the three treatments within a single cultivar.

3.6 Disease Evaluation

A separate group of plants were grown under the same conditions to be used for disease evaluation as the tissue harvesting for gene expression analysis resulted in destroyed plants that could not be rated for disease. Plants were rated for disease symptoms at 2, 3, 4, and 5 weeks post-inoculation. The plants were rated according to leaf symptoms visually observed. Visual ratings were based on a qualitative scale of 0-5 as defined by Alkher et al. (2009). The ratings were based on leaf area percentages affected as follows: 0 – no necrosis or chlorosis, 1 – visible chlorosis with <1% necrosis, 2 – up to 40% chlorosis and 1-20% necrosis, 3 – 40-65% chlorosis and 20-35% necrosis, 4 – 65-100% chlorosis and 35-70% necrosis, 5- 100% chlorosis and 70-100% necrosis. The total number of leaves and total number of infected leaves was also recorded. From these results percent infection and disease severity were calculated as follows:

Percent Infection = $(I_L/T_L)\ x\ 100$ where I_L is the number of leaves on a given plant exhibiting Verticillium wilt symptoms (chlorosis, necrosis, wilting) and T_L is the total number of leaves on the given plant

Disease Severity = $[\sum_{i=0}^{n}(n\ x\ b)]\ x\ 100/T\ x\ (N\text{-}1)$ where b is the chlorosis/necrosis rating (according the scale described above), and n is the number of leaves with that rating, N is the total number of ratings used on the scale (5), and T is the total number of leaves on the given plant.

Based on the percent infection and disease severity ratings over time, the area under the disease progress curve (AUDPC) was calculated as follows:

AUDPC = $\sum_{i=1}^{n}[\left(\frac{yi+yi+1}{2}\right)(t_{i+1}-t_i)]$, where n is the total number of assessments in weeks, yi is the percent infection or disease severity at the i^{th} assessment week, and the term $t_{i+1}-t_i$ is time duration between two assessments (Campbell & Madden 1990).

Data from the disease evaluations was statistically analyzed using a a two-way ANOVA and differences between the means were tested using Fisher's LSD test ($P<0.05$). The analysis was performed on SigmaPlot 11.1 (Systat Software Inc., Chicago, IL). Each treatment was tested separately to detect differences between the cultivars. A second two-way ANOVA was performed on each cultivar separately to test for differences between the treatments within a single cultivar.

4.0 RESULTS

4.1 Gene Expression Analysis

The list of genes included in Table 3.1 were analyzed for the level of gene expression in both the roots and leaves of potato plants after being wounded (control treatment) (WC), inoculated with the weakly aggressive (WA) isolate of *V. dahliae*, or inoculated with the highly aggressive (HA) isolate of *V. dahliae*. The major goal of the study was to determine genes differentially expressed between the susceptible and the moderately resistant (MR) cultivar, therefore the two cultivars were compared within each treatment individually. The ANOVA ran on each treatment determined significant differences between the cultivars and also determined if there were differences between timings within each cultivar. In addition, a second test was conducted determine if there were differences between treatments within an individual cultivar.

4.1.1 Differences between Cultivars

4.1.1.1 Roots

4.1.1.1.1 SA-Related Genes. Phenylalanine ammonia lyase (PAL) is an enzyme involved in SA synthesis that is encoded for by a multigene family in potato. The gene expression of *PAL1* and *PAL2* was analyzed in this study. Within the roots, *PAL1* showed greater expression in the MR cultivar than in the susceptible cultivar (Fig. 4.1). This was true at all timings for each treatment. Within the MR cultivar the expression of *PAL1* was greatest at the early timings in all treatments (Fig. 4.1). Conversely, *PAL2* showed few differences in expression between cultivars and treatments within the roots (Fig. 4.2).

PR-1 and *PR-2* are pathogenesis-related (PR) genes that are associated with the SA-pathway. *PR-1* encodes a protein with anti-fungal activity and *PR-2* encodes an endo-1,3-β-D-glucanase. Similar to the expression of *PAL1* in the roots, the MR cultivar showed significantly higher expression of *PR-1* (Fig. 4.3) and *PR-2* (Fig. 4.4) than the susceptible cultivar. This difference was not as consistent as it was with *PAL1* and with *PR-1* was only visible after the wounding/inoculation event (Fig. 4.5). In addition, the difference in expression of *PR-1* between the two cultivars was not observed when the plants were inoculated with the HA isolate of *V. dahliae* (except at 7 dpi) (Fig. 4.3c). These two genes differ from *PAL1* in their expression within the MR roots over time. Expression levels of *PAL1* peaked at early sampling timings (Fig. 4.1) whereas expression of *PR-1* peaked at 7 dpi in all treatments (Fig. 4.3). The expression of *PR-2* remained relatively steady over time (Fig. 4.4).

PR-5 encodes an thaumatin-like protein that is linked to the SA pathway. This gene differed from the other genes linked to this pathway in that there were very few differences in the expression of this gene between cultivars (Fig. 4.5) similar to what was observed with *PAL2* (Fig. 4.2). However there were some differences in the expression of *PR-5* over time. Within the roots of the susceptible cultivar inoculated with the HA isolate the expression of *PR-5* increased at 14 dpi significantly above the expression at 0 hpi (Fig. 4.5c).

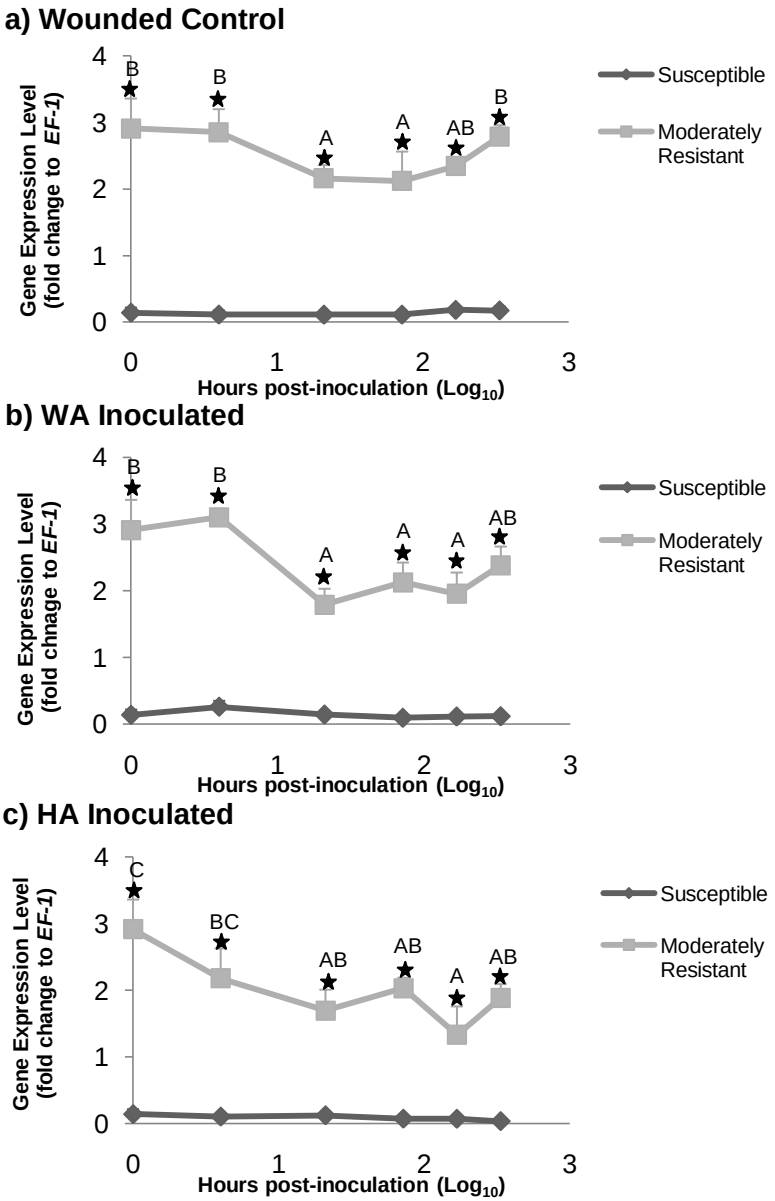


Figure 4.1. Gene expression analysis of *PAL1* in the roots of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★ = significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within the MR cultivar using Fisher's LSD test).

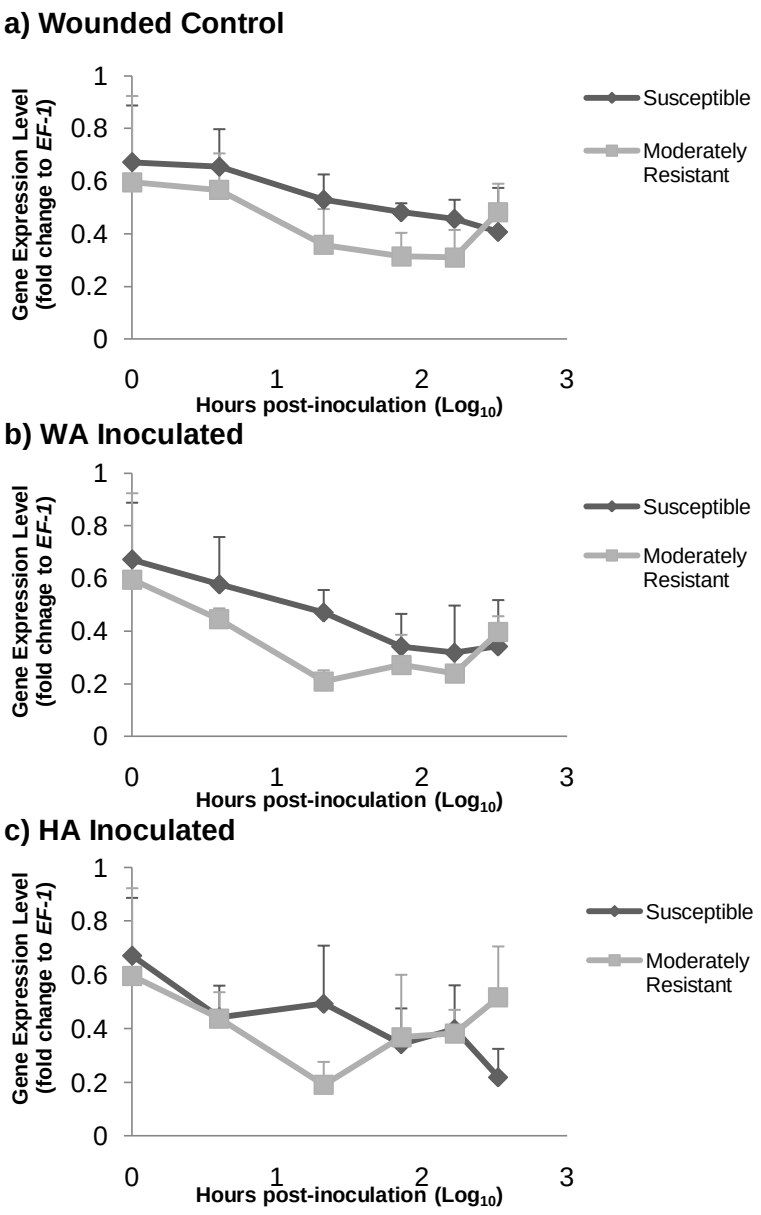


Figure 4.2. Gene expression analysis of *PAL2* in the roots of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (No significant differences ($P<0.05$) detected using Fisher's LSD test).

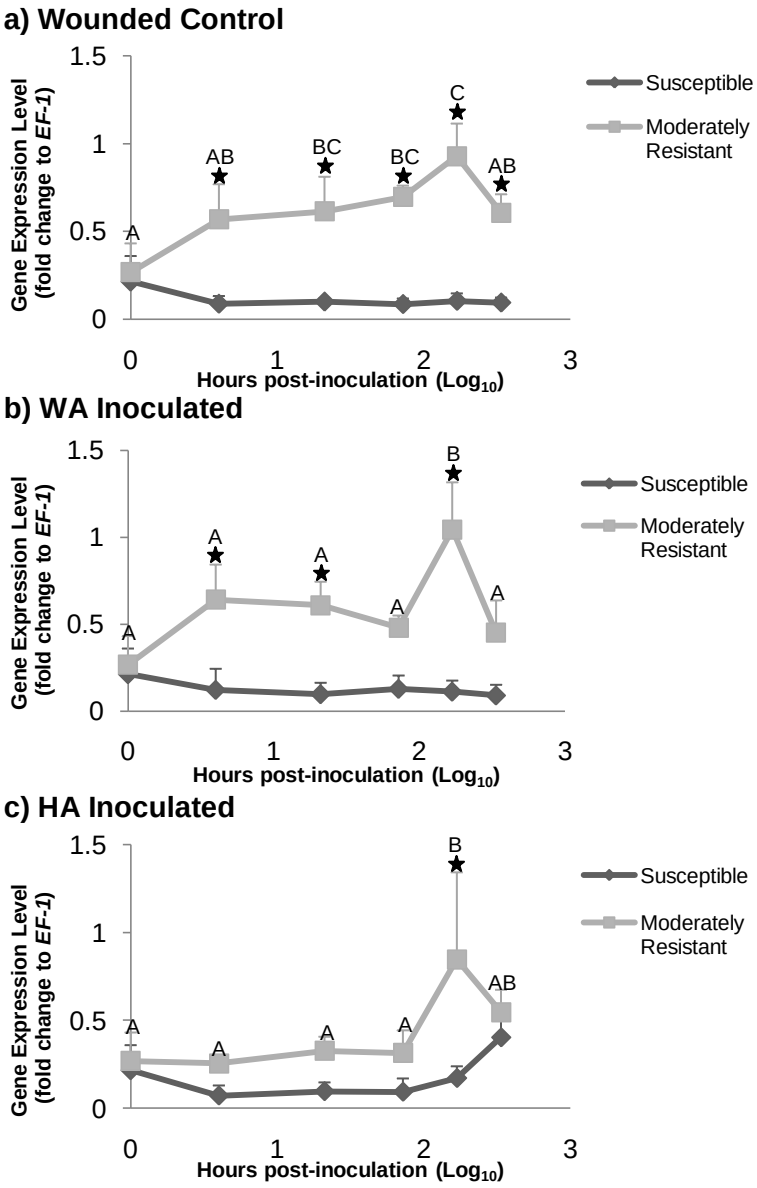


Figure 4.3. Gene expression analysis of *PR-1* in the roots of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★= significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within the MR cultivar using Fisher's LSD test).

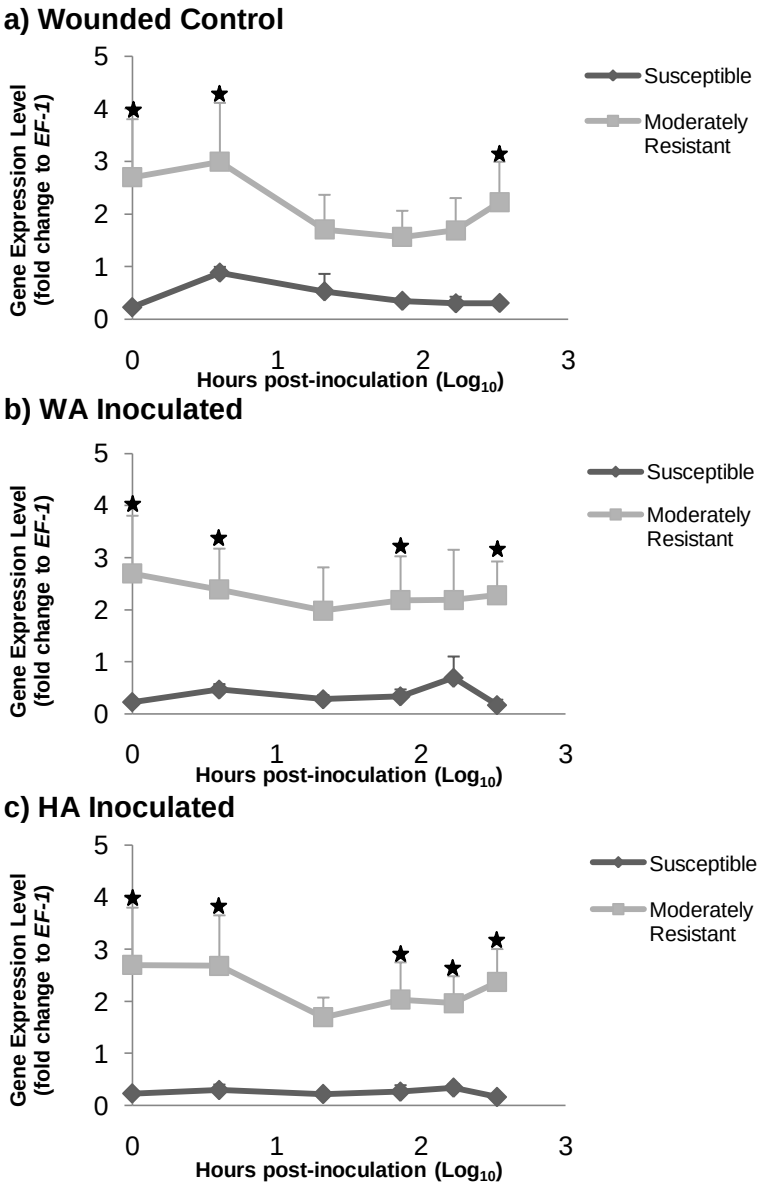


Figure 4.4. Gene expression analysis of *PR-2* in the roots of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★ = significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing using Fisher's LSD test. There were no significant differences (P<0.05) among sampling timings within a single cultivar using Fisher's LSD test).

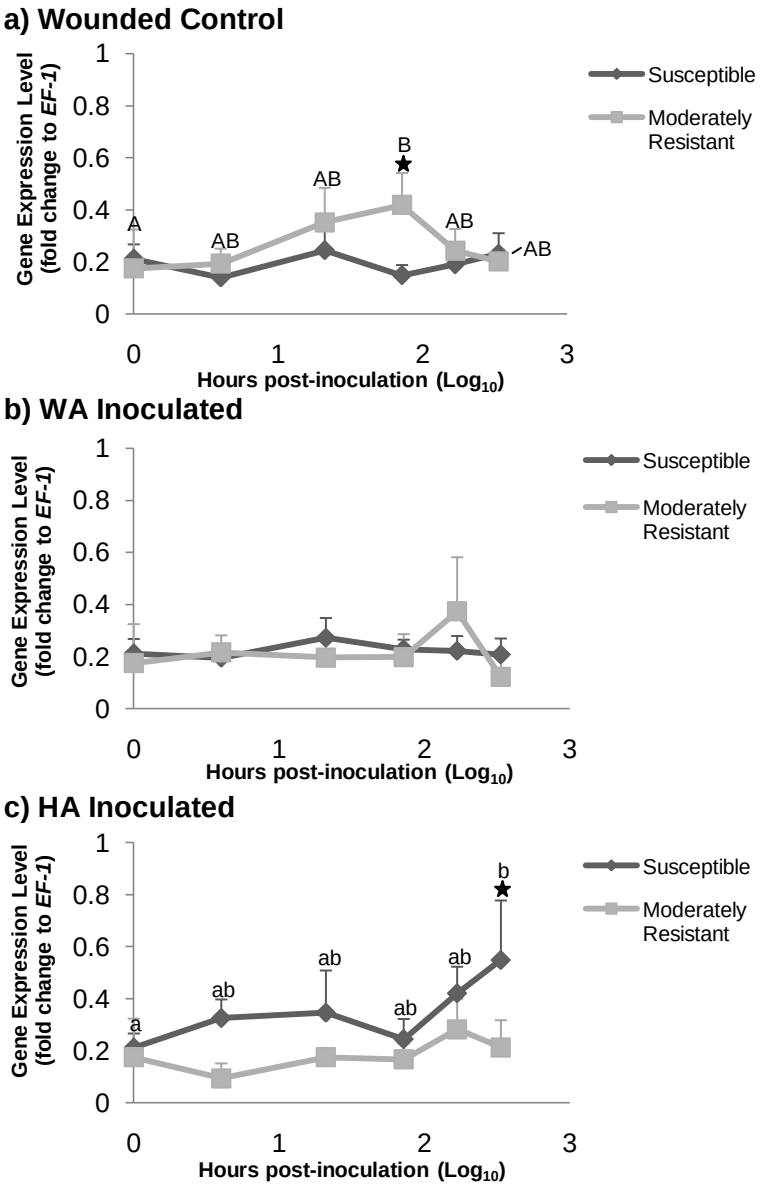


Figure 4.5. Gene expression analysis of *PR-5* in the roots of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★ = significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within a single cultivar, lowercase – susceptible, UPPERCASE – MR using Fisher's LSD test).

4.1.1.1.2 ET-Related Genes. For each hormone pathway at least one enzyme involved in the hormone's synthesis was analyzed for gene expression. *1-Aminocyclopropane-1-Carboxylate Synthase* (*ACCS*) is an important enzyme involved in the synthesis of ET. There were high levels of variation in the expression of this gene, therefore very few significant differences were observed over time and there were no significant differences between cultivars (Fig. 4.6).

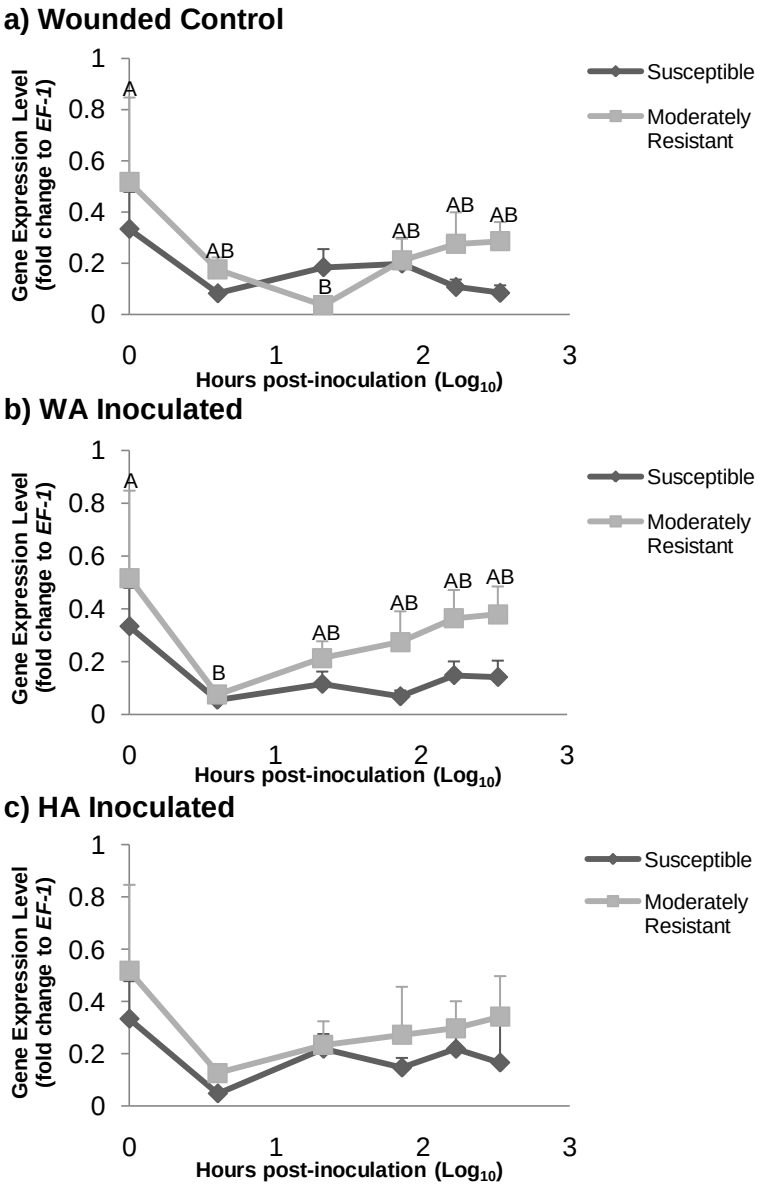


Figure 4.6. Gene expression analysis of *ACCS* in the roots of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (Letters indicate significant differences (P<0.05) among sampling timings within the MR cultivar using Fisher's LSD test; there were no significant differences (P<0.05) between susceptible and moderately resistant cultivars at any timing using Fisher's LSD test).

4.1.1.1.3 JA/ET-Related Genes. It is known that the JA and ET pathways often work together in hormone defense signaling. Therefore, there are some genes that are known to be related to both pathways. The genes analyzed for the purposes of this research included PR-genes, *PR-3* and *WIN2*, and a transcription factor, *ERF-1*. *PR-3* encodes a chitinase and *WIN2* (*Wound-Induced Protein2*) encodes a protein that is homologous to the PR-4 family of pathogenesis-related proteins.

Within the roots, the expression of *ERF1* and *WIN2* did not show significant differences between the cultivars in general (Fig. 4.7, Fig. 4.8). However, at 14 dpi with the WA isolate there was significantly higher expression of both *ERF1* (Fig. 4.7b) and *WIN2* (Fig. 4.8b) in the MR cultivar. Interestingly, at this same time point in the WA treatment there was a decrease in expression of *PR-3* in the MR cultivar leading to expression levels comparable to those observed in the susceptible cultivar at this time point (Fig. 4.9b). In the WC and the WA treatments, the expression of *PR-3* increased immediately after the wounding/inoculation event in the MR cultivar. As mentioned previously, in the WA treatment the expression decreased at 14 dpi to 0 dpi levels.

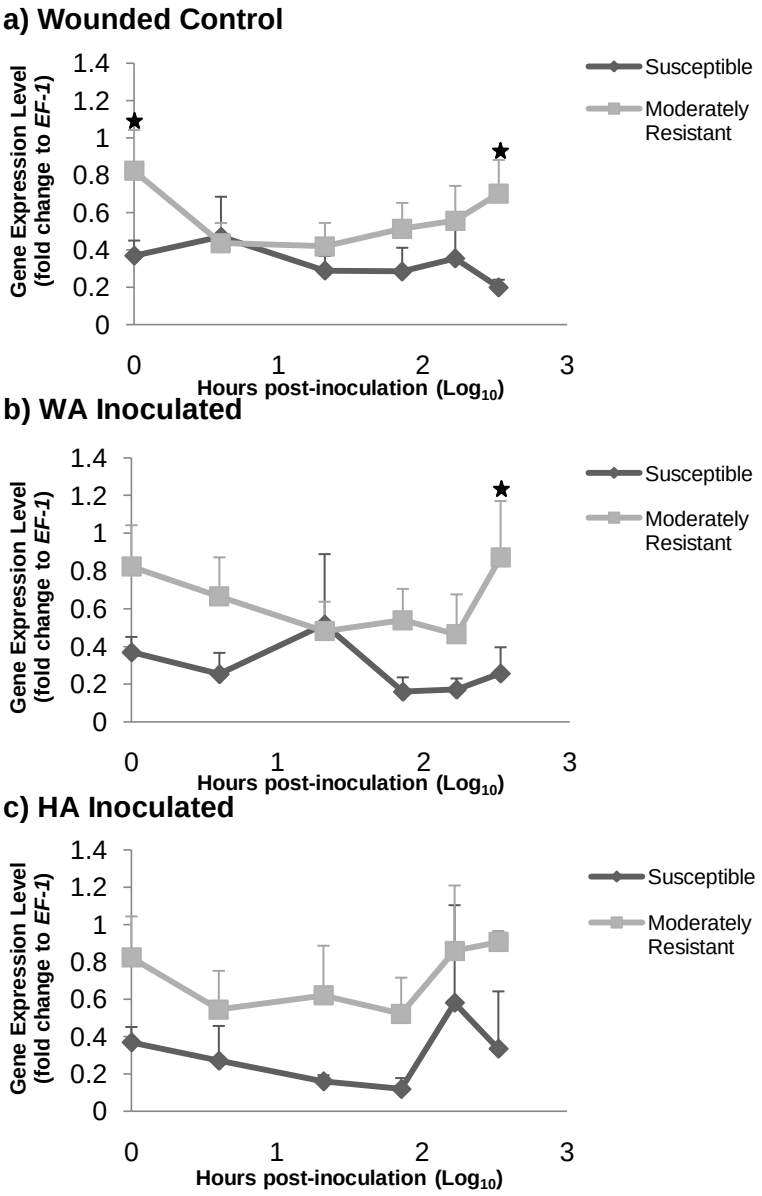


Figure 4.7. Gene expression analysis of *ERF1* in the roots of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★= significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing using Fisher's LSD test. There were no significant differences (P<0.05) among sampling timings within a single cultivar using Fisher's LSD test).

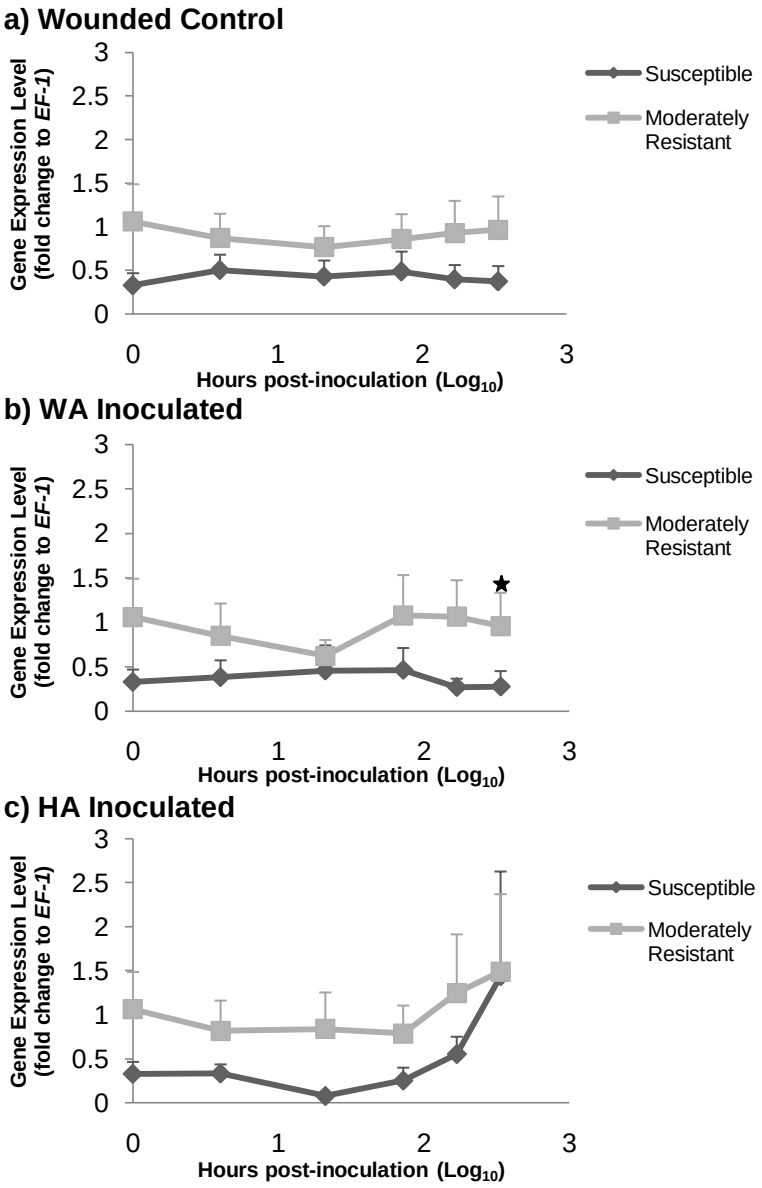


Figure 4.8. Gene expression analysis of *WIN2* in the roots of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★ = significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing using Fisher's LSD test. There were no significant differences (P<0.05) among sampling timings within a single cultivar using Fisher's LSD test).

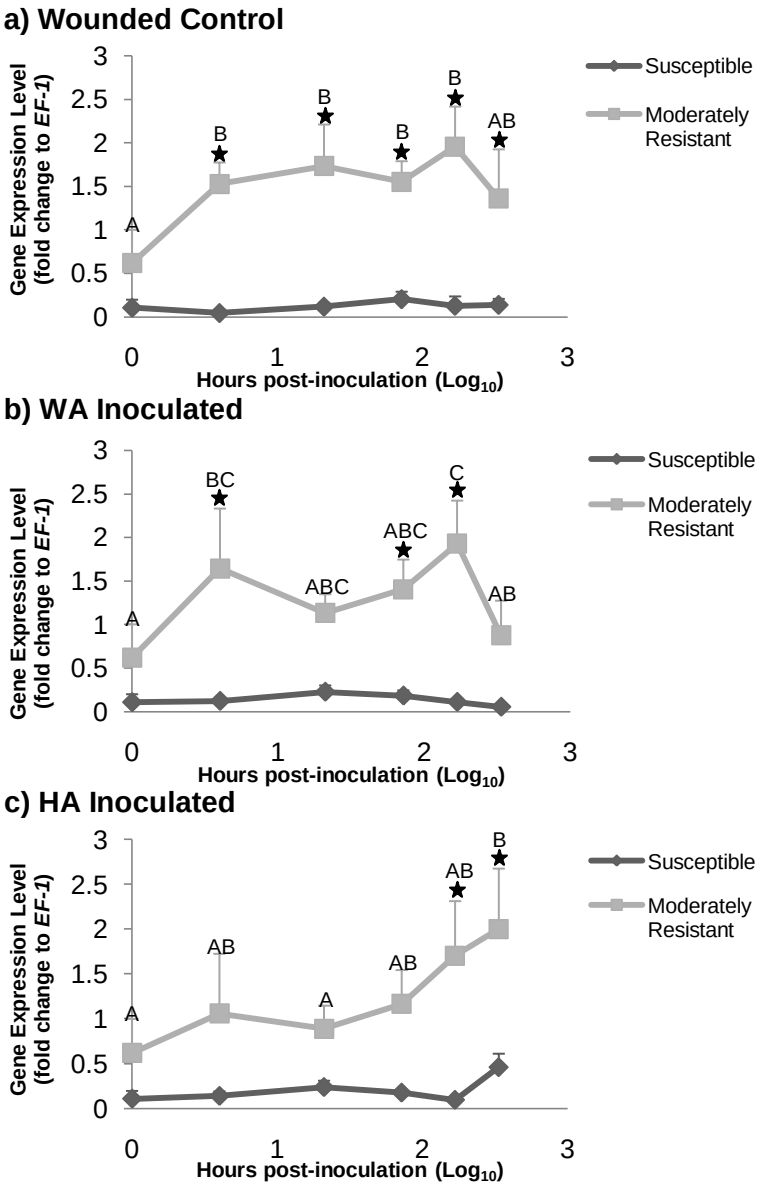


Figure 4.9. Gene expression analysis of *PR-3* in the roots of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★= significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within the MR cultivar using Fisher's LSD test).

4.1.1.1.4 JA-Related Genes. Lipoxygenase (*POTLX3)* is an enzyme involved in the synthesis of JA. In addition, two PR-genes known to be related to the JA pathway were tested. *INH1* encodes a proteinase inhibitor which is part of the PR-6 family of pathogenesis-related proteins and *PR-9* encodes a peroxidase.

Very few similarities were observed between the root expression profiles of these three genes. *INH1* showed higher expression in the MR cultivar than the susceptible cultivar in all treatments with the only exception being 4 hpi with the HA isolate in which there was a decrease in expression in the MR cultivar (Fig. 4.10). *POTLX3* also showed higher expression in the MR cultivar than the susceptible cultivar, but only at 0 hpi in all treatments and at later sampling times in the WC treatment (Fig. 4.11). After inoculation, the expression of *POTLX3* was similar between the susceptible and MR cultivars (Fig. 4.11b,c). In contrast, the expression of *PR-9* was similar between the two cultivars in all treatments in all timings except 14 dpi with the HA isolate where the susceptible cultivar showed significantly higher expression than the MR cultivar (Fig. 4.12).

Within the MR cultivar, the expression of *INH1* showed increases and decreases throughout time with the HA treatment showing the most variation (Fig. 4.10). The expression of *POTLX3* in the MR cultivar also varied throughout time, but only in the WC treatment (Fig. 4.11a). The expression of both *PR-9* and *POTLX3* showed the same trend in the susceptible cultivar inoculated with the HA isolate in that the expression increased at later timings and peaked at 14 dpi (Fig. 4.11c, Fig. 4.12c). This pattern of expression is similar to what was observed with *PR*-5 in the susceptible roots inoculated with the HA isolate (Fig.

4.5c). In all of these genes, there was an increase in expression measured at 14 dpi and this increase was only observed after inoculation with the HA isolate in the susceptible roots.

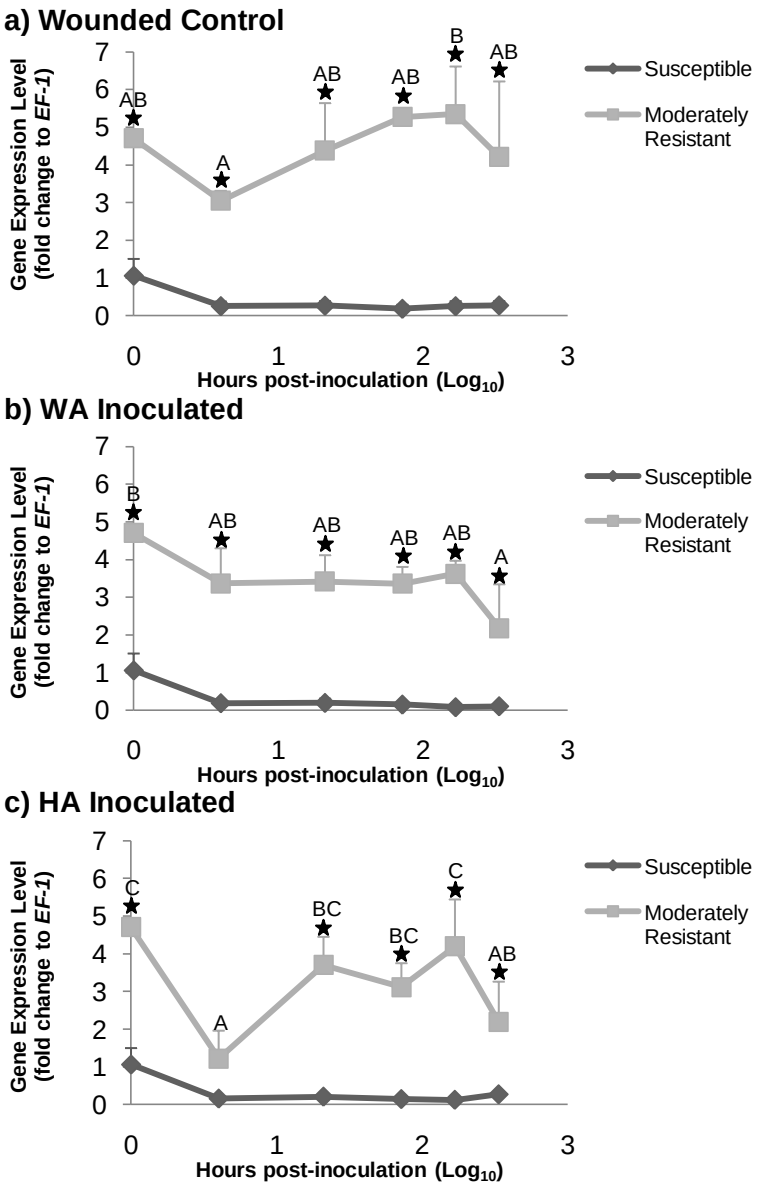


Figure 4.10. Gene expression analysis of *INH1* in the roots of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★ = significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within the MR cultivar using Fisher's LSD test).

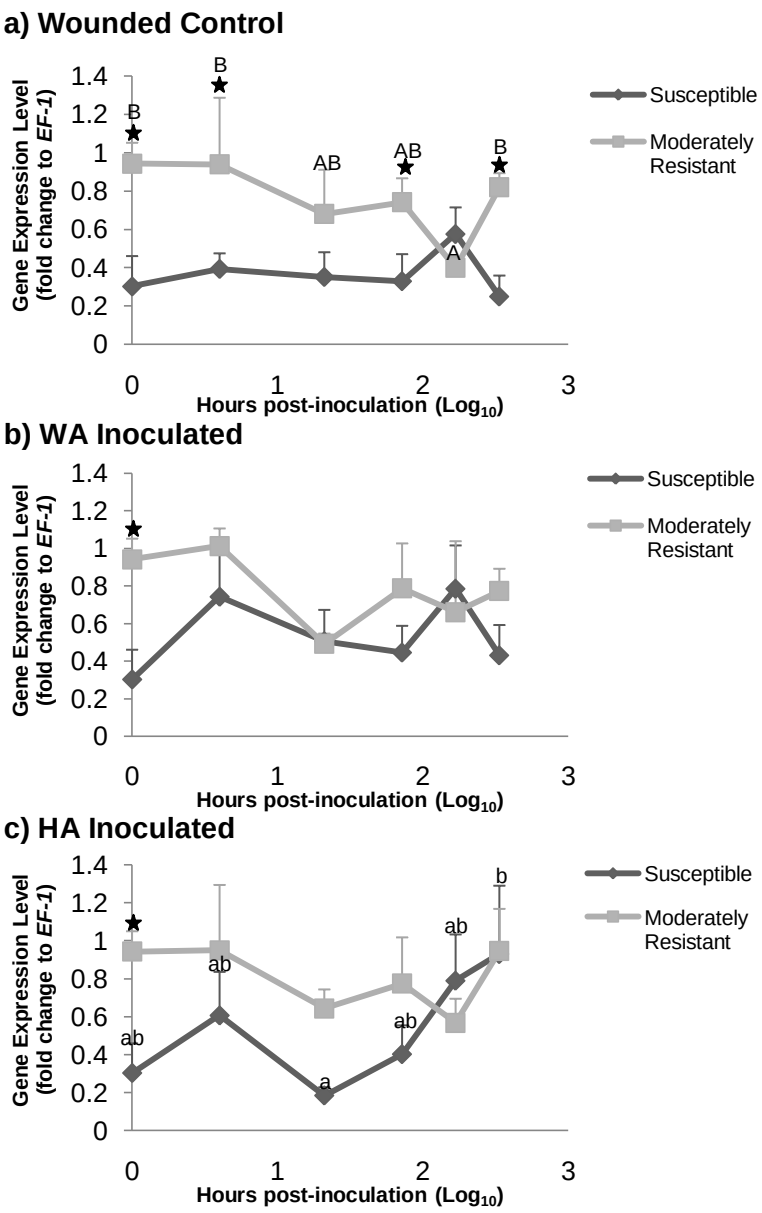


Figure 4.11. Gene expression analysis of *POTLX3* in the roots of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★ = significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within a single cultivar, lowercase – susceptible, UPPERCASE – MR using Fisher's LSD test).

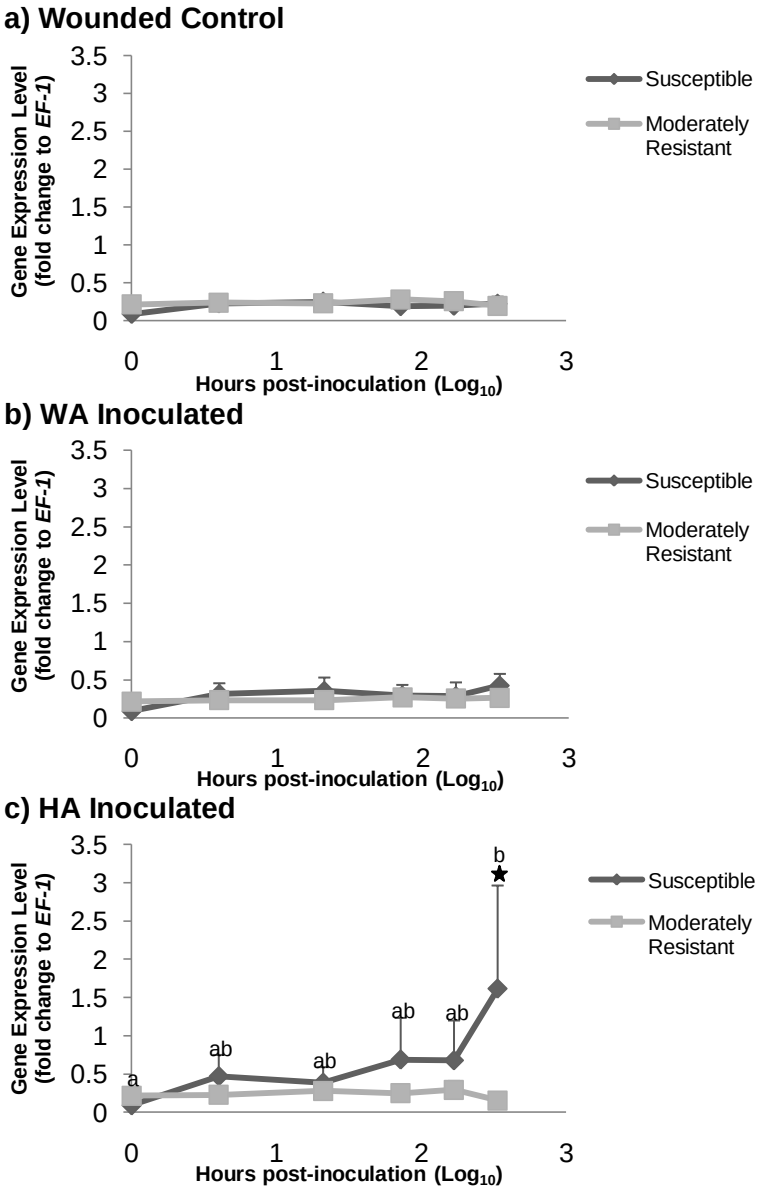


Figure 4.12. Gene expression analysis of *PR-9* in the roots of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★= significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within the susceptible cultivar using Fisher's LSD test).

4.1.1.1.5 ABA-Related Genes. Two genes involved in ABA synthesis were analyzed in this study, zeaxanthin epoxidase (*ZEP*) and neoxanthin synthase (*NXS*). The final gene analyzed encodes an ABA-related transcription factor, *AREB*.

The gene expression profiles of *NXS* and *AREB* were very similar whereas the profile for *ZEP* was much different. In almost all timings in each treatment the MR cultivar showed significantly higher expression of *ZEP* than the susceptible cultivar although the expression in the HA treatment was the most variable (Fig. 4.13). In addition, in the inoculated treatments the MR cultivar showed a decrease in expression after the inoculation event. These decreases led to expression levels similar to those observed in the susceptible cultivar in some instances (Fig. 4.13b,c). In contrast, the levels of *NXS* and *AREB* in the roots of the potato plants were very similar between the cultivars except at 0 hpi in which the susceptible cultivar showed higher levels of expression than the MR cultivar (Fig. 4.14, Fig. 4.15). The susceptible cultivar showed decreased expression of *NXS* and *AREB* after the inoculation/wounding event; although this decrease in expression of *AREB* was more gradual in the WC treatment (Fig. 4.15a).

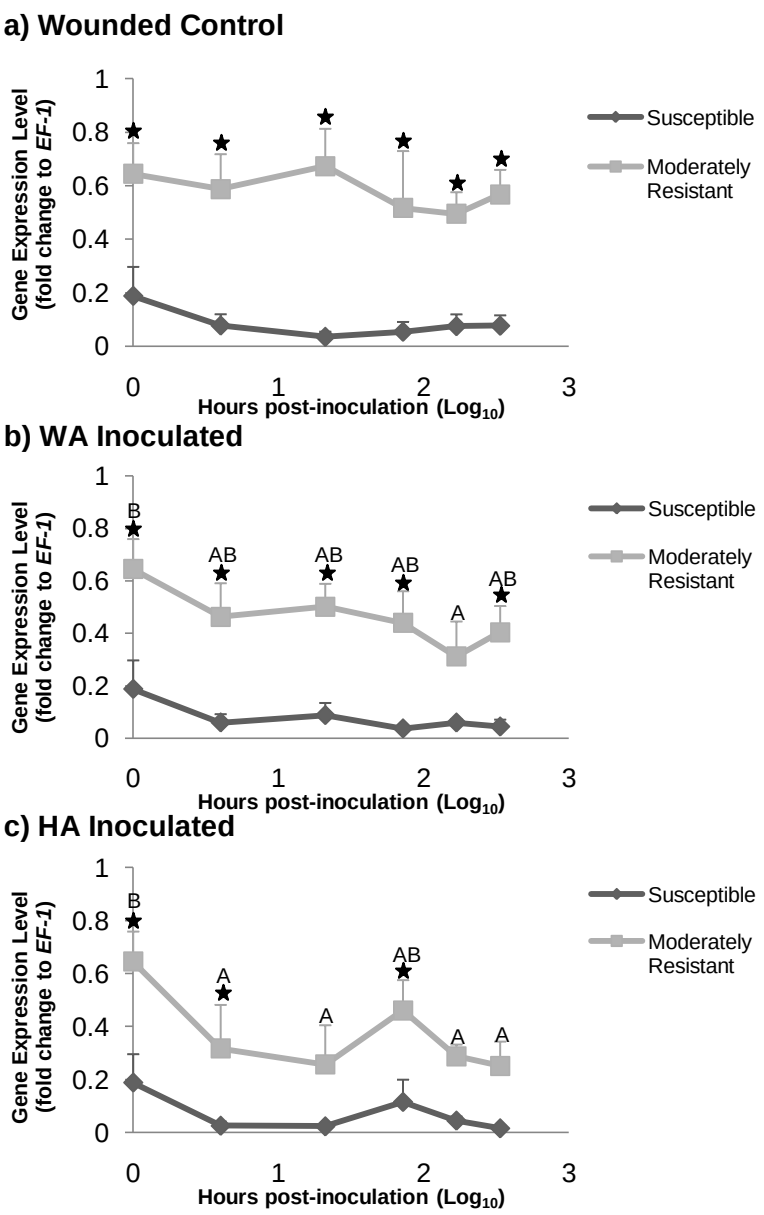


Figure 4.13. Gene expression analysis of *ZEP* in the roots of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★ = significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within the MR cultivar using Fisher's LSD test).

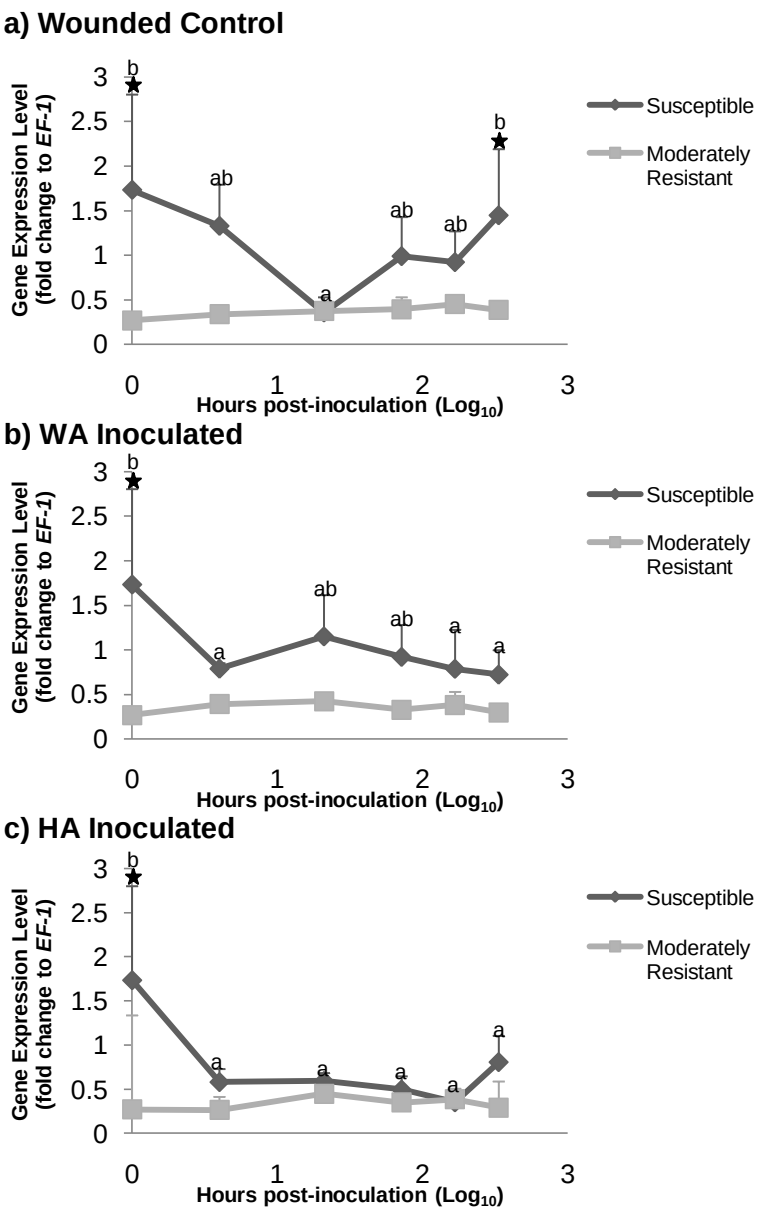


Figure 4.14. Gene expression analysis of *NXS* in the roots of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★ = significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within the susceptible cultivar using Fisher's LSD test).

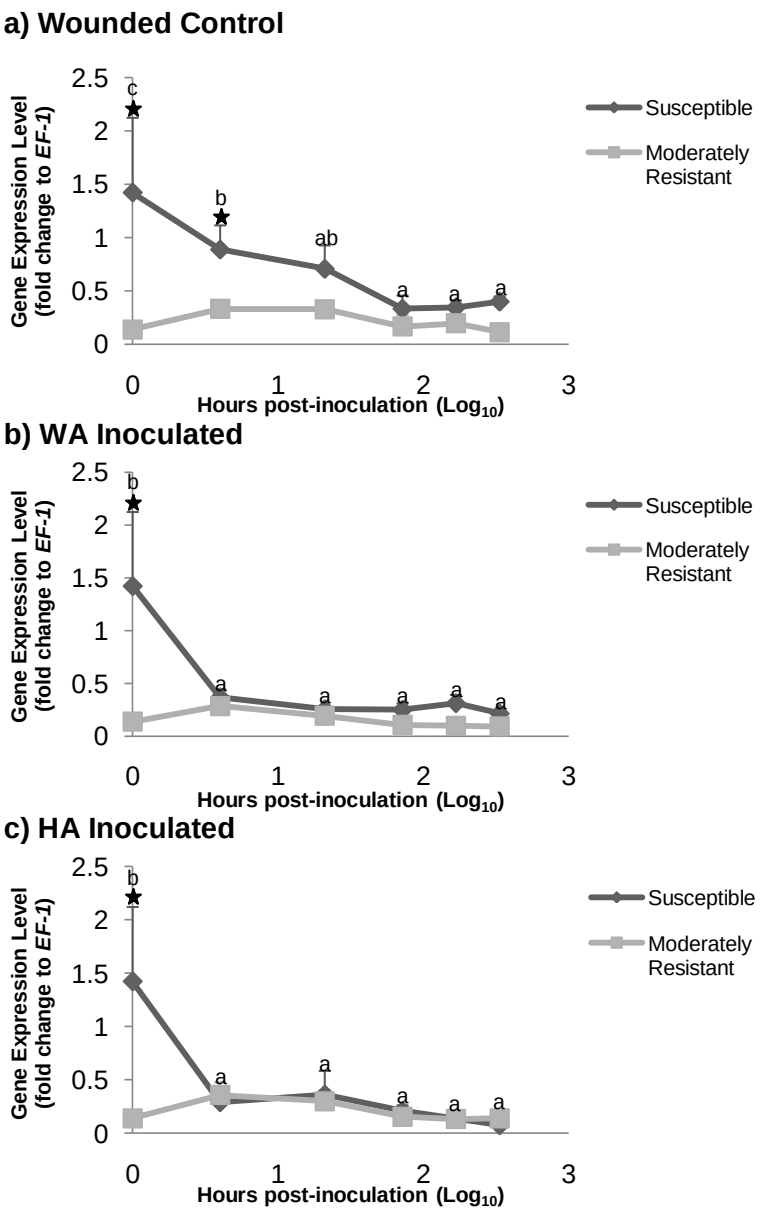


Figure 4.15. Gene expression analysis of *AREB* in the roots of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★= significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within the susceptible cultivar, using Fisher's LSD test).

4.1.1.2 Leaves. A gene expression analysis was done in the leaves on all of the same genes that were analyzed in the roots. Many genes did not follow the same trends in both organs.

4.1.1.2.1 SA-Related Genes. *PAL2* had higher expression levels in the MR leaves than the susceptible leaves in most instances (Fig. 4.16). This difference was visible in all treatments although at 4 hpi with either isolate the susceptible cultivar showed temporarily increased expression to levels similar to those found in the MR cultivar (Fig. 4.16b,c). *PAL1* was also expressed higher in the MR cultivar than the susceptible cultivar (Fig. 4.17), but this difference was only significant in a few timings in the WC and WA treatments and in the HA treatment the expression of *PAL1* was similar between the treatments (Fig. 4.17). *PR-1* and *PR-5* showed similar expression levels in both cultivars in all treatments (Fig.4.18, Fig.4.19) whereas the expression of *PR-2* was variable overall, but in some instances was higher in the susceptible cultivar than the MR cultivar (Fig.4.20).

Within the MR cultivar, the expression of *PR-1* in the leaves varied throughout time in all treatments and followed a similar trend in the WC and the HA treatment peaking at 4 hpi and at later timings (Fig. 4.18a,c), but was more variable in the WA treatment (Fig. 4.18b). The expression of *PR-1* in the MR cultivar inoculated with the WA isolate followed a similar trend to the expression of *PR-5* under the same conditions (Fig. 4.19b).

Within the susceptible cultivar, the expression of *PAL2* spiked at 4 hpi in the inoculated treatments, but dropped again at 21 hpi to levels similar to those

measured at the time of inoculation (Fig. 4.16b,c). In contrast, the expression of *PR-2* in the susceptible leaves was more variable following the same trend in the WC and the HA treatments, remaining relatively steady over time and then increasing at 14 dpi (Fig. 4.20a,c). Interestingly, the expression of *PR-2* in the susceptible leaves inoculated with the WA isolate peaked at 4 hpi then decreased at 14 dpi (Fig. 4.20b).

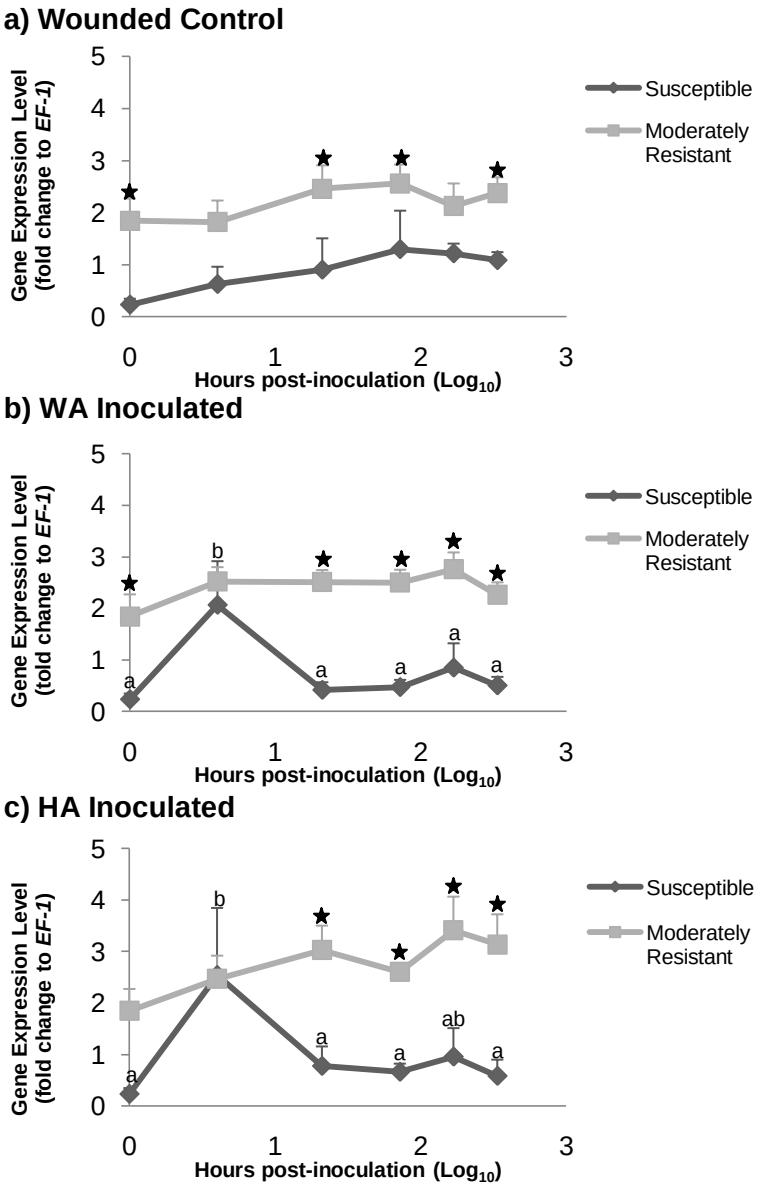


Figure 4.16. Gene expression analysis of *PAL2* in the leaves of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★ = significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within the susceptible cultivar using Fisher's LSD test).

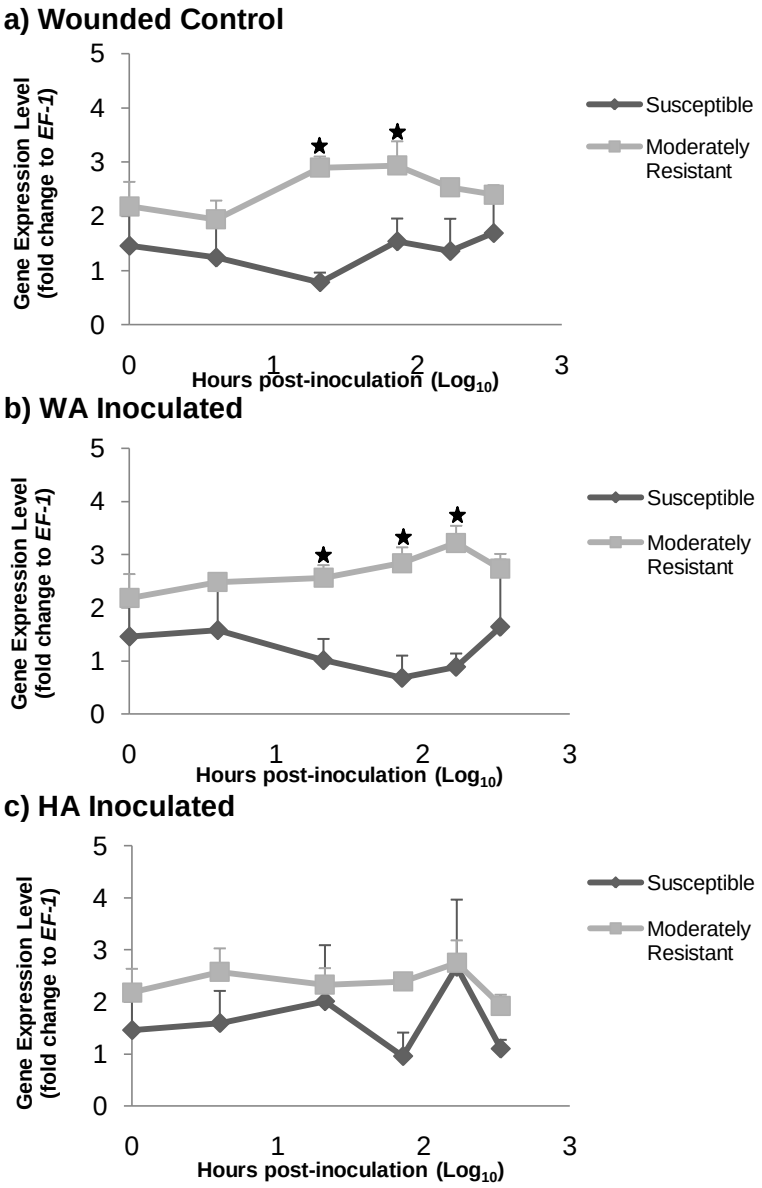


Figure 4.17. Gene expression analysis of *PAL1* in the leaves of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★ = significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing using Fisher's LSD test.There were no significant differences (P<0.05) among sampling timings within a single cultivar using Fisher's LSD test).

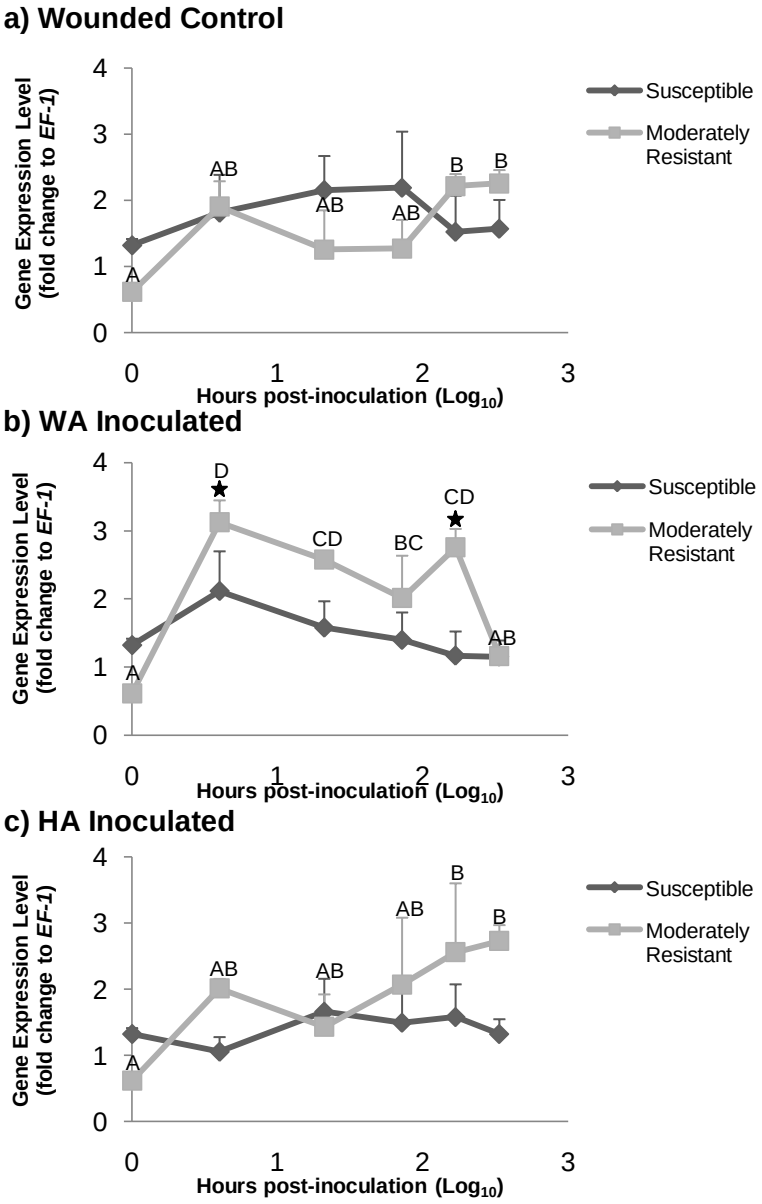


Figure 4.18. Gene expression analysis of *PR-1* in the leaves of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★ = significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within the MR cultivar using Fisher's LSD test).

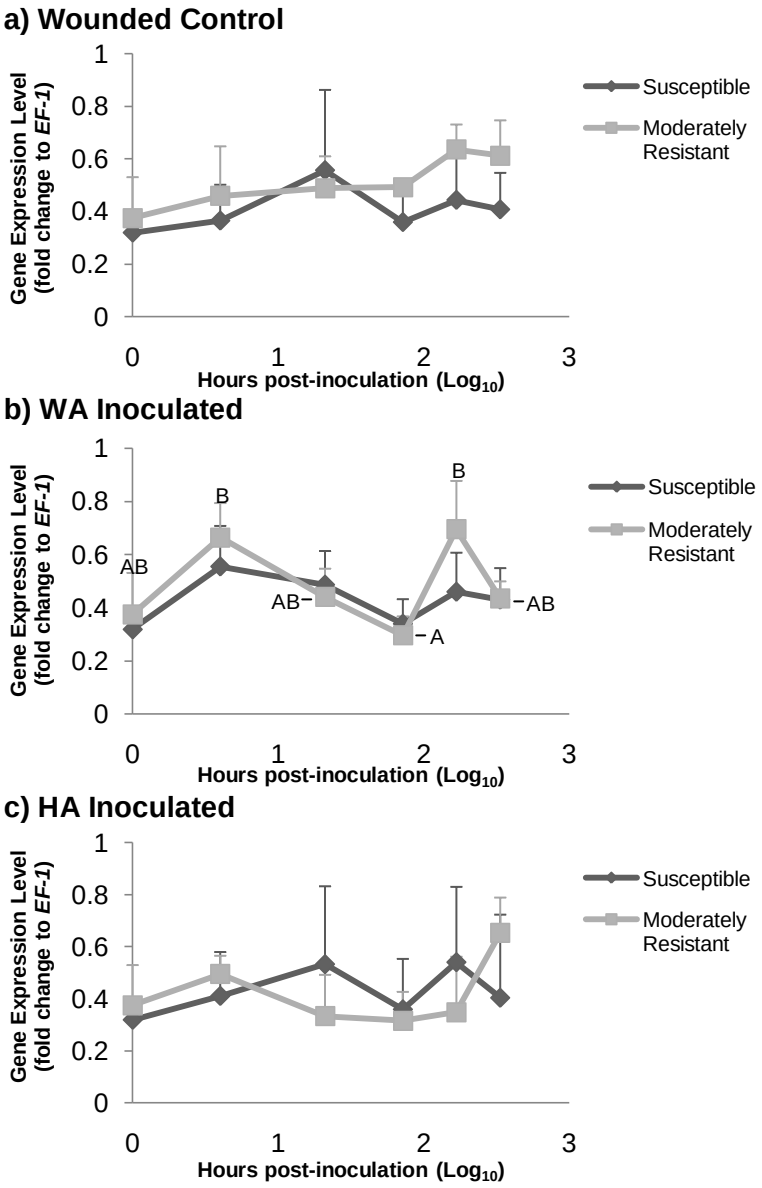


Figure 4.19. Gene expression analysis of *PR-5* in the leaves of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (letters indicate significant differences (P<0.05) among sampling timings within the MR cultivar using Fisher's LSD test; there were no significant differences (P<0.05) between susceptible and moderately resistant cultivars at any timing using Fisher's LSD test).

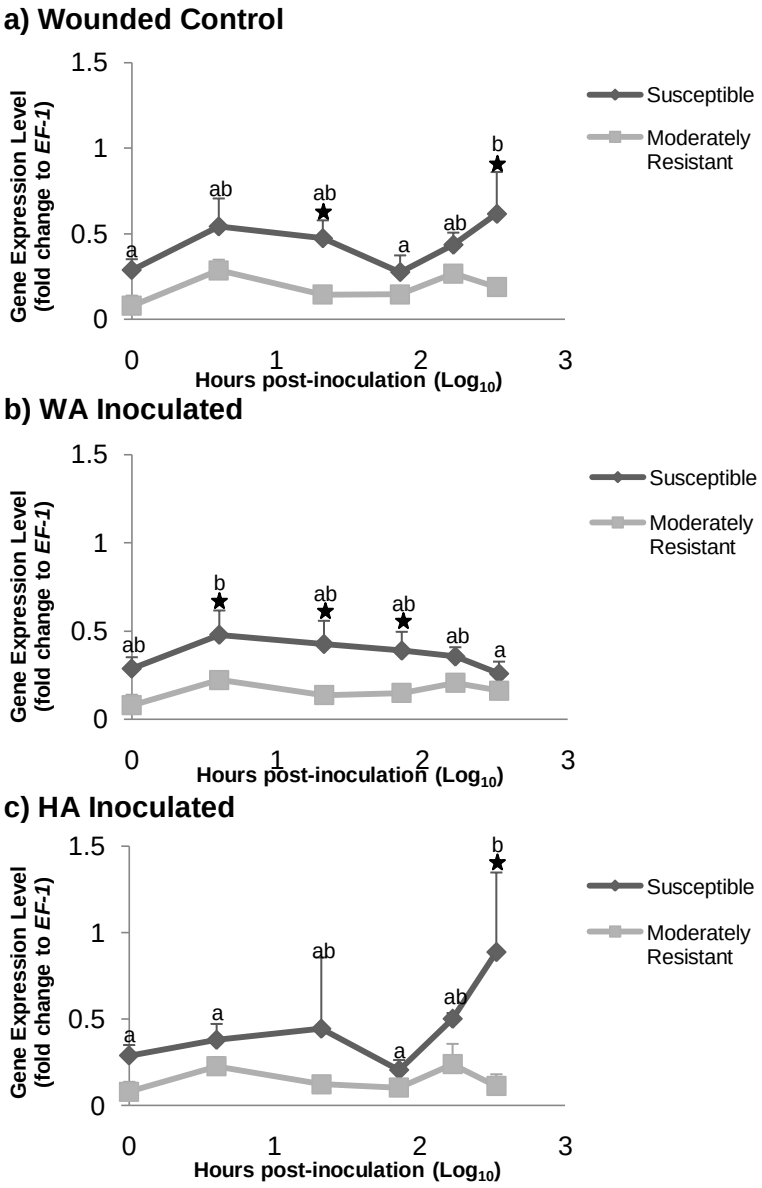


Figure 4.20. Gene expression analysis of *PR-2* in the leaves of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★ = significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within the susceptible cultivar using Fisher's LSD test).

4.1.1.2.2 ET-Related Genes. The expression of *ACCS* in the leaves of potato plants showed only one significant difference between cultivars. At 21hours post wounding, the susceptible cultivar showed significantly higher expression of *ACCS* than the MR cultivar (Fig. 4.21a). There were no differences in expression of this gene among sampling timings.

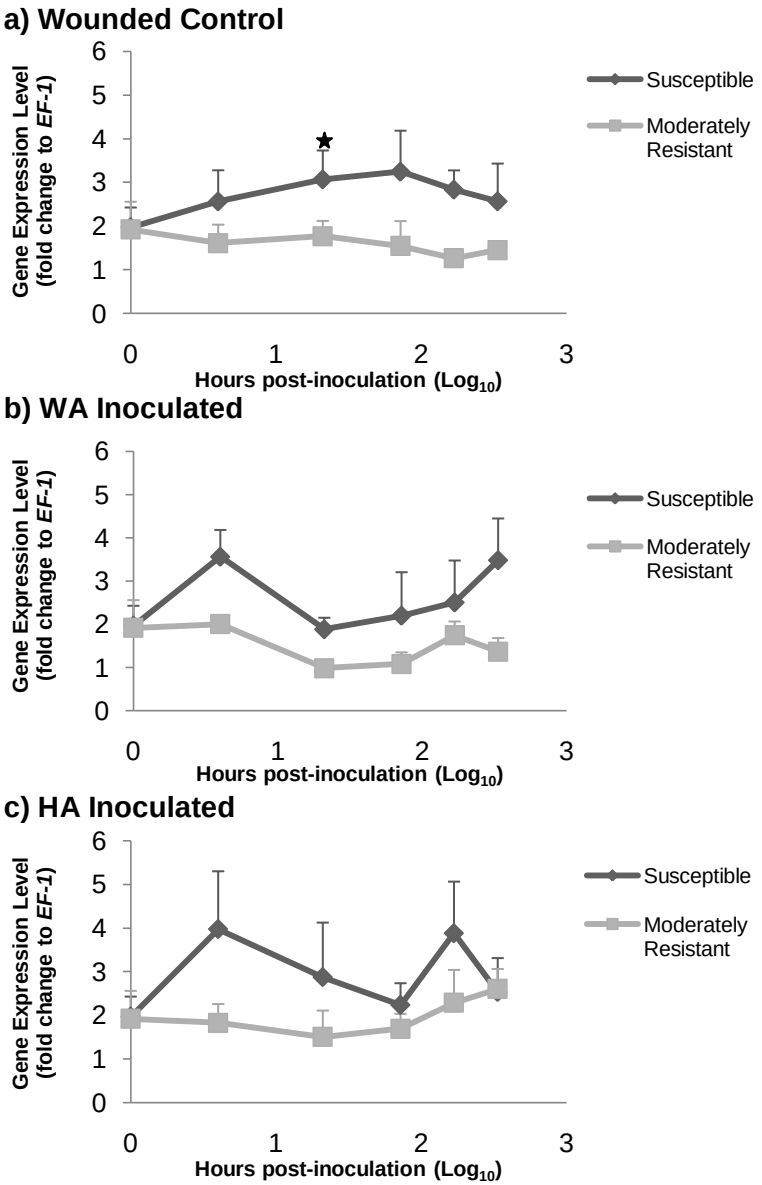


Figure 4.21. Gene expression analysis of *ACCS* in the leaves of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★= significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing using Fisher's LSD test. There were no significant differences (P<0.05) among sampling timings within a single cultivar using Fisher's LSD test).

4.1.1.2.3 JA/ET-Related Genes. The genes related to the cooperative action between the JA and ET pathways showed varying results in their expression in the potato leaves.

WIN2 had higher expression levels in the MR cultivar than the susceptible cultivar in all treatments at most timings following the inoculation/wounding event (Fig. 4.22). In contrast, both *ERF1* and *PR-3* showed similar levels of expression between the two cultivars in all treatments (Fig. 4.23, Fig. 4.24). However, at 14 dpi with the HA isolate the susceptible cultivar experienced an increase in expression of both these genes and at this time point the expression of *PR-3* was significantly higher in the susceptible cultivar (Fig.4.23c, Fig.4.24c).

The expression of *WIN2* in the leaves of the MR cultivar followed the same trend in all of the treatments. This trend included a decrease in expression at 3 dpi followed by a peak in expression at 7 dpi (Fig. 4.22). As mentioned previously, the susceptible leaves showed an increase in expression of *PR-3* at 14 dpi with the HA treatment. This peak in expression was significantly higher than the levels at all other timings within this treatment (Fig. 4.24c). Interestingly, a similar trend was seen with the expression of *WIN2* in the susceptible leaves except that this peak in expression occurred at 7 dpi and was only significantly higher than the expression at 4 and 21 hpi (Fig. 4.22c).

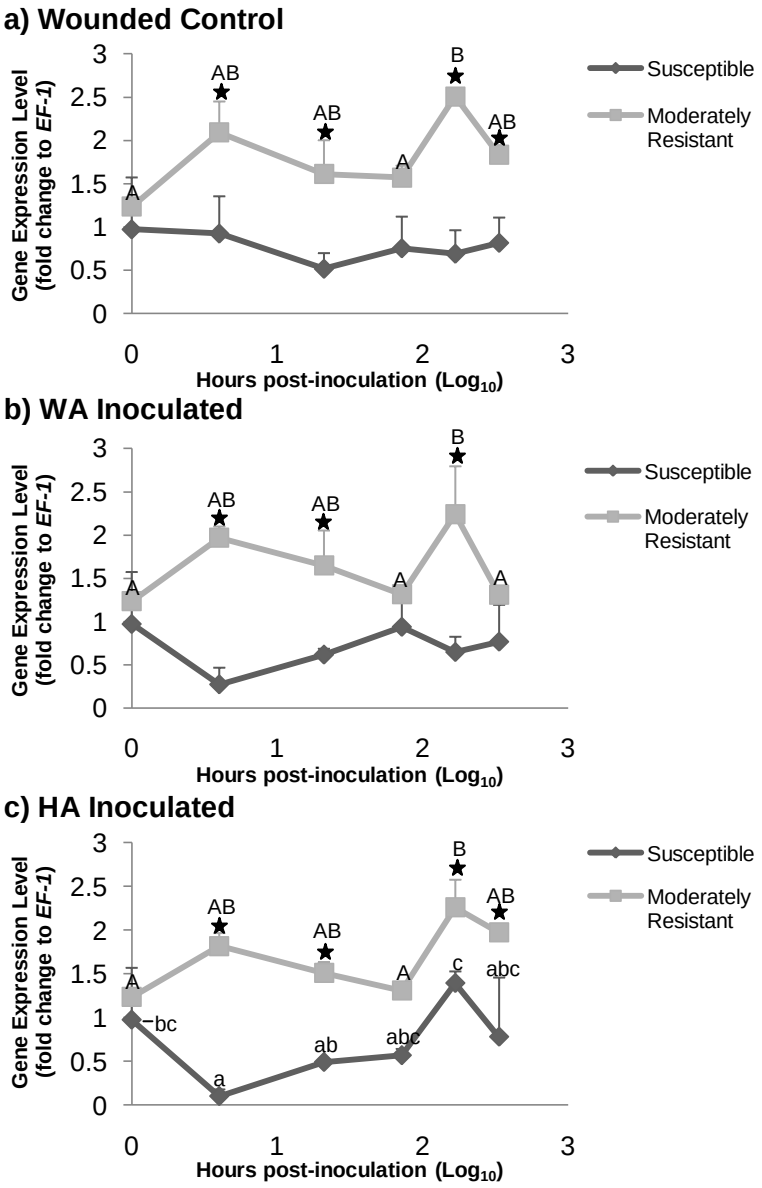


Figure 4.22. Gene expression analysis of *WIN2* in the leaves of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★ = significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within a single cultivar, lowercase – susceptible, UPPERCASE – MR using Fisher's LSD test).

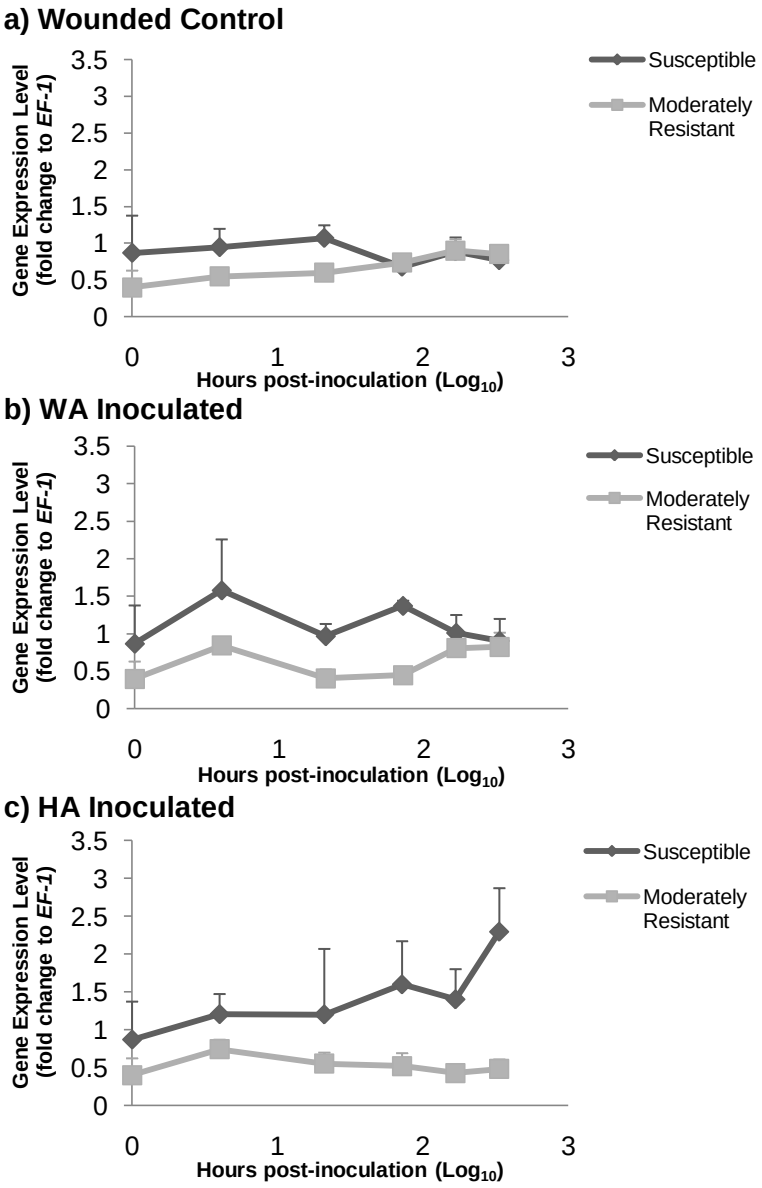


Figure 4.23. Gene expression analysis of *ERF1* in the leaves of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (There were no significant differences ($P<0.05$) detected using Fisher's LSD test).

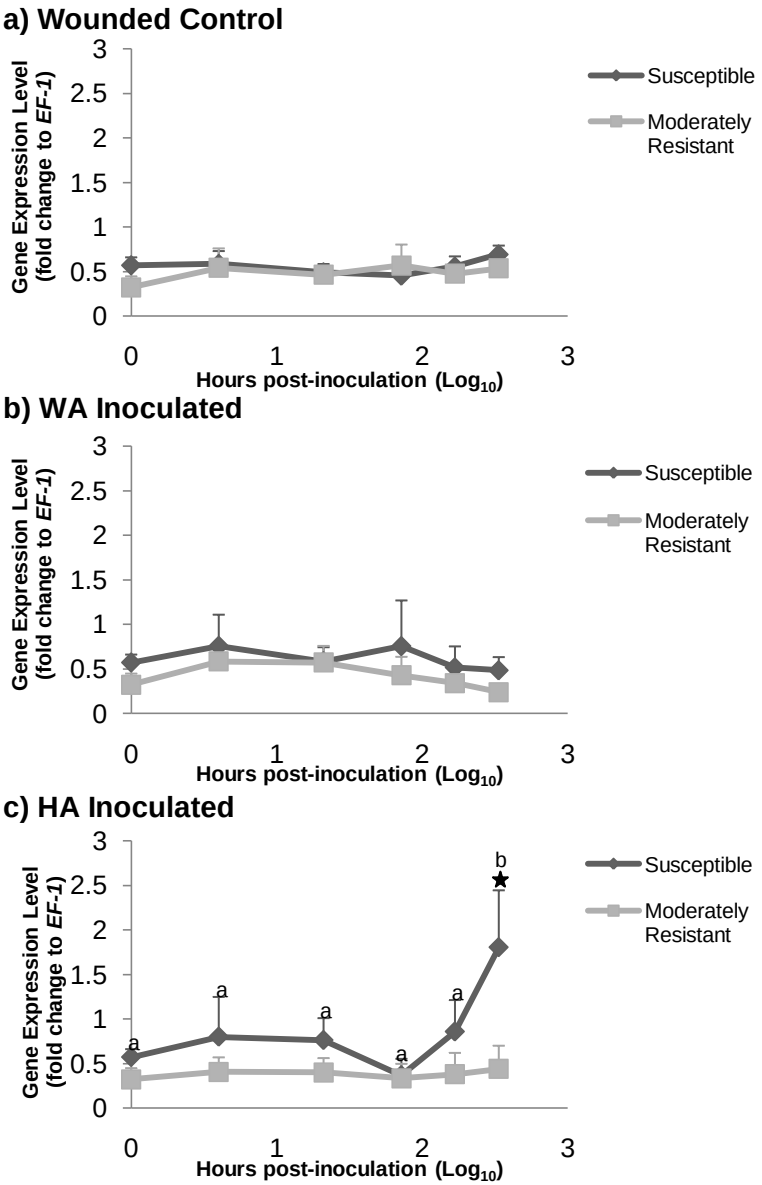


Figure 4.24. Gene expression analysis of *PR-3* in the leaves of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★ = significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within the susceptible cultivar using Fisher's LSD test).

4.1.1.2.4 JA-Related Genes. Overall, the MR cultivar showed higher expression than the susceptible cultivar of *POTLX3* (Fig. 4.25) and *PR-9* (Fig. 4.26) although this difference was more consistent with *PR-9*. Higher expression levels of *POTLX3* in the MR cultivar were significant at later timings in all treatments as well as at 4 hpi with the WA isolate (Fig. 4.25). The expression of *PR-9* was higher in the MR cultivar than the susceptible cultivar consistently in the WC and WA treatments (Fig. 4.26a,b). But the expression of *PR-9* in the MR cultivar decreased in the HA treatment to levels similar to those of the susceptible cultivar in some instances (Fig. 4.26c). On the other hand, the expression of *INH1* in the potato leaves was similar between the cultivars with only two instances showing higher expression in the MR cultivar (Fig. 4.27).

The expression of *PR-9* in the MR leaves followed a similar trend in the inoculated treatments. This trend included steady expression from 0 to 4 hpi followed by a decrease in expression at 21 hpi (Fig. 4.26). This level of expression remained relatively steady in the WC and the WA treatments (Fig.4.26a,b). Interestingly, an increase in expression of *POTLX3* in the MR leaves was also observed at 7 dpi, but in all treatments (Fig. 4.25). Unlike *PR-9*, the expression of *POTLX3* was the lowest at the time of inoculation (Fig. 4.25).

The expression of *INH1* in the leaves of the susceptible plants inoculated with the HA isolate followed a trend that has been previously observed peaking at 14 dpi (Fig. 4.27c). Interestingly, both the WA and the HA treatments showed significant decreases in expression of *INH1* in the susceptible leaves at 4 hpi (Fig. 4.27b,c).

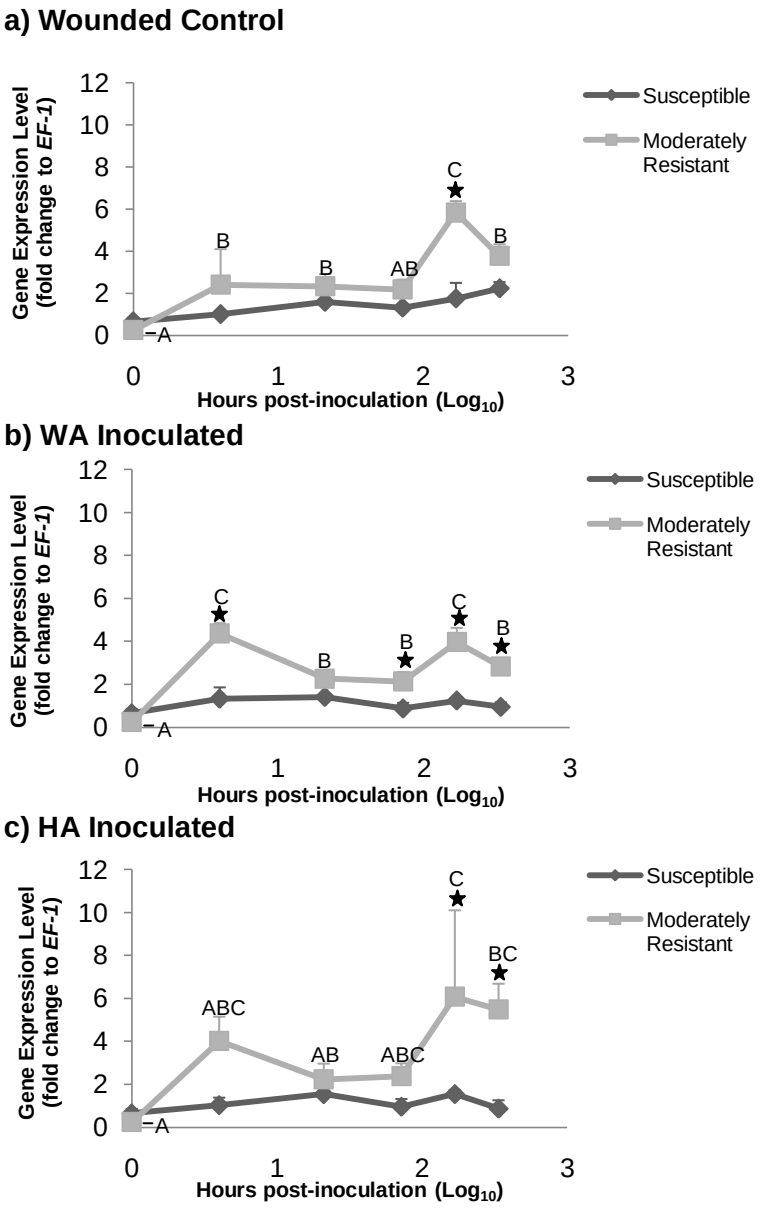


Figure 4.25. Gene expression analysis of *POTLX3* in the leaves of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★ = significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within the MR cultivar using Fisher's LSD test).

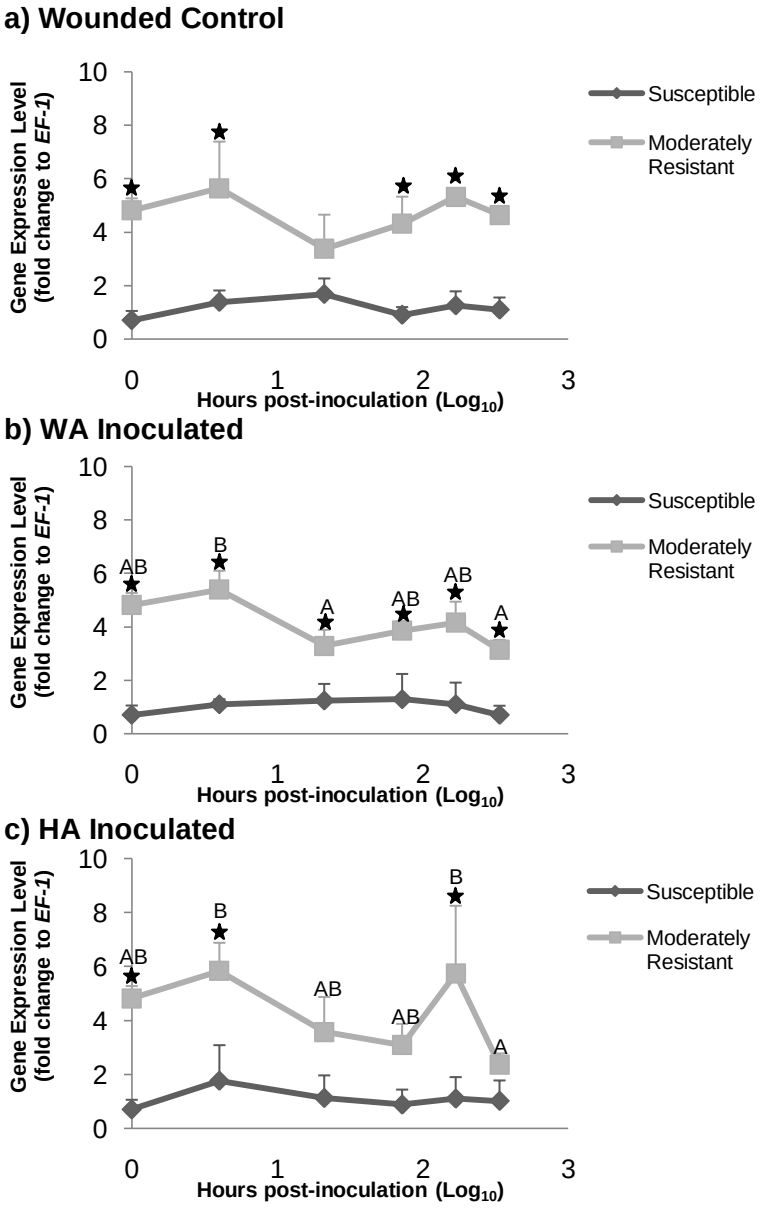


Figure 4.26. Gene expression analysis of *PR-9* in the leaves of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★ = significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within the MR cultivar using Fisher's LSD test).

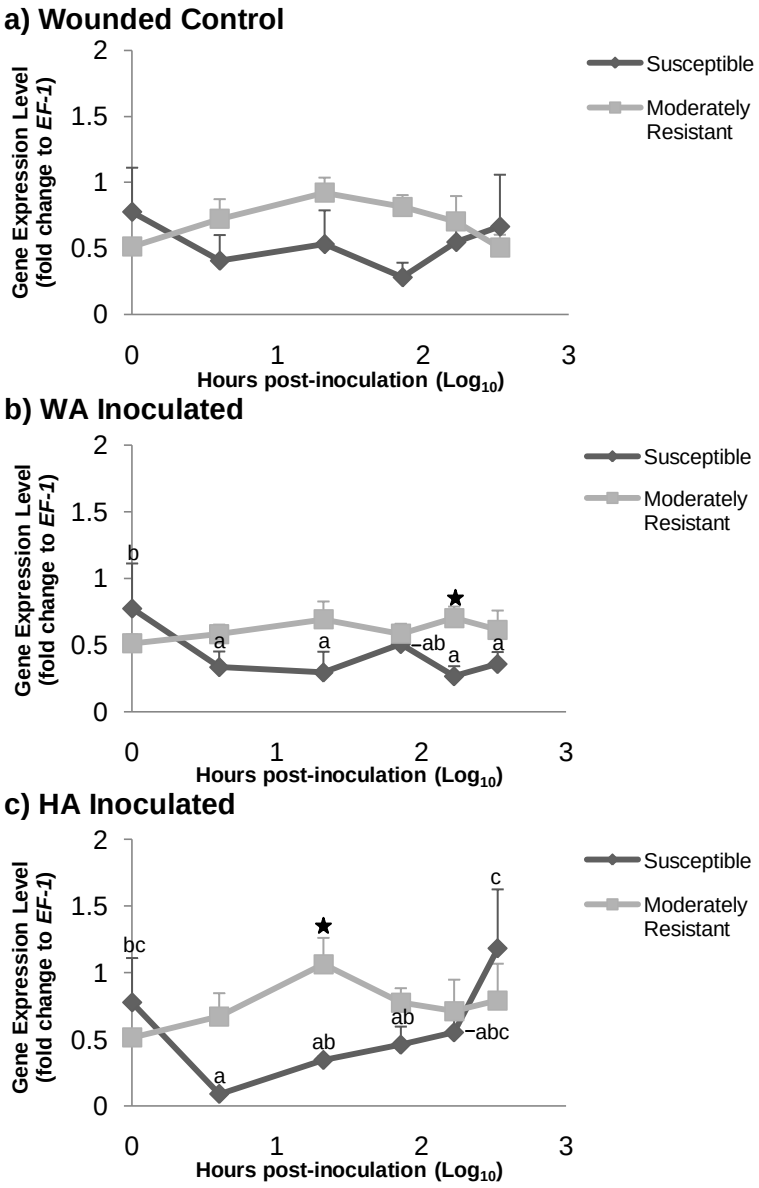


Figure 4.27. Gene expression analysis of *INH1* in the leaves of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★ = significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within the susceptible cultivar using Fisher's LSD test).

4.1.1.2.5 ABA-Related Genes. Both of the genes related to the ABA synthesis pathway (*NXS*, *ZEP*) showed higher expression in the MR cultivar than the susceptible cultivar (Figs. 4.28, 4.29). Expression of *NXS* was not detected in the leaves of the susceptible cultivar in this study, but due to variability of expression in the MR leaves the levels were not significantly higher at all timings. The MR cultivar showed significantly higher expression of *NXS* than the susceptible cultivar after the wounding/inoculation and due to decreases in expression in the MR cultivar this difference was not observed at all timings (Fig. 4.28). In regards to *ZEP*, this difference was visible at 0 hpi and levels remained relatively steady in the MR cultivar through to 14 dpi (Fig. 4.29). In the HA treatment, the expression of *ZEP* in the MR cultivar was variable and therefore the expression was not considered significantly higher than the susceptible cultivar at all timings (Fig. 4.29c). Although these two genes were not directly compared to one another it is important to note that the expression of *ZEP* in the MR cultivar was much higher than *NXS* relative to the internal control (*EF-1*).

The expression of *AREB* in the leaves was higher in the susceptible cultivar than the MR cultivar although the levels were highly variable and this difference was not statistically significant for the majority of the timings (Fig. 4.30).

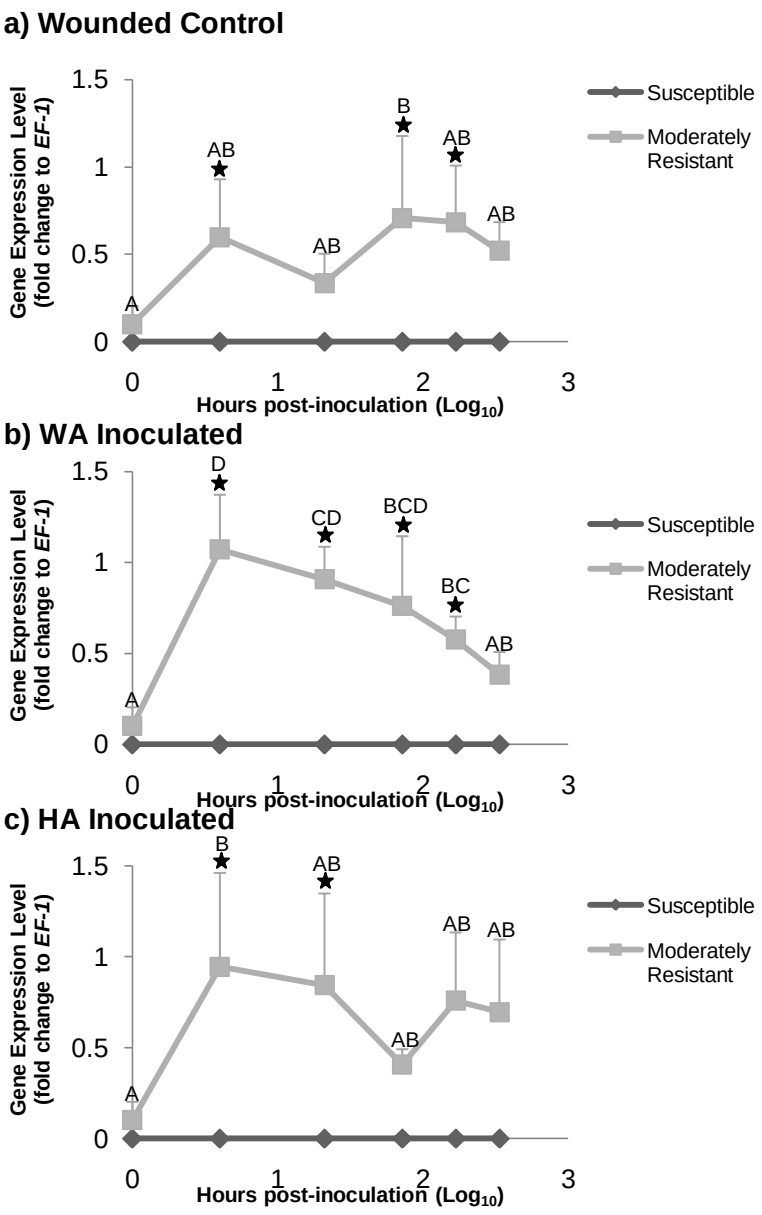


Figure 4.28. Gene expression analysis of *NXS* in the leaves of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★= significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within the MR cultivar using Fisher's LSD test).

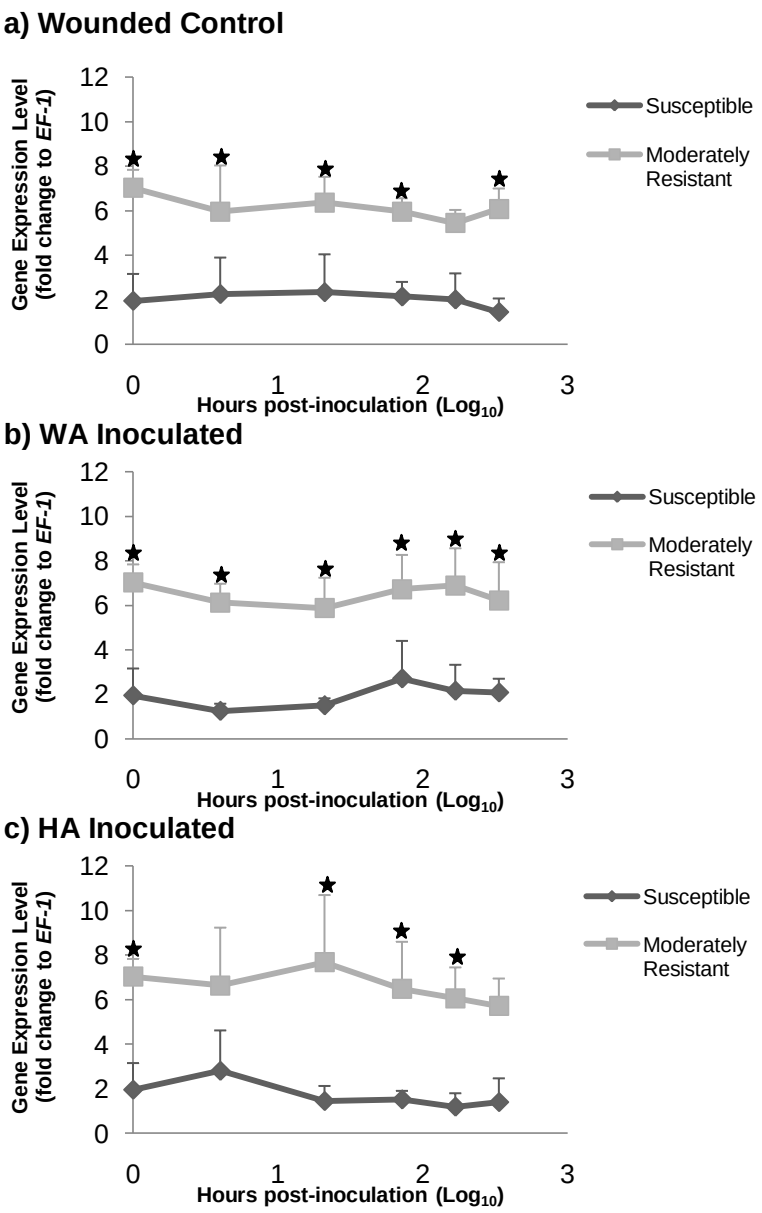


Figure 4.29. Gene expression analysis of *ZEP* in the leaves of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★ = significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing using Fisher's LSD test. There were no significant differences (P<0.05) among sampling timings within a single cultivar using Fisher's LSD test).

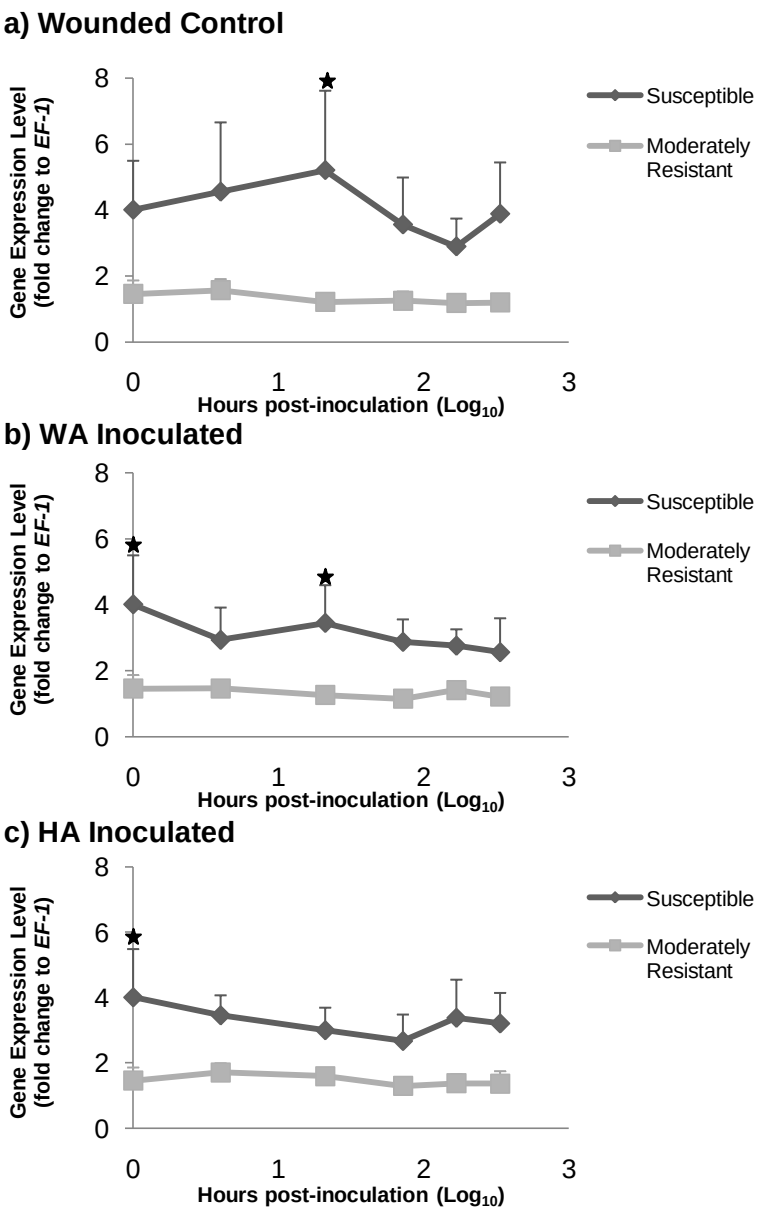


Figure 4.30. Gene expression analysis of *AREB* in the leaves of two cultivars at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★ = significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing using Fisher's LSD test. There were no significant differences (P<0.05) among sampling timings within a single cultivar using Fisher's LSD test).

Table 4.1. Summary of genes with higher expression in the MR cultivar in comparison with the susceptible cultivar.

Organ	Pathway	Genes
Roots	SA	*PAL1*, *PR-1*, *PR-2*
Roots	JA/ET	*PR-3*
Roots	JA	*INH1*
Roots	ABA	*ZEP*
Leaves	SA	*PAL2*
Leaves	JA/ET	*WIN2*
Leaves	JA	*PR-9*
Leaves	ABA	*NXS*, *ZEP*

Table 4.2. Summary of genes with similar expression in the MR and susceptible cultivars.

Organ	Pathway	Genes
Roots	SA	*PAL2*, *PR-5*
Roots	JA/ET	*WIN2*, *ERF1*
Roots	JA	*PR-9*
Roots	ET	*ACCS*
Leaves	SA	*PAL1*, *PR-1*, *PR-5*
Leaves	JA/ET	*ERF1*, *PR-3*
Leaves	JA	*POTLX3*, *INH1*
Leaves	ET	*ACCS*
Leaves	ABA	*NXS*, *ZEP*

4.1.2 Differences between Treatments

4.1.2.1 Roots

4.1.2.1.1 Susceptible Cultivar. Within the roots of the susceptible cultivar there were differences in gene expression between the treatments.

A sharp increase in expression of a number of genes in the susceptible roots was observed 14 dpi with the HA isolate. These genes include SA-related genes (*PR-1*, *PR-5*) (Figs. 4.31, 4.32), JA/ET-related genes (*PR-3*, *WIN2*) (Figs. 4.33, 4.34), and a JA-related gene (*PR-9*) (Fig. 4.35). In all of these genes this increase in expression at 14 dpi was only visible with the HA treatment and led to

expression levels significantly higher in the HA treatment than the other treatments at this time. Likewise, the expression of *POTLX3* (JA pathway) was significantly higher in the susceptible roots 14 dpi with the HA isolate in comparison with the WC treatment (Fig. 4.36). In the case of *POTLX3*, however, this increase in the HA treatment was more gradual whereas the other two treatments showed a decrease in expression at 14 dpi.

In contrast, there were also examples of the WC treatment showing significantly higher expression of some genes than the HA treatment in the susceptible roots. This was true of an ABA-related gene (*AREB*) (Fig. 4.37) and a SA-related gene (*PR-2*) (Fig. 4.38) at 4 hpi. In the case of *PR-2*, this difference was due to an increase in expression in the WC treatment (Fig. 4.38). In contrast, the expression of *AREB* quickly decreased after the inoculation/wounding event in all treatments, but was slightly more gradual in the WC treatment leading to *AREB* expression significantly higher in the WC treatment than the HA treatment at (Figs. 4.15, 4.37).

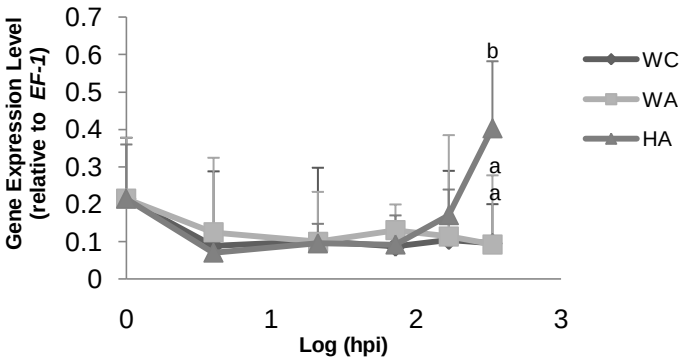


Figure 4.31. Gene expression analysis of *PR-1* in the roots of a susceptible cultivar at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments using Fisher's LSD test.

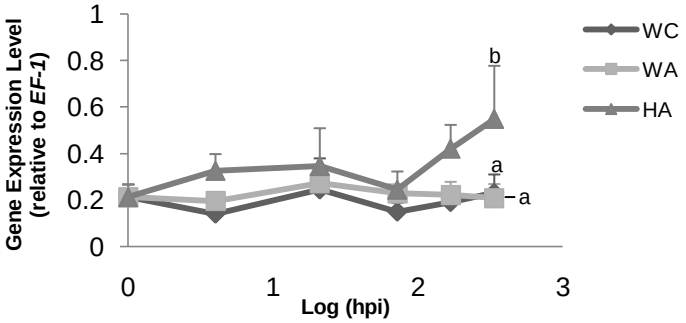


Figure 4.32. Gene expression analysis of *PR-5* in the roots of a susceptible cultivar at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments using Fisher's LSD test.

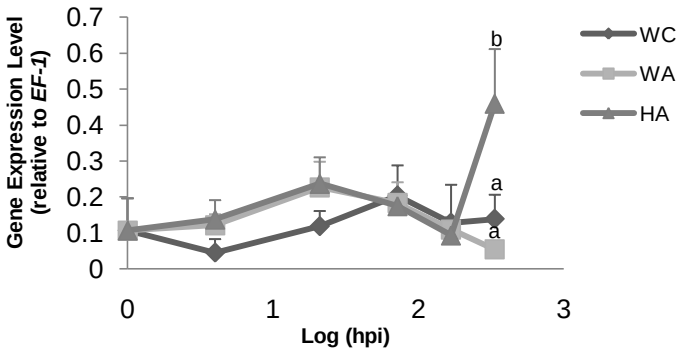


Figure 4.33. Gene expression analysis of *PR-3* in the roots of a susceptible cultivar at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments using Fisher's LSD test.

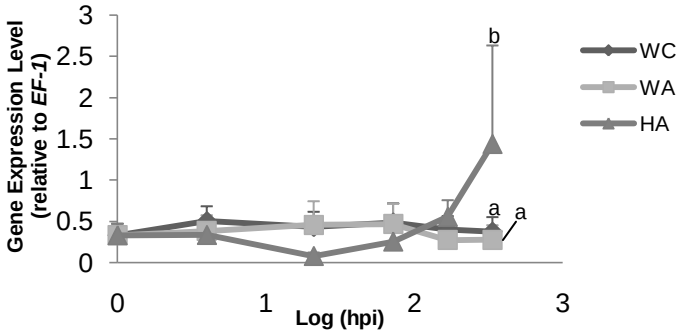


Figure 4.34. Gene expression analysis of *WIN2* in the roots of a susceptible cultivar at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments using Fisher's LSD test.

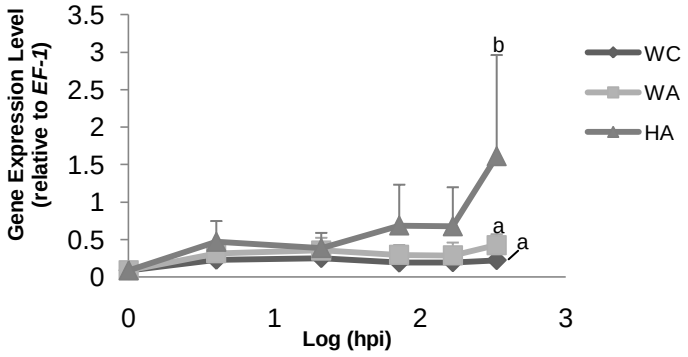


Figure 4.35. Gene expression analysis of *PR-9* in the roots of a susceptible cultivar at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments using Fisher's LSD test.

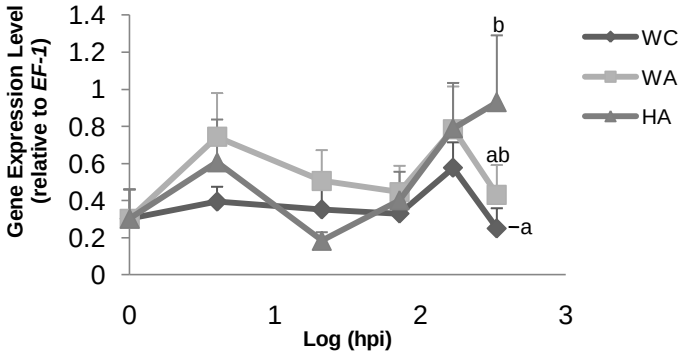


Figure 4.36. Gene expression analysis of *POTLX3* in the roots of a susceptible cultivar at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments using Fisher's LSD test.

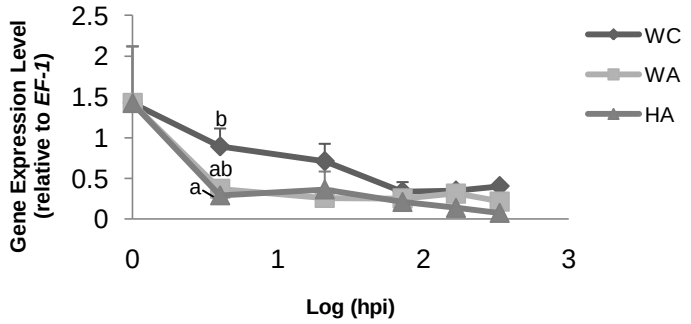


Figure 4.37. Gene expression analysis of *AREB* in the roots of a susceptible cultivar at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments using Fisher's LSD test.

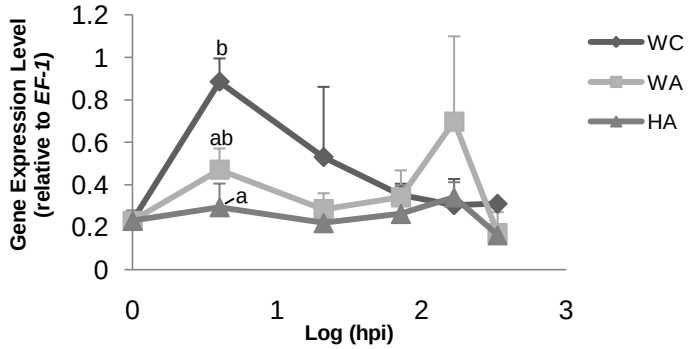


Figure 4.38. Gene expression analysis of *PR-2* in the roots of a susceptible cultivar at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments using Fisher's LSD test.

4.1.2.1.2 Moderately Resistant Cultivar. *ZEP* (ABA-related) was the only gene that showed significant differences between the treatments within the MR cultivar. At 21 hpi the WC treatment showed significantly higher expression of *ZEP* than the HA inoculated treatment (Fig. 4.39). This difference between the treatments was due to decreased expression within the HA treatment (Fig. 4.13).

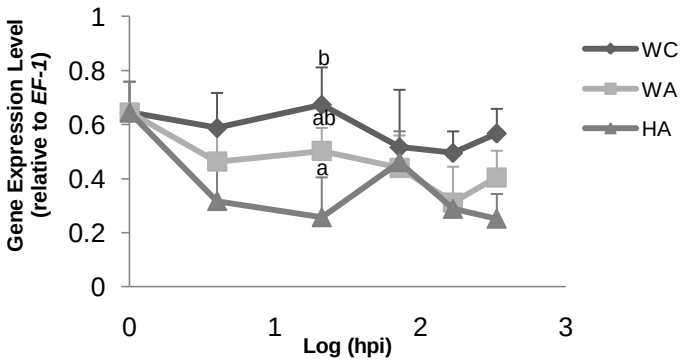


Figure 4.39. Gene expression analysis of *ZEP* in the roots of a moderately resistant cultivar at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments using Fisher's LSD test.

4.1.2.2 Leaves

4.1.2.2.1 Susceptible Cultivar. In the leaves of the susceptible cultivar, there were differences between treatments in regards to expression of some genes. Interestingly, the delayed expression of some genes that was observed in the susceptible roots inoculated with the HA isolate was also observed in the susceptible leaves inoculated with the HA isolate. Three genes showed an increase in expression at 14 dpi with the HA isolate. These genes included one related to the SA pathway (*PR-2*) (Fig. 4.40), one related to the JA/ET-pathway (*PR-3*) (Fig. 4.41), and one related to the JA pathway (*INH1*) (Fig. 4.42). Only one of these genes (*PR-3*) behaved similarly in the susceptible roots (Fig. 4.33). At 14 dpi, *PR-3* showed significantly higher expression in the HA treatment than the other treatments in the leaves (Fig. 4.41). In contrast, at 14 dpi *PR-2* (SA) and *INH1* (JA) showed significantly higher expression in the HA treatment compared to the WA treatment, but similar levels to the WC treatment (Fig. 4.40, Fig. 4.42). This was due to a slight increase in expression in the WC treatment at 14 dpi. The expression of *POTLX3* (JA) was the first example of a difference between treatments that mirrored this common trend. At 14 dpi the expression of *POTLX3* in the WC treatment was significantly higher than those observed in the inoculated treatments (Fig. 4.43). This was due to both a steady increase in expression in the WC treatment and a decrease in expression from 7 to 14 dpi in the inoculated treatments (Fig. 4.25).

One other gene showed differences between the treatments within the susceptible leaves. The expression of *PAL2* (SA) increased at 4 hpi with the

inoculated treatments leading to levels significantly higher than those observed in the WC treatment at this timing (Fig. 4.44).

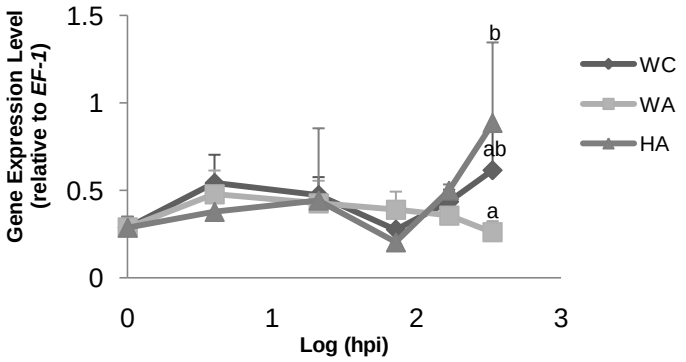


Figure 4.40. Gene expression analysis of *PR-2* in the leaves of a susceptible cultivar at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments using Fisher's LSD test.

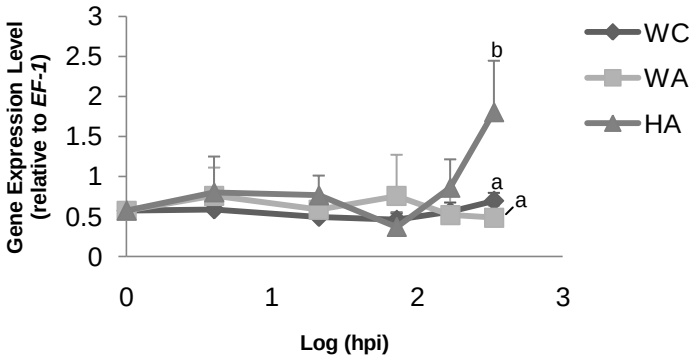


Figure 4.41. Gene expression analysis of *PR-3* in the leaves of a susceptible cultivar at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments using Fisher's LSD test.

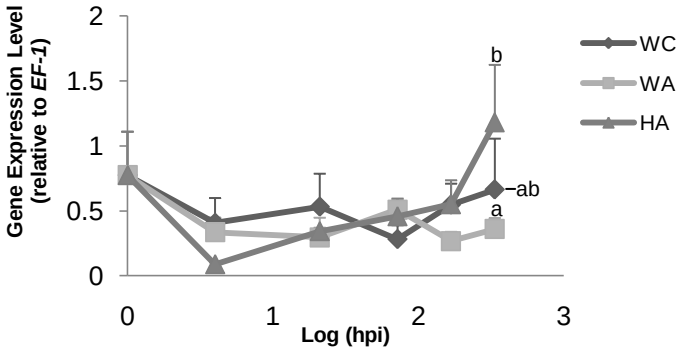


Figure 4.42. Gene expression analysis of *INH1* in the leaves of a susceptible cultivar at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments using Fisher's LSD test.

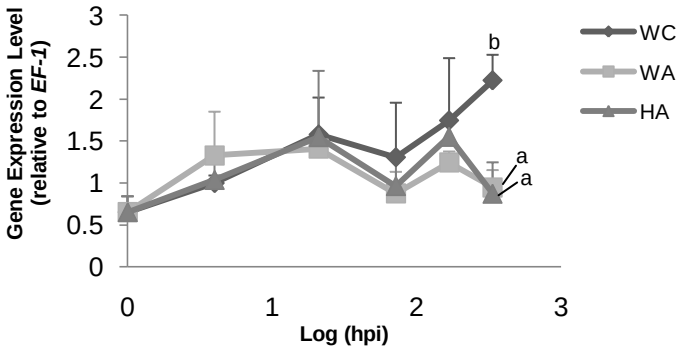


Figure 4.43. Gene expression analysis of *POTLX3* in the leaves of a susceptible cultivar at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments using Fisher's LSD test.

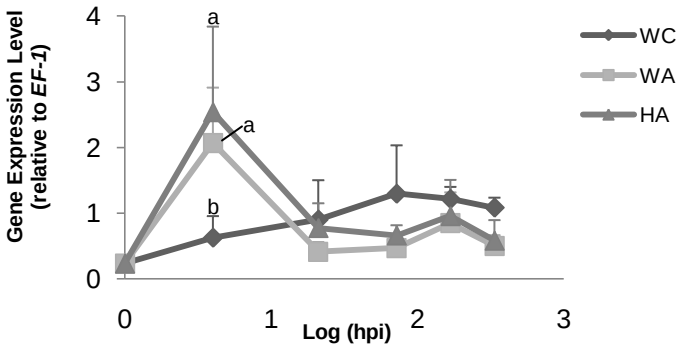


Figure 4.44. Gene expression analysis of *PAL2* in the leaves of a susceptible cultivar at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments using Fisher's LSD test.

4.1.2.2.2 Moderately Resistant Cultivar. The expression of the target genes in the leaves of the MR cultivar did not show many differences between the treatments as only four genes showed significant differences. The expression of *PAL2* (SA) was significantly higher in the HA treatment in comparison with the WC treatment at 7 dpi (Fig. 4.45). In contrast, the expression of *ERF1* (JA/ET) was significantly higher in the WC treatment than the HA treatment at the same time point (Fig. 4.46). The expression of both *WIN2* (JA/ET) and *PR-1* (SA) was significantly higher in the HA treatment than the WA treatment at 14 dpi (Figs. 4.47, 4.48). For *PR-1* and *WIN2*, there was only a decrease in expression in the WA treatment at 14 dpi (Figs. 4.18, 4.22). Interestingly, the expression of *PR-1* in the WA treatment was significantly higher than the WC treatment at 21 hpi (Fig.4.48).

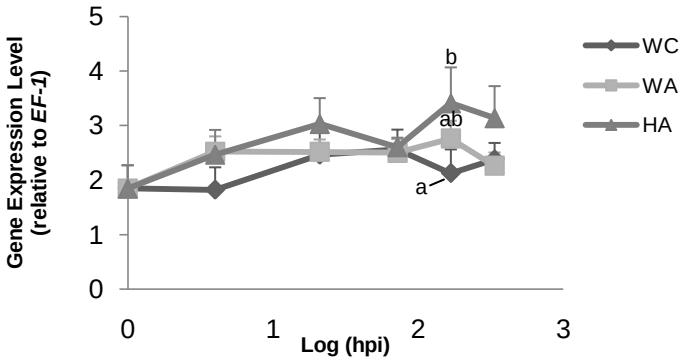


Figure 4.45. Gene expression analysis of *PAL2* in the leaves of a moderately resistant cultivar at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments using Fisher's LSD test.

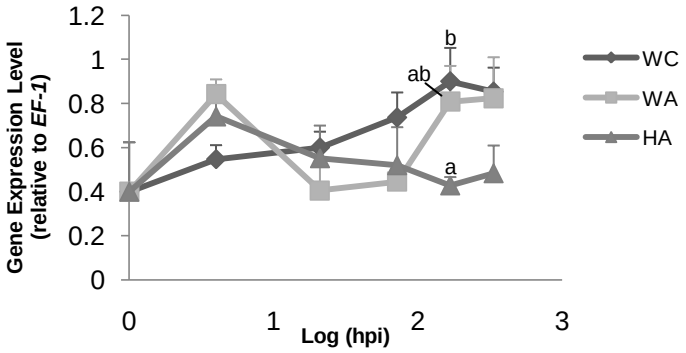


Figure 4.46. Gene expression analysis of *ERF1* in the leaves of a moderately resistant cultivar at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments using Fisher's LSD test.

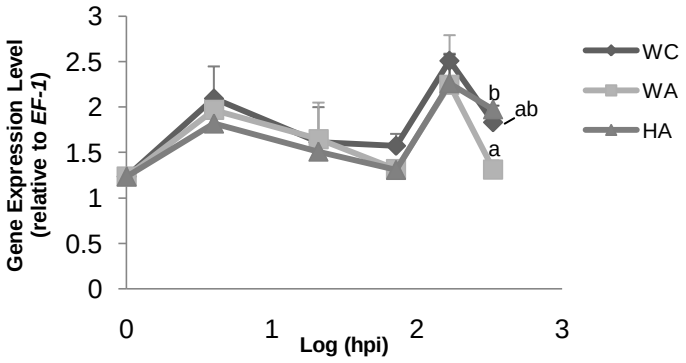


Figure 4.47. Gene expression analysis of *WIN2* in the leaves of a moderately resistant cultivar at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments using Fisher's LSD test.

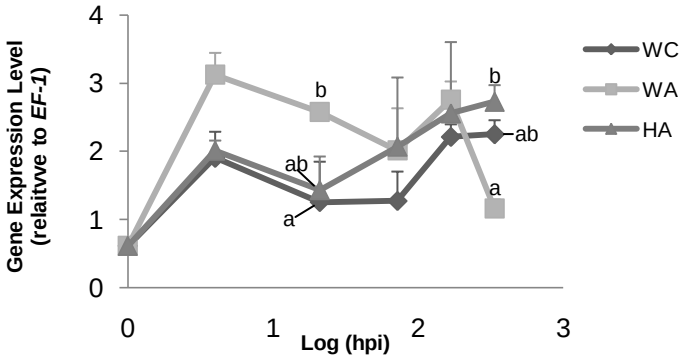


Figure 4.48. Gene expression analysis of *PR-1* in the leaves of a moderately resistant cultivar at 0 hpi (hours post inoculated) to 14 dpi (days post inoculation) under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments using Fisher's LSD test.

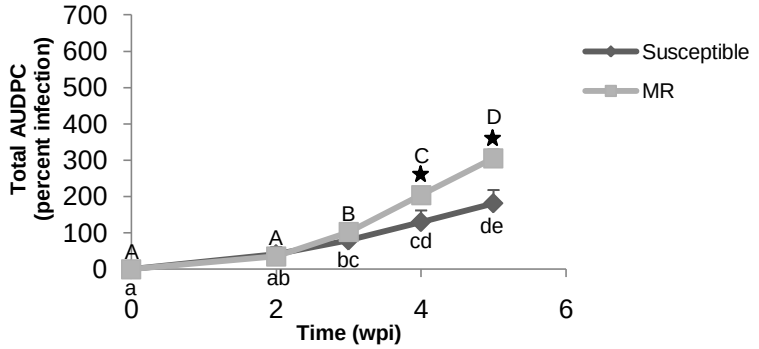
Table 4.3. Summary of genes showing delayed induction in the susceptible cultivar inoculated with the HA isolate.

Organ	Pathway	Genes
Roots	SA	*PR-1*, *PR-5*
Roots	JA/ET	*PR-3*, *WIN2*
Roots	JA	*PR-9*
Leaves	SA	*PR-2*
Leaves	JA/ET	*PR-3*
Leaves	JA	*INH1*

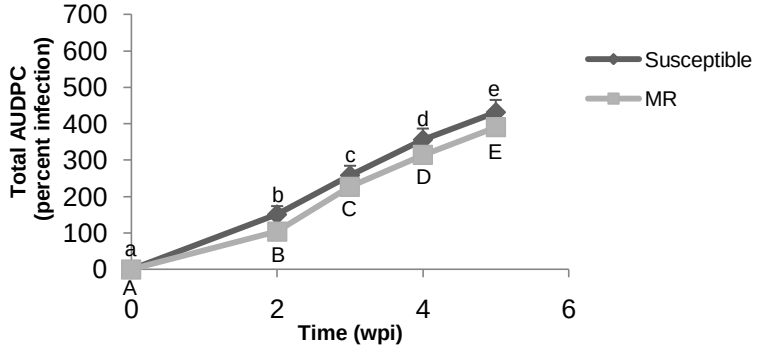
4.2 Disease Evaluation

The disease evaluation ratings showed very few differences between the cultivars. The increase in total AUDPC for percent infection (Fig. 4.49) and disease severity (Fig. 4.50) over time was very similar between the two cultivars in the inoculated treatments. In the WC treatment, the MR cultivar showed significantly higher levels of total AUDPC for both ratings at 4 and 5 wpi (weeks post-inoculation) (Figs. 4.49a, 4.50a). For total AUDPC of percent infection the susceptible cultivar shows significant differences between all three treatments at 4 and 5 wpi (Fig. 4.51). The MR cultivar also shows significant differences at these timings (Fig. 4.52), but the difference between the HA treatment and the WC treatment is not as great in the MR cultivar as it is in the susceptible cultivar. Likewise, the total AUDPC for disease severity shows a large significant difference between the HA and the WC treatment in the susceptible cultivar (Fig. 4.53) and this difference is not as large in the MR cultivar (Fig. 4.54).

a) Wounded Control



b) WA Inoculated



c) HA Inoculated

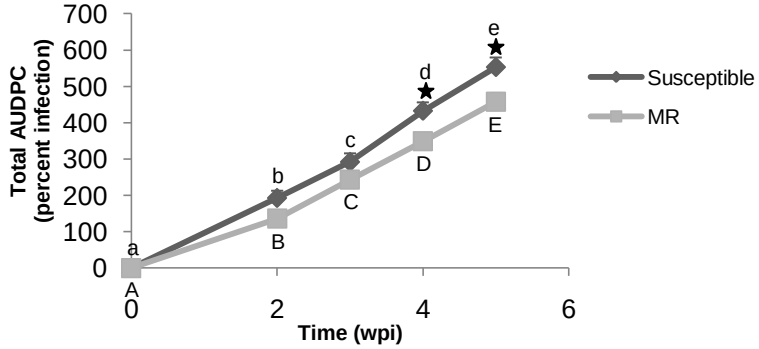


Figure 4.49. Percent of potato leaves showing symptoms of Verticillium wilt measured as Total AUDPC at 2 wpi (weeks post inoculated) to 5 wpi under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★= significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within a single cultivar, lowercase – susceptible, UPPERCASE – MR using Fisher's LSD test).

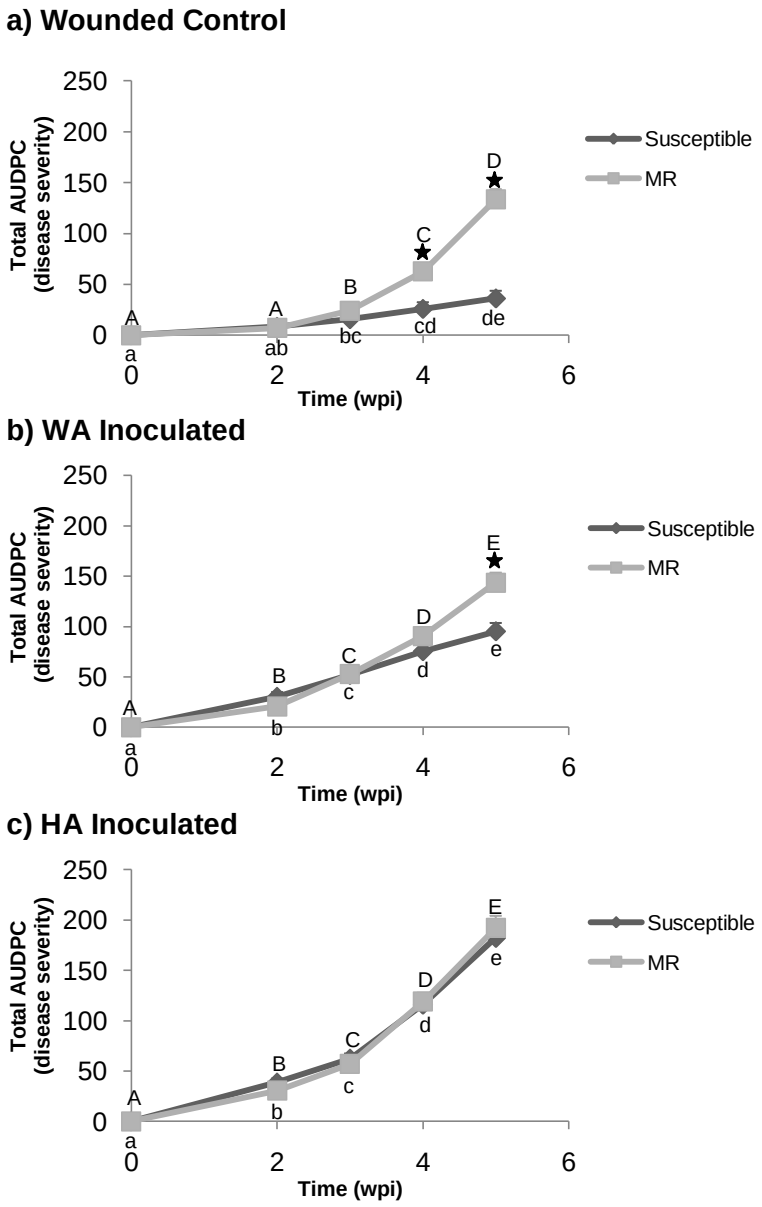


Figure 4.50. Verticillium wilt disease severity in potato leaves measured as Total AUDPC at 2 wpi (weeks post inoculated) to 5 wpi under the following treatments: a) wounded control; b) inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; c) inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. (★= significant difference (P<0.05) between susceptible and moderately resistant cultivar at this timing, letters indicate significant differences (P<0.05) among sampling timings within a single cultivar, lowercase – susceptible, UPPERCASE – MR using Fisher's LSD test).

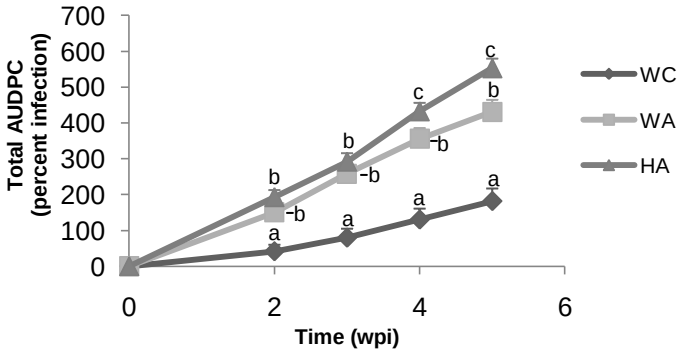


Figure 4.51. Percent of susceptible potato leaves showing Verticillium wilt symptoms as Total AUDPC at 2 wpi (weeks post inoculated) to 5 wpi under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments at a given timing using Fisher's LSD test.

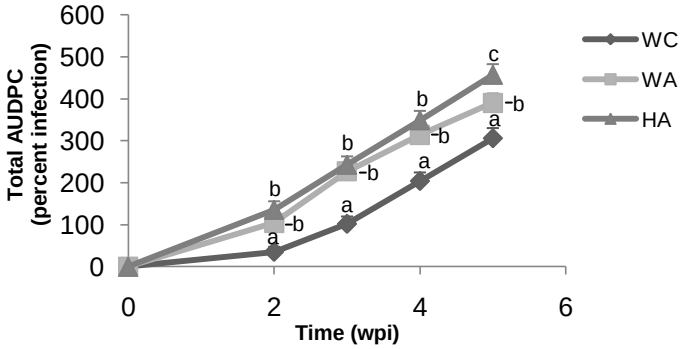


Figure 4.52. Percent of moderately resistant potato leaves showing Verticillium wilt symptoms as Total AUDPC at 2 wpi (weeks post inoculated) to 5 wpi under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments at a given timing using Fisher's LSD test.

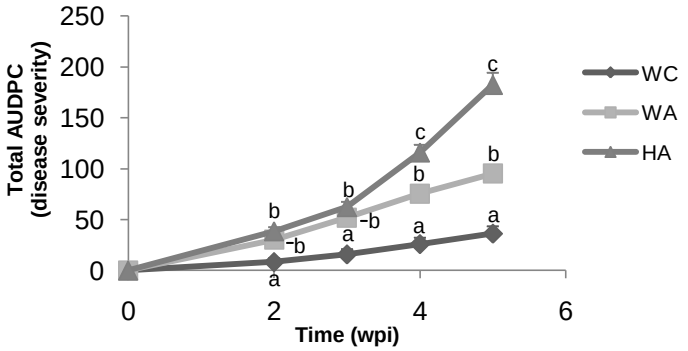


Figure 4.53. Verticillium wilt disease severity of susceptible potato leaves as Total AUDPC at 2 wpi (weeks post inoculated) to 5 wpi under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments at a given timing using Fisher's LSD test.

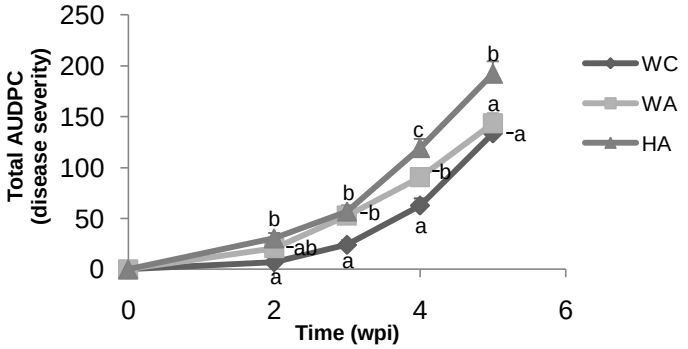


Figure 4.54. Verticillium wilt disease severity of moderately resistant potato leaves as Total AUDPC at 2 wpi (weeks post inoculated) to 5 wpi under the following treatments: wounded control (WC); inoculated with a weakly aggressive (WA) isolate of *Verticillium dahliae*; inoculated with a highly aggressive (HA) isolate of *Verticillium dahliae*. Letters indicate significant differences (P<0.05) between treatments at a given timing using Fisher's LSD test.

5.0 DISCUSSION

Verticillium dahliae is the causal agent of Verticillium wilt in potato and is also a contributing factor to the potato early dying complex. This disease complex can cause yield losses of up to 50% and is considered a limiting factor of potato growth (Barker 2008). Currently there are no commonly accepted or widespread strategies for fully controlling this pathogen. It is known that hormone signaling plays a role in plant defenses against pathogens (as reviewed by Bari & Jones 2009). The role of hormone signaling in the defense of potato against *V. dahliae* has not been fully elucidated. Previous research has indicated that the jasmonic acid (JA) signaling pathway plays a role in the interaction of *V. dahliae* with the Solanaceae family as JA-deficient tomato plants showed decreased plant growth and increased plant mortality after inoculation with *V. dahliae* (Thaler et al. 2004). In addition, researchers have suggested that the hormone signaling pathway involved in a defense response depends on the lifestyle of the pathogen (McDowell & Dangl 2000). The lifestyle of *V. dahliae* is defined somewhere between biotrophic and necrotrophic as the pathogen initially acts as a biotroph and later acts in a necrotrophic fashion. The pathogen initially attacks living tissue and then travels to the xylem. *Verticillium dahliae* infects further tissues after they have wilted and died due to the plugging of the vascular system (Thaler et al. 2004). If the lifestyle of the pathogen plays a role in this interaction, it would indicate that the plant defends itself via the salicylic acid (SA) and jasmonic acid/ethylene (JA/ET) pathways (McDowell & Dangl 2000).

Within this research, genes known to be associated with different signaling pathways were analyzed for expression levels. A potato cultivar moderately resistant (MR) to *V. dahliae* was compared to a susceptible cultivar to determine if any signaling genes contributed to increased resistance or susceptibility. In addition, this research also compared a highly aggressive (HA) isolate of *V. dahliae* to a weakly aggressive (WA) isolate to determine if varying aggressiveness elicited different responses in the host plants. Wounding was used as the control treatment. Many genes involved in pathogen defense signaling are also involved in stress defense signaling (Cheong et al. 2002) and therefore wounding also had an effect on the expression of many genes.

Genes related to the salicylic acid (SA), jasmonic acid/ethylene (JA/ET), and abscisic acid (ABA) pathways showed higher expression in the MR cultivar than the susceptible one, indicating that all three pathways may be involved in defense against *V. dahliae*. In addition, a small number of genes showed the opposite effect suggesting that they may contribute to potato susceptibility to *V. dahliae*. In some genes (*PR-1*, *PR-5*, *PR-3*, *WIN2*, *PR-9*, *PR-2*, *INH1*), increased expression was delayed in the susceptible cultivar inoculated with the HA isolate. This could indicate that these genes are important in defense signaling but that the susceptible cultivar takes longer to respond to the infection, therefore allowing the pathogen to invade. The analysis of these findings is divided into different sections, depending on the expression trend that was observed.

5.1 MR > Susceptible

A number of genes showed increased levels of expression in the MR cultivar versus the susceptible one. In the leaves, these genes include *PAL2* (SA), *PR-9* (JA), *WIN2* (JA/ET), *ZEP* (ABA), and *NXS* (ABA). In the roots, this trend was observed with *PAL1* (SA), *PR-1* (SA), *PR-2* (SA), *PR-3* (JA/ET), *INH1* (JA), and *ZEP* (ABA). It is important to separate genes into those that show higher levels of expression in the MR cultivar in response to stress as opposed to those that have higher background expression levels in the MR cultivar. These two expression trends represent different information regarding their role in potato defense against *V. dahliae*.

5.1.1 Background Levels

In the roots, *PAL1*, *INH1*, *PR-2*, and *ZEP* showed inherently higher expression levels in the MR cultivar than in the susceptible cultivar. *PAL1* is related to the SA pathway and encodes an enzyme involved in SA synthesis (Mauch-Mani & Slusarenko 1996). *PR-2* is also related to the SA pathway (Uknes et al. 1992) and encodes an endo-1,3-β-D-glucanase (Kauffmann et al. 1987). *INH1* encodes a proteinase inhibitor which is part of the PR-6 family of pathogenesis-related proteins (van Loon et al. 1994, Doares et al. 1995) related to the JA pathway. *ZEP* encodes an enzyme involved in ABA synthesis (Marin et al. 1996). The difference between the two cultivars in the expression of these genes was evident before the plant response to the pathogen or to wounding (i.e., at 0 hpi). In addition, this difference was visible in the inoculated treatments as well as the wounded control (WC) plants. Taken together, these data indicate

that the expression of these genes in the MR cultivar is not a specific response to pathogen or wounding stress, but they may still contribute to the defense reaction. This trend was also observed in the leaves with the expression of *PAL2*, *PR-9*, and *ZEP*. *PAL2* represents another gene which encodes an enzyme involved in SA synthesis. *PR-9* encodes a peroxidase related to the JA signaling pathway (van Loon et al. 1994, Curtis et al. 1997). These observations could indicate that the MR cultivar is predisposed to defense against *V. dahliae* due to increased expression levels of the aforementioned genes. It is important to note that these genes are not all related to the same hormone signaling pathway which is an indication that more than one pathway contributes to defense against *V. dahliae* in potato.

Of the genes listed above there were some inconsistencies where the MR cultivar did not show significantly higher expression levels than the susceptible one. In particular, these instances were often observed in plants inoculated with the HA isolate of *V. dahliae*. In general, after inoculation with the HA isolate the expression levels in the MR cultivar would decrease to levels statistically similar to those observed in the susceptible cultivar. Examples of this observation can be seen in the expression profiles for *INH1* and *ZEP* in the roots and *PR-9* in the leaves. The HA isolate may have developed the ability to suppress or inhibit the expression of these defense genes. Hormone signaling pathways are known to negatively interact with one another and it is possible that a pathogen may trigger the activation of one pathway and this activation results in an inhibition of another pathway. Previous studies have indicated that in the *Arabidopsis-Pseudomonas*

syringae interaction the pathogen produces a phytotoxin which activates that JA pathway resulting in an inhibition of SA-related defense responses and increased susceptibility in the plant (Kloek et al. 2001). *INH1* and *PR-9* are related to the JA-pathway and *ZEP* plays a role in the synthesis of ABA. It is tempting to speculate that the JA-pathway is being inhibited by the SA-pathway, which is a well-documented antagonistic interaction (Dewdney et al. 2000, Gupta et al. 2000, Brodersen et al. 2006, Spoel et al. 2007). However this research did not provide any evidence to support this conclusion as the expression of the SA-related genes did not indicate that they are antagonizing the expression of the JA-related genes. Future research should be done in this area through the use of mutagenesis or exogenous application of hormones. Alternatively, the decrease in expression of *INH-1*, *ZEP*, and *PR-9* in the MR cultivar inoculated with the HA isolate may allow the pathogen to escape detection by plant so that the plant does not respond by inducing or maintaining expression of these defense genes. This could be an example of counter-defense by the HA isolate of *V. dahliae* in the constantly evolving potato-*V. dahliae* interaction. Many previous studies have been conducted regarding the co-evolution of plants with the pathogens that attack them (reviewed by Anderson et al. 2010). Plants develop defense strategies and pathogens evolve methods to overcome the plant's defenses. This back-and-forth relationship is ongoing and in this relationship it is possible that the HA isolate of *V. dahliae* is one evolutionary "step" ahead of the WA isolate.

The expression of *PAL2* in the leaves shows a unique response in the susceptible cultivar after inoculation with *V. dahliae*. A sharp increase in

expression was observed at 4 hpi in the inoculated treatments and not observed in the wounded control plants. These increases resulted in expression levels in the susceptible cultivar similar to those observed in the MR cultivar. However, this increased expression was not maintained and returned to levels significantly lower than those observed in the MR cultivar at 21 hpi through to 14 dpi. These results can be compared to the research performed by Joos and Hahlbrock (1992) on the expression of *PAL* genes in potato after inoculation with *Phytophthora infestans*. In their research, a sharp increase in the expression of *PAL1* and *PAL2* was observed immediately following infection with *P. infestans* in the compatible interaction, but this was not observed in the incompatible interaction. In addition, Joos and Hahlbrock (1992) observed an increase in expression of both of these genes immediately following wounding of the potato plant (cv. Datura). These results are comparable to what was seen in the current study and the differences that were observed (re: wounding) may be cultivar-specific responses. It is important to note that in this research the increase in *PAL2* in the susceptible cultivar resulted in expression levels in this cultivar similar to those that were measured in the MR cultivar. Therefore, this gene may play a role in defense and the inability of the susceptible cultivar to maintain increased expression of this gene may contribute to its susceptibility to *V. dahliae*.

5.1.2 Response to Stress

In contrast to the group of genes described in the previous section, this section describes genes that showed significantly higher expression in the MR

cultivar versus the susceptible cultivar post-wounding/inoculation with *Verticillium dahliae*. In other words, this difference between the cultivars was due to the presence of a stress event. This was observed in the roots with *PR-1* and *PR-3*, related to the SA and JA/ET pathway, respectively (Uknes et al. 1992, Hennig et al. 1993, Lorenzo et al. 2003). This was also observed in the leaves with *WIN2* (JA/ET-related) and *NXS* (ABA-related) (Friedrich et al. 1991, Al-Babili et al. 2000). Once again, this trend indicates that more than one pathway may be involved in potato defense against *V. dahliae*.

The difference between the cultivars was not as prominent with the HA isolate in comparison with the other treatments, specifically with the expression of *PR-1* and *PR-3* in the roots and *NXS* in the leaves. The two genes in the roots showed delayed expression increases when inoculated with the HA isolate compared to the WC and WA treatments. This could indicate that the pathogen is suppressing this increase in expression or escaping detection by the plant in order to become more established in the host. The ability of the HA isolate, and not the WA isolate, to do this implies that it may contribute to the aggressiveness of the isolate. In addition, the decreased expression of these genes in the HA treatment further suggests that these genes are candidates for involvement in defense by potato if the pathogen counter-defends against them. In contrast, the expression of *NXS* in the leaves showed immediate increases in the MR cultivar after the inoculation/wounding event in all treatments. However, in the HA treatment the expression of this gene decreased at later timings to levels not significantly different than those measured in the susceptible cultivar. Once

again, this difference in the HA treatment as opposed to the other treatments could be attributed to counter-defense by the HA isolate. It is important to note that no expression of this gene was detected in the susceptible leaves in any of the treatments at any of the timings. This is not to say that this gene is not expressed in the leaves of this cultivar, it is just not expressed at levels detectable by the methods used in this research. Future research should employ techniques that are more sensitive to low levels of gene expression such as real-time PCR (Heid et al. 1996).

The fourth gene that showed significantly higher expression levels in the MR cultivar after wounding/inoculation was unique from the other three genes. After inoculation with *V. dahliae, WIN2* showed an increase in expression in the leaves of the MR cultivar and decrease in expression in the leaves of the susceptible cultivar. It is important to note that the WC treatment also resulted in increased expression of *WIN2* in MR cultivar, but no response was observed in the susceptible cultivar. The fact that this gene was induced in all treatments in the MR cultivar is an indication that it plays a role in the general defense response in this cultivar. *WIN2* is a wound-induced PR-gene that has been determined to play a role in defense interactions (Friedrich et al. 1991). It is interesting that this gene was not induced in the susceptible cultivar since it is a wound-induced gene. This could be due to the fact that the wounding of the potato plants occurred in the roots and this trend was observed in the leaves. A wound response in a plant may be localized to the organ where the wounding occurred (Creelman et al. 1992). Overall, this is an important expression trend to

note as this is the first example of opposite responses to infection with *V. dahliae* by the two cultivars. This differential response could be a contributor to the different levels of resistance between these two cultivars.

In summary, a variety of genes showed higher expression in the MR cultivar than in the susceptible one, indicating their potential role in potato defense against *V. dahliae*. The expression of ten genes followed this trend, of which only *ZEP* was identified in both the leaves and the roots of potato plants. It is not unusual that organ-specific gene expression is observed in response to abiotic and/or biotic stresses (Catoni et al. 2009). In addition, all of the hormone pathways were represented in this trend except for the ET pathway acting alone; although the JA/ET cooperative pathway was represented in both the leaves and the roots. At this point, it appears that a number of hormone signaling pathways are associated with defense, but whether these interactions are synergistic or occur via parallel pathways remains to be determined.

It is also important to note that the genes identified in this section represent hormone synthesis genes and genes that occur further downstream in the hormone signalling pathways (ie. PR-genes). *PAL1* (roots) and *PAL2* (leaves) identified in this section are part of the PAL SA synthesis pathway (Mauch-Mani & Slusarenko 1996) and *NXS* (leaves) and *ZEP* (leaves and roots) are part of the ABA synthesis pathway (Al-Babili et al. 2000, Marin et al. 1996). These results indicate that the effects of the SA and the ABA pathway are possibly due to increased levels of these hormones whereas the effects of the JA and JA/ET pathways are more likely the result of induction of particular genes in these

pathways. It is important to note that the PAL pathway is only one of the pathways that lead to SA synthesis and that the PAL pathway has alternate functions, such as synthesis of phytoalexins (Ward et al. 1989), flavonoids, and lignins (Rohde et al. 2004, Yao et al. 1995). Therefore, although the increased levels of expression of PAL genes may represent a defense response not directly related to SA, research has shown that the PAL pathway is the major contributor to SA synthesis in potato (Coquoz et al. 1998).

5.2 MR = Susceptible

A multitude of genes showed similar expression profiles in both the moderately resistant (MR) and the susceptible cultivars. This trend encompassed a wide range of genes from all of the different pathways and included both synthesis genes and genes occurring further downstream in the pathways. There were more genes that showed similar expression between the two cultivars in the leaves than in the roots. This is not surprising as *V. dahliae* is a soil-borne pathogen and infects via the roots. Therefore, it is expected that defense gene expression in the roots is where most of the differences between the MR and the susceptible cultivar would be observed (Gunawardena & Hawes 2002). This pathogen travels through the plant via the vascular system and the leaves also respond to its presence with defense gene expression, but it appears the leaves respond to a lesser extent than the roots.

Some of the genes that fell into this category showed significant differences in regards to the changes in gene expression over time. For example, expression of *PR-1* and *POTLX3* were induced post-inoculation in the leaves of

the MR cultivar. These two genes are not believed to be directly related as *POTLX3* (lipoxygenase) plays a role in the synthesis of JA (Li et al. 2005) and *PR-1* is a PR-gene related to the SA pathway (Penninckx et al. 1996). The increase in expression of these genes was the most prominent in the WA treatment and was more gradual in the HA treatment. The increase in the WA treatment resulted in significantly higher expression in the MR cultivar versus the susceptible cultivar at 4 hours post-inoculation. Due to the fact that this significant difference was only initially observed in the WA treatment may suggest that the HA isolate manages to suppress the expression of this gene or initially avoid detection by the plant. This initial evasion of defense responses by the HA isolate allows the pathogen to establish its infection before the defense responses are triggered. Taken together, these data suggest that *POTLX3* and *PR-1* play a role in defense against *V. dahliae*, and the ability of the HA isolate of *V. dahliae* to prevent their early induction contributes to its aggressiveness on potato.

In contrast, some of the genes that showed similar expression between the cultivars showed decreases in expression after the wounding/inoculation event. Within the roots, the expression of *ACCS* decreased in both cultivars after wounding/inoculation. Within the leaves, the expression of *INH1* decreased in the susceptible cultivar after wounding/inoculation. *ACCS* encodes an enzyme involved in the synthesis of ethylene (Wang et al. 2002) and *INH1* encodes a proteinase inhibitor in the PR-6 family (van Loon et al. 1994, Doares et al. 1995). It is possible that these genes are not involved in the defense response in potato

against this particular pathogen and that the host is sacrificing expression of these genes in order to mount a defense against the invading pathogen. This type of trade-off is well-documented in the evolutionary history of plants. Plants have a limited amount of resources to allot to growth and development, reproduction, competition, and stress responses. Therefore, higher level plants have developed the plasticity to alter gene expression to what is required in its current environment (Cipollini 2004, Walters & Heil 2007).

5.3 Susceptible > MR

There were a few examples where the susceptible cultivar showed significantly higher expression of a target gene than the MR cultivar. These trends were not consistent over time, but they are of interest as they are showing the opposite of what is expected of defense genes. This type of trend could represent genes that contribute to susceptibility to *V. dahliae* – potentially through inhibiting defense pathways. Three genes were identified as falling into this category: *PR-2* (leaves), *NXS* (roots), and *AREB* (roots & leaves).

In the roots, *NXS* and *AREB* (both ABA-related) showed significantly higher expression in the susceptible cultivar at the time of inoculation/wounding. Therefore, the background expression levels of these genes were significantly higher in the susceptible cultivar. *NXS* encodes neoxanthin synthase which is involved in ABA synthesis (Marin et al. 1996). *AREB* encodes a transcription factor is known to play a role in the ABA-related pathway in response to abiotic stresses (Agarwal & Jha 2010).In a natural infection, the interaction between potato and *V. dahliae* occurs previous to any physical contact. Root exudates of

potato stimulate microsclerotia in the soil to germinate and eventually infect surrounding host plants (Mol 1995). Due to this early interaction, it may be possible that gene expression levels prior to physical contact may play a role in the susceptibility of the plant. Mol (1995) observed that in the presence of a cultivar of potato susceptible to *V. dahliae* ('Element') the highest levels of microsclerotia germination were measured. However, it is important to note that the inoculation method employed in this research did not mimic natural infection. This high level of expression of *NXS* and *AREB* immediately decreased after the wounding/inoculation event although this decrease was more gradual in the WC treatment. If these two genes contribute to the increased susceptibility of the susceptible cultivar then they would have to be related to the early interaction before the first sampling timing as at that point the expression of these genes had already decreased. It is more likely that the expression of these genes is sacrificed in this cultivar in order for the plant to employ alternate defense strategies. This would be similar to what was observed with *INH1* and *ACCS*. Regardless of the cause for this decrease in expression, it appears that *NXS* and *AREB* are not involved in the defense response in either cultivar. This is an indication that the ABA signaling pathway may not be directly involved in defense signaling in the roots.

In the leaves, *PR-2* and *AREB* were identified as having significantly higher expression levels in the susceptible cultivar. In the leaves, the susceptible cultivar had consistently higher expression levels of *AREB* than the MR cultivar, but this difference was not always statistically significant. Once again, this gene

represents an ABA-related gene further suggesting that ABA does not play a large role in the defense response against *V. dahliae* in potato. In contrast, the expression of *PR-2* in the leaves is significantly higher in the susceptible cultivar at some of the timings after the inoculation/wounding event. In this case, the susceptible cultivar appears to respond to the wounding event and the inoculation by the WA isolate. It is possible that this gene is involved in the weaker defense response of the susceptible cultivar, and not induced in the leaves of the MR cultivar. Alternatively, this could represent a method of defense that has been overcome through counter-defense by the pathogen and the different responses by the two cultivars represent differences in their evolutionary response to *V. dahliae*. In the HA treatment, the susceptible cultivar shows significantly higher expression of *PR-2* at 14 dpi. This could be part of a delayed defense response in the susceptible cultivar that will be discussed in the next section.

5.4 Delayed Response in the Susceptible Cultivar

When comparing the different treatments within a single cultivar an interesting trend was observed in the susceptible cultivar 14 dpi with the HA isolate of *V. dahliae*. An increase in the expression of a number of defense genes was observed 14 dpi with the HA isolate in both the susceptible leaves and roots. This led to levels of expression in the HA treatment significantly higher than the WC treatment, WA treatment, or both. In some cases (*PR-1*, *WIN2*, and *INH1*) this increase resulted in the susceptible cultivar expressing the gene at similar levels to the MR cultivar. In other cases (*PR-5*, *PR-9*, *PR-2*, and *PR-3*(leaves))

this increase led to significantly higher expression in the susceptible cultivar. *PR-3* did not fit into either of these trends, as although there was an increase in the expression *PR-3* in the roots of the susceptible cultivar, the roots of the MR cultivar remained to have significantly higher expression levels. It is important to note that the significantly higher expression levels at 14 dpi with the HA isolate of *V. dahliae* was exclusively due to increased expression in the HA treatment and not due to decreased expression levels in the other treatments. This indicates that the susceptible cultivar specifically recognizes the HA isolate of *V. dahliae* and responds to it at approximately 14 dpi. Previous studies have indicated that susceptible cultivars can have a delayed defense reaction to invading pathogens which may contribute to their susceptibility (Kalde et al. 2007, Gayoso et al. 2010).

The expression of *POTLX3* in the susceptible leaves also showed differences between the treatments that are worth noting. The three treatments follow a similar expression trend over time until 14 dpi where an increase in expression in the WC treatment is observed coupled with a decrease of *POTLX3* expression in the inoculated treatments. It would be tempting to conclude that this decrease in *POTLX3* expression directly relates to the increased expression of *PR-2*, *PR-3*, and *INH1* in the HA treatment in the susceptible leaves. This does not seem likely to be the case as *POTLX3* is related to the JA-pathway as are two of the genes (*PR-3* and *INH1*) showing the opposite trend. On the other hand, previous research has indicated that negative feedback loops in hormone signalling occur in some plant-pathogen interactions (Lorenzo et al. 2004, Chini

et al. 2007). It is also of importance that the decrease in *POTLX3* expression is observed in both inoculated treatments whereas the increase in *PR-3* is only observed in the HA treatment.

5.5 Symptom Development

The differences in percent infection and disease severity between the MR and susceptible cultivars of potato were not as prominent as expected. This was mainly due to higher ratings in the MR cultivar than what was anticipated. The MR cultivar, 'Ranger Russet', appears to develop necrotic lesions under greenhouse conditions that are not in response to *V. dahliae* infection. However, these necrotic lesions are difficult to distinguish from Verticillium wilt symptoms and therefore, the symptom development ratings between the cultivars may be biased. The high levels of symptom development in the MR cultivar do not reflect *V. dahliae* infection and the susceptible cultivar is much more affected by the presence of the pathogen. In addition, the difference between the isolates is visible when comparing differences between the treatments in the susceptible cultivar. The HA isolate showed significantly higher percent infection and disease severity than the WA isolate at 7 and 14 dpi. This could indicate that gene expression at these later timings may represent important differences that the plant uses to defend against the varying levels of aggressiveness of *V. dahliae* isolates such as what was discussed in the previous section.

6.0 GENERAL DISCUSSION AND CONCLUSIONS

6.1 Hormone Pathways Involved in Potato-*V. dahliae* Interaction

Verticillium dahliae has the potential to become a devastating pathogen in potato crops around the world. Due to the wide range of host species for *V. dahliae* and overwintering structures that can survive for multiple years many commonly accepted control methods are not effective (Powelson et al. 1993, Powelson & Rowe 1993, Rowe & Powelson 2002, Johnson & Dung 2010). It is known that phytohormones, including salicylic acid (SA), jasmonic acid (JA), and ethylene (ET), play a role in pathogen defense in many plant-pathogen interactions. More recently, abscisic acid (ABA) has been added to this list of defense signaling hormones, although its role in many plant-pathogen interactions remains to be determined.

Previous research has indicated that JA plays a role in the interaction between *V. dahliae* and tomato, a plant closely related to potato (Thaler et al. 2004). Other studies have indicated that the lifestyle of the pathogen is a good predictor as to which hormone signaling pathways are employed in the plant's defense (McDowell & Dangl 2000). *V. dahliae* is not defined solely as a biotroph or as a necrotroph, but falls somewhere between the two indicating that plants may defend against this pathogen via both the SA and JA/ET pathways (McDowell & Dangl 2000). This research supports the latter hypothesis in that more than one pathway seems to be involved in potato's defense against *V. dahliae*. A wide-range of genes showed higher expression in the moderately resistant (MR) cultivar than the susceptible cultivar in both the leaves and roots.

These included genes related to the SA-pathway, the JA/ET-pathway, the JA alone-pathway, and the ABA-pathway.

6.2 Defense Genes May Contribute to Resistance via Two Methods

Two distinct trends were observed with genes that showed differing expression levels between the cultivars. Some of the genes showed higher expression levels in the MR cultivar before the plant had responded to the pathogen or the stress of wounding. In contrast, other genes showed higher expression levels in the MR cultivar in response to a stress. Both of these trends were observed in the roots and the leaves, but the same gene did not always follow the same trend in both organs. The first trend indicates that the MR cultivar may be predisposed to have a higher level of resistance to *V. dahliae* whereas the second trend suggests that the MR cultivar also induces genes in response to stress to add to its resistance level. The latter trend is more commonly accepted as a method of determining genes involved in pathogen defense as the majority of research in plant-pathogen interactions studies genes that are induced post pathogen infection (Ward et al. 1991, Reymond et al. 2000). In contrast, most research regarding a plant's predisposition to pathogen defense involves either systemic acquired resistance or induced systemic resistance where there is a previous event that primes the plant to protect itself against future infections (Ward et al. 1991, Pieterse et al. 1998). There was no previous wounding or pathogen attack in this research and for that reason this phenomenon of a plant's predisposition to resistance needs to be investigated further.

6.3 Delayed Response in Susceptible Cultivar

A number of defense genes showed delayed expression in the susceptible cultivar after inoculation with the highly aggressive isolate of *V*. *dahliae*. This delayed defense response may be a contributor to the susceptibility of this cultivar. The induction of these defense genes only occurred 7 to 14 days after inoculation and interestingly, this induction was only observed with the HA isolate of *V. dahliae*. It is hypothesized that the levels of disease in plants inoculated with the HA isolate are higher than the levels in the plants inoculated with the WA isolate and there may be a threshold disease level that has to be reached before the susceptible cultivar detects the pathogen and responds by inducing defense genes. This delay in a defense response has previously been reported for susceptible cultivars (Kalde et al. 2007, Gayoso et al. 2010). Specifically, Gayoso et al. (2010) observed a delay in *PAL* expression in tomato plants susceptible to *V. dahliae* in comparison with resistant plants. In contrast, in this research *PAL1* and *PAL2* did not follow this trend, but genes potentially related to the same pathway such as *PR-1*, *PR-2*, and *PR-5* showed the delayed response.

The genes involved in the delayed response in the susceptible cultivar inoculated with the HA isolate all represented genes further downstream in the signaling pathways. In other words, there were no synthesis genes detected to have followed this trend. This may indicate that hormone levels are not changing, but specific genes downstream in the pathway are induced as part of the delayed response. Once again this trend represented genes from more than one pathway with the SA-, JA/ET-, and JA-pathway all being represented. These observations

reiterate that more than one signaling pathway appears to play a role in the defense response of potato against *V. dahliae*.

6.4 Conclusions and Future Perspectives

Gaining an understanding of how a plant naturally defends against a pathogen allows researchers to develop more effective control strategies. Very few studies have been conducted regarding the role of hormone signaling in the potato-*Verticillium dahliae* interaction; therefore the purpose of this research was to lay the groundwork for future study in this area. The results of this investigation suggest that both the SA- and JA/ET-pathways play a role in defense by potato against *V. dahliae*. Although a small number of genes related to the ABA pathway were tested there was no clear response of these genes to *V. dahliae* and consequently, the role of ABA is this interaction still remains to be determined.

This research is just a stepping stone in elucidating the interaction between potato and *V. dahliae* in regards hormone defense signaling. The next step in this line of investigating would be to perform real-time PCR to measure gene expression levels under similar conditions as to what were presented here. There are limitations to semi-quantitative PCR based on some of the conditions that must be met. During amplification, the PCR product entered a log phase before reaching a plateau. PCR conditions are optimized for each gene so that the expression levels are measured during this log phase. Both the target gene and the normalization gene (in this case *EF-1α*) should be measured in their log phase. The log phase was targeted for each gene by running a PCR at different

cycle numbers to detect what number of cycles most accurately represents amplification during this phase (Heid et al. 1996). This is an accurate method, but it was measured visually and was therefore subject to human error. In addition, the actual measurement of gene expression was performed through gel electrophoresis analysis. This required post-PCR manipulation which allows for contamination or loss of some of the sample. Real-time PCR allows the user to removes the steps of visual assessments and post-PCR manipulation. Fluorescence is used to measure the levels of the amplified regions of DNA during the PCR reaction (Heid et al. 1996). The computer records the levels of fluorescence and therefore, the levels of the target gene present (Heid et al. 1996). This permits the user to easily and accurately identify the log phase as well as prevents post-PCR manipulation.

Further studies could include mutagenesis, measurement of hormone levels, and exogenous application of hormones. Genes that showed potential for playing a contributing role to potato resistance to *V. dahliae* could be used in mutagenesis studies. Researchers could study whether the silencing or overexpression of these genes has the expected response. Techniques to measure the hormone levels could be used after subjecting plants to wounding or infection by the different isolates of *V. dahliae*. In addition, symptoms of potato plants susceptible to *V. dahliae* could be measured after applying SA, JA, ET, and ABA exogenously to determine if these hormones will trigger a resistant response. The application of the hormones could take place previous to infection, concurrently with the inoculation, or after *V. dahliae* has infected the plants to

measure preventative or curative methods of defense signaling. While most studies following hormone defense signaling look at early responses in the plant this research indicates that delayed responses in the susceptible cultivar could be of interest and future studies should focus on both early and late responses of the plant.

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