THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

Molecular characterization of defense responses of potato to different genotypes of *Phytophthora infestans* (Mont.) de Bary

by

Xiben Wang

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

of

Doctor of Philosophy

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FORWORD

This thesis is written in manuscript style. A general introduction and review of the literatures precedes the four manuscripts that comprise the main part of the thesis. Each manuscript consists of an abstract, introduction, materials and methods, results and discussion. The first manuscript has been published in Physiological and Molecular Plant Pathology (65:157-167) and the second manuscript has been accepted for publication by Environmental and Experimental Botany (in press). The third manuscript has been submitted to Physiological and Molecular Plant Pathology. A general discussion and a list of references cited follow the manuscripts.

ABSTRACT

Late blight, caused by *Phytophthora infestans,* is one of the most devastating plant diseases on potato. In the last two decades, it re-emerged as a serious threat to potato production due to dramatic changes of *P. infestans* population and appearance of new more aggressive genotypes worldwide. To effectively control this disease, it is important to have a better understanding of the responses that potato has developed with US-8 (currently predominant genotype, highly aggressive) versus US-1 (previously predominant genotype, mildly aggressive) of the pathogen. In our study, isolates from *P. infestans* genotypes US-1 and US-8, were included. Defense responses of cultivar Russet Burbank (susceptible) and Kennebec (moderately tolerant) in response to *P. infestans* were studied using three approaches: i) microscopic observations of histological responses related to the hypersensitive reaction (HR) in potato leaf discs inoculated with *P. infestans*; ii) analysis of the expression pattern of defense-related genes encoding 5 pathogenesis-related proteins (*pr-1*, *pr*-2, *pr-3, pr-5* and *pr-9*) as well as genes encoding phenylalanine ammonia-lyase (*pal*) and 3-hydroxy, 3-methylglutaryl CoA reductase (*hmgr*) in three leaf strata and different times after inoculation using northern blot and quantitative SYBR real-time PCR analyses; and iii) studying the accumulation of defense-related secondary metabolites (e.g. sesquiterpene phytoalexin rishitin) in infected potato plants based on thin layer chromatography and HPLC analyses. Our results demonstrated that trailing HR and the expression of seven defense-related genes were induced earlier in tolerant Kennebec than in susceptible Russet burbank in response to *P. infestans* infection. In both

cultivars, trailing HR and the expression of these defense-related genes were induced earlier when potato was inoculated with *P. infestans* US-1 as compared to the US-8 genotype. The expression of these defense-related genes was first induced in local followed by proximal then distal leaflets. Furthermore, induction of *pal* and *hmgr* as well as the synthesis of rishitin were found to be suppressed at the site of infection while such suppression was not observed in the expression of five *pr* genes. Our study demonstrated the quantitative nature of resistance *to P. infestans* in two tested potato cultivars and the outcome of the interaction is affected by both activation and suppression of plant defense responses by *P. infestans*.

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1.0. Introduction

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is one of the most devastating diseases on potato. The disease management is heavily dependant on the application of fungicides, which is expensive and has adverse effects on the environment (Platt & Tai 1998). There is a clear economical and environmental need for new approaches which would lead to a more sustainable management of this disease. The natural or "engineered" host resistance has been considered as a potential alternative capable of replacing at least some of the chemical inputs used to control this disease (Inglis et al. 1999). Up to date, two types of resistance to late blight have been described in potato, race-specific resistance and non race-specific resistance. The race-specific resistance to *P. infestans* conferred by dominant resistance genes (R) has been demonstrated to be short-lived due to the rapid adaptation of *P. infestans* to its hosts (Ballvora et al. 2002). On the contrary, non race-specific, also known as partial resistance, is believed to be more durable and effective against all races of *P. infestans* (Kamoun et al. 1999; Turkensteen 1993).

In the last two decades, the population structure of *Phytophthora infestans* in North America has changed dramatically. The previously predominant US-1 genotype (A1 mating type) has been replaced by more aggressive and fungicide-insensitive populations such as US-8 (A2 mating type) and US-11 (A1 mating type) genotypes (Daayf et al., 2001). The appearance of new genotypes has resulted in a new wave of severe and destructive epidemic and is challenging the durability of resistance in current potato cultivars (Goodwin et al., 1997). Up to date, few studies have thoroughly

investigated interactions between potato and strains from different genotypes of *P. infestans*. Almost none have investigated the defense response of potato newly appeared *P. infestans* genotypes such as US-8 or US-11. A better understanding of the defense mechanisms involved in potato cultivars to different populations of *P. infestans* (previously and currently predominant genotypes) will be beneficial for the utilization of natural host resistance and will provide insights for the development of more effective management of this disease.

The main objective of our study is to characterize the potato defense responses to isolates from different genotypes of *Phytophthora infestans* and to study the activation of defense mechanisms in potato cultivars with different level of resistance to *P. infestans*. For this purpose, two commercial potato cultivars with different levels of resistance to *P. infestans* were selected for this study. Russet Burbank is a widely used cultivar and highly susceptible to late blight while Kennebec is a potato cultivar with moderate tolerance to late blight. *P. infestans* isolates FA-1 and D1901, belonging to US-1 and US-8 genotypes respectively, were used to represent the previously and currently predominant population of *P. infestans*. The timing of the activation of potato defense responses was compared between cultivars and between genotypes.

Hypersensitive response is one of the most common defense responses that often associate with incompatibility (Dangl et al. 1996). Recently, it has been demonstrated that hypersensitive response is associated with both race-specific and non race-specific resistance, which suggests that hypersensitive response plays an important role in the resistance of potato to late blight (Vleeshouwers et al. 2000a). However, no data is

available on the induction of hypersensitive response and its involvement in potato resistance to the new *P. infestans* populations (eg. US-8). Therefore, we will first study some histological responses related to hypersensitive response in potato foliage inoculated with different *P. infestans* isolates using microscopy. The timing and frequency of the induction of hypersensitive response will be compared between cultivar Kennebec and Russet burbank in response to *P. infestans* genotypes US-1 and US-8.

Furthermore, plant disease resistance also involves differential activation of countless defense-related genes. The induction of these defense-related genes is often associated with the level of resistance in the host (Bertini et al. 2003; Graham et al. 2003; van Loon & van Strien 1999; van Loon 1999). However, such a relationship has not been established between potatoes and previously predominant *versus* currently predominant populations of *P. infestans.* Therefore, we will analyze the induction pattern of several key defense-related genes (genes encoding PR proteins and key enzymes involved in the biosynthesis of defense-related secondary metabolites) in the two potato cultivars after inoculation. This will be completed both qualitatively and quantitatively using northern blot and quantitative real time RT-PCR analyses. Furthermore, we will investigate the induction of defense-related genes in different parts of inoculated plants to verify whether the systemic acquired resistance is involved in potato resistance to old and new genotypes of *P. infestans*.

The function of defense-related phenolic compounds in disease resistance has been related to the formation of physical and biochemical barriers against pathogen infection

(Dixon et al. 2003). Phytoalexins also are related to plant disease resistance. In potato, the best characterized phytoalexin is rishitin, a sesquiterpene antimicrobial compound (Lyon 1972; Lyon 1980). It has been previously shown that the biosynthesis of rishitin can be induced/suppressed in potato tubers by treatments with elicitors/suppressors from *P. infestans* (Preisig & Kuc 1985; Andreu et al. 1999). However it is not clear yet whether the production of rishitin is related to the resistance of potato to the new genotypes of *P. infestans*. Therefore, we will also study the accumulation of defense-related secondary metabolites (e.g. rishitin) in potato leaves inoculated with different genotypes of *P. infestans* and compare such accumulation with the expression of related genes such as *pal* and *hmgr*.

2.0 Literature review

2.1 Potato (*Solanum tuberosum L.*), host plant

Potato (*Solanum tuberosum L.*) is an annual plant belonging to the genus *Solanum* in the family *Solanaceae*. It is a leafy plant with underground tubers which are developed from the swelling of the subapical portion of the stolon with reserve materials.

The basic chromosome number of tuber-bearing *Solanum spp.* is x=12 and diploid, tetraploid and hexaploid exist in the genus. Most potato cultivars are highly heterozygous tetraploids (Li et al. 1998).

2.1.1 History of potato

The potato originated in the highlands of South America and had been grown in high flat valleys of Columbia, Chile and the North of Argentina before the time of European exploration (Dodds 1962). The European history of potato cultivation began with the introduction of tetraploid cultivar, Andigena, into Spain around 1570 while potato was not present in North America until 1613 (Correll 1962).

2.1.2 Economical importance of potato

Today, potato is the fourth largest grown crop worldwide, with the annual production approaching 300 million tons. The Canadian potato acreage stands at 434,400 acres and produces 4.6 million tons of potato annually (Statistic Canada, 2003). Manitoba is the second largest potato producer in Canada.

Over 75 potato varieties are grown across Canada. Most of them are susceptible to late blight. The variety Russet burbank, which occupies over 50 percent of the acreage

under potato in Canada (Statistic Canada, 2003), is the most common cultivar used for processing due to its favorable marketing and processing qualities. However this cultivar is highly susceptible to late blight. Kennebec is a high-yielding, oblong, white-skinned variety with relative tolerance to late blight.

2.1.3 Important diseases of potato

Potato is the host of multiple diseases including bacterial, fungal, viral, and phytoplasmal origins. Some of the common diseases on potato are listed in Table 2.1.

Table 2.1 Common diseases on potato

	Disease	Pathogen
Bacterial	Bacterial wilt	*Pseudomonas solanacearum* (Smith 1896)
	Blackleg	*Erwinia. carotovora* subsp. *carotovora* (Jones 1901)
	Pink eye	*Pseudomonas fluorescens* (Migula 1895)
	Ring rot	*Clavibacter michiganensis* subsp. *sepedonicus*
	Common scab	(Spieckermann 1914)
		Streptomyces acidiscabies (Lambert and Loria 1989)
Fungal	Black dot	*Colletotrichum coccodes* (Wallr.) S. J. Hughes
	Early blight	*Alternaria solani* (E&M) Jones & Grout
	Fusarium wilt	*Fusarium* spp.
	Late blight	*Phytophthora infestans* (Mont.) de Bary
	Pink rot	*Phytophthora* spp.
	Rhizoctonia	*Rhizoctonia solani* Kühn
	canker	*Helminthosporium solani* Dur. & Mont
	Silver scurf	*Verticillium albo-atrum* Reinke & Berthier
	Verticillium wilt	*V. dahliae* Kleb.
Parasitical (Nematode)	Lesion nematode	*Pratylenchus* spp.
	Potato rot	*Ditylenchus destructor* Thorne
	nematode	*Meloidogyne* spp.
	Root knot	*Belonolaimus longicaudatus* Rau
	nematode	
	Sting nematode	
Viral	Potato Mosaic	genus *Carlavirus, Potato latent virus*
	virus	genus *Potexvirus, Potato virus X*
	Potato latent virus	genus *Potyvirus, Potato virus Y*
	Potato virus X	genus *Sobemovirus, Sowbane mosaic virus*

	Potato virus Y	
Phytoplasmal	Aster yellows	Aster yellows group of phytoplasmas
	Witches'-broom	Witches' broom phytoplasma

2.1.4 Potato late blight

Late blight is the most destructive disease on potatoes currently. It causes dark brown lesions on almost every part of potato plants and results in severe defoliations, tuber rots and yield losses on susceptible crops (Hooker 1986). The main characteristics of late blight are summarized in Table 2.2.

Table 2.2 Main characteristics of potato late blight pathogen, *P. infestans*

	P. infestans
Host	Potato, tomato and related species
Tissue	Leaves, stems and tubers
Penetration of plant tissue	Intracellular penetration of an epidermal cell through appressoria formation
Infection process	Early biotrophic phase with haustora followed by saprophytic growth
Sporangia	Deciduous, dislodge easily form the sporangiophores
Main habitat	Aerial plant parts
Sexual behavior	Self-sterile or heterothallic
Genome size	250 Mb
Haploid chromosome count	8-10

Late blight is notorious for the Great Irish famine (1845-1847) which led to a mass starvation in history. Today, it has become a major threat to potato industries worldwide following a series of severe late blight epidemics that coincided with the migration of aggressive A2 mating type isolates into Europe and North America in the late 1980s (Fry & Goodwin 1997b; Goodwin et al. 1998). The worldwide losses in the

potato production due to late blight and its management are estimated to be more than US$5 billion annually (Duncan 1999).

2.2 *Phytophthora infestans,* the causal agent of late blight

2.2.1 Taxonomy

Phytophthora infestans was first placed by DeBary (1876) in the genus *Phytophthora* based on the features of its sporangia (typical lemon-shape, with a papilla at sporangia tip). Currently, *P. infestans* is placed in Kingdom *Chromista*, Phylum *Oomycota*, Order *Peronosporales*, Family *Peronosporaceae*, and Genus *Phytophthora* (Kamoun 2003).

2.2.2 Life cycle

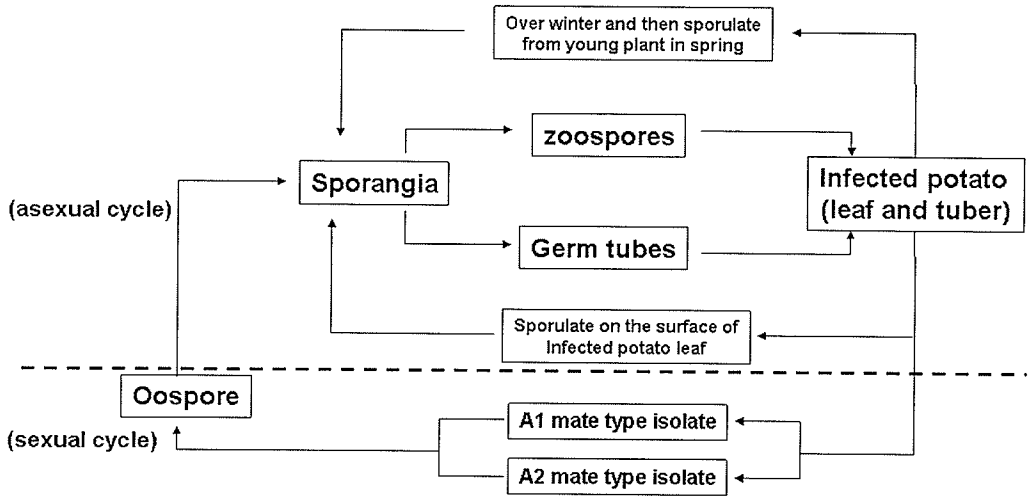
The life cycle of *P. infestans* is comprised of different stages: sexual and asexual reproduction, propagule dispersal, spore germination, host penetration, biotrophic and necrotrophic phases of infection (Avrova et al. 2003). In every stage, specialized cell types are produced and cell differentiation requires the up and down regulation of different genes which will be mentioned later (Figure 2.1).

2.2.3 Unique biological features of *P. infestans*

Phytophthora infestans produces microscopic, asexual sporangia, which are hyaline, lemon-shaped and formed on specialized sporangiophores. The branched sporangiophore with swellings at the points where sporangia are attached is one of the distinctive characteristics for the classification of *P. infestans* (Erwin et al. 1983).

Phytophthora infestans is sterol- and thiamine-auxotroph. It requires β-hydroxyl sterol for the sporulation and thiamine for its growth (Erwin et al. 1983).

Figure 2.1 The disease cycle of *P. infestans*



Phytophthora infestans is a heterothallic microorganism with two mating types (A1 and A2). The presence of both mating types is required for the production of sexual spores (oospores), while asexual sporangia are produced by each single mating type.

Unlike true fungi (Eumycota), cell walls of *P. infestans* (Oomycota) are mainly composed of β-1,3-glucan polymers and cellulose with very little chitin (Erwin & Ribeiro 1996). However, recently it has been suggested that chitins might be also important for the growth of *P. infestans* due to the fact that some oomycetes posses chitin synthase genes (Mort-Bontemps et al. 1997) and chitin synthase inhibitors have negative effects on the development of oomycete cell walls (Bulone et al. 1992).

2.2.4 Pathogenesis of *P. infestans*

Up to date, studies on *P. infestans* pathogenesis have been focused on spore germination, zoospore formation, spore encystment and appressoria formation. Meanwhile, attention has also been paid to the isolation and identification of genes in *P. infestans* that are up or down regulated during early or late stages of infection *in planta.*

P. infestans sporangia can produce either germ tubes (direct germination) or zoospores (indirect germination) (Erwin et al. 1983). The formation of zoospores in *P. infestans* is achieved through a process called sporangial cleavage in which the cytokinesis is initiated with a cold shock that eventually compartmentalizes single nuclei within each zoospore (Tyler 2002). This process is under the influence of some divalent cations, in particular Ca^{2+}, and a protein kinase with similarity to a calmodulin-regulated protein kinase (Judelson & Roberts 2002). Furthermore, genes encoding G-protein α and β subunits were recently isolated from *P. infestans* and found to play an important role in the differentiation of sporangia. Both Ca^{2+} and G-proteins are also found to be important for the spore encystment and the appressoria formation (Laxalt et al. 2002).

Considerable efforts have been made to identify genes in *P. infestans* related to its pathogenesis which are up or down regulated in either early stages or late stages of infections using methods of differential display or the subtractive hybridization (Gornhardt et al. 2000; Kramer et al. 1997; Avrova et al. 1999; Avrova et al. 2003; Birch et al. 1999; Klooster et al. 2000; Latijnhouwers et al. 2002; Marshall et al. 2001; Tani et al. 2005; Tani et al. 2004, Pieterse et al. 1994a; Pieterse et al. 1995; Kamoun et al. 1997b; Pieterse et al. 1994b; van West et al. 1998).

Rrecent progress made in large scale sequencing projects for *P. infestans* genome (*Phytophthora* Genome Consortium: http://www.pfgd.org and Syngenta *Phytophthora* Consortium: https://xgi.ncgr.org/spc), some genes related to its pathogenesis have been recently identified by scanning sequences in databases such as genes encoding

phytotoxin, extra-cellular effectors or avirulence factors (Liu et al. 2005; Torto et al. 2003; Whisson et al. 2001).

2.2.5 Genome of *P. infestans*

Phytophthora infestans has 8-10 chromosomes (Sansome et al. 1975) and its total genome size is estimated to be approximately 237 MB (Tooley & Therrien 1987). Over 50% of its genome is covered with repeated DNA sequences and genes related to pathogenesis are found to be clustered on several 'gene islands' (Judelson & Randall 1998).

2.2.6 Diversity of *P. infestans* populations

Phytophthora infestans is commonly known to exhibit vast phenotypic and genetic variations (Goodwin 1997). Meanwhile, increases in the complexity of *P. infestans* population have been recently reported in many countries through out the world (Daayf & Platt 2000; Day et al. 2004; Goodwin et al. 1998; Cooke et al. 2003; Day et al. 2004). The situation is likely to get worse due to the migration of A2 mating type outside Mexico which makes sexual recombination possible.

Although the genetic basis of the diversity in *P. infestans* population is still not clear, it has been proposed that it might have resulted from transposable elements, gene conversions, mitotic recombinations and/or dispensable chromosomes (Kamoun 2003).

Since a clear understanding of *P. infestans* population structure is essential for the development of an effective control strategy, the population genetics of *P. infestans* has been an area under extensive study. With the vast diversity of *P. infestans* population, a single marker system alone is apparently not adequate enough (Cooke & Lees 2004).

Therefore, multiple marker systems have been developed which are summarized in Table 2.3.

Table 2.3 Phenotypic and genetic markers used for classification of *P. infestans* populations.

	Description	Reference
Phenotypic		
Mating type	Based on the pairing with tester A1 or A2 strains and screening for oospore production	(Brurberg et al. 1999) (Cooke et al. 2003)
Virulence	Determined by inoculating a series of 11 differential potato cultivar carrying specific R gene	(Malcolmson & Black 1966) (Hermansen et al. 2000)
Fungicide resistance	Agar or in vivo test resistance against well documented phenylamide fungicide (Metalaxyl)	(Daayf & Platt 1999) (Daayf & Platt 2000)
Others	Aggressiveness, polidy levels and antibiotic resistance and temperature response	(Kato et al. 1997) (Therrien 1989) (Shattock & Shaw 1975) (Mizubuti & Fry 1998) (Daayf & Platt 2003)
Genomic		
Isozyme	Glucosephosphate isomerase and peptidase are wide used and codominant markers	(Daayf & Platt 2000) (Daayf et al. 2001)
mtDNA haplotype	Based on RFLP finger printing of mitochondrial genome of *P. infestans*	(Griffith & Shaw 1998) (Gavino & Fry 2002) (May & Ristaino 2004)
Random amplified polymorphism DNA (RAPD)	DNA fingerprinting methods based on PCR with short random primers	(Whisson et al. 1995) (Mahuku et al. 2000) (Daayf et al. 2001)
Amplified Fragment Length Polymorphism (AFLP)	DNA fingerprinting method involves restriction enzyme digestion and PCR amplification of digested DNA fragments.	(Knapova & Gisi 2002b; Cooke et al. 2003) (Flier et al. 2003)
Simple sequence repeat (SSR)	Microsatellite DNA based markers in which motifs of 1-6 bases occurs in tandem repeats	(Knapova & Gisi 2002a)

2.2.6.1 *P. infestans* genotypes

Currently, "the genotype" is the most widely used system in the classification of *P. infestans* population. It has been used widely to analyze the movement and displacement of *P. infestans* populations worldwide (Fry et al. 1993).

"The genotype" is mainly established on the information collected from mating types (Mating type A1/2), random fragment length polymorphism probe RG57 and isozyme analysis (glucose-6-phosphate isomerase). The naming of the new genotype obeys the rule of a country code followed by a unique number (Forbes et al. 1998). For example, 17 *P. infestans* genotypes have been described in the United State and Canada and designated as US-1 to US-17 (Goodwin et al. 1998).

2.2.6.2 Shifting in the structure of *P. infestans* population

Prior to 1980, the world population of *P. infestans* was long dominated by a single US-1 genotype belonging to A1 mating type while isolates belonging to A2 mating type were confined to Mexico (Fry et al. 1993). The appearance of the single genotype around the world was the result of the original migration of *P. infestans* out of Mexico (Goodwin & Lee 1993).

In 1984, A2 mating type isolates were first discovered in Europe and subsequently worldwide including in US and Canada (Fry & Goodwin 1997c). Since then, various new genotypes belonging to either A1 or A2 mating types have been identified in the United States or Canada (Daayf & Platt 1999; Daayf & Platt 2000; Daayf et al. 2001; Goodwin 1997; Peters et al. 2001; Peters et al. 1999b; Goodwin et al. 1998).

In the survey of *P. infestans* population in the United States and Canada from 1994-1996, it was shown that previously dominant US-1 genotype only made up 8% or

less of total samples analyzed (Goodwin et al. 1998). From 1996-1998, *P. infestans* population in Canadian provinces was found to be mainly comprised of US-8 and US-11 genotypes. Genotypes US-7, US-10, US-14 and US-17 were also found in the survey (1996-1998) however they were less common comparatively (Daayf & Platt 1999; Daayf & Platt 2000; Daayf et al. 2001).

2.2.6.3 Problems caused by the shifting of *P. infestans* populations

With the rapid change of *P. infestans* population and the occurrence of new genotypes, late blight incidence and severity have increased in most potato-growing areas worldwide (Goodwin & Fry 1994). Meanwhile, studies on the resistance of *P. infestans* to fungicide metalaxyl have shown that the new genotypes (e.g. US-8) are more resistant than old US-1 genotype (Daayf et al. 2000). Therefore the shifting of *P. infestans* population structure from less to more aggressive *P. infestans* genotype has posed a severe threat to the potato production worldwide. Meanwhile, the occurrence of new A1 and A2 populations together (Daayf & Platt 1999; Daayf & Platt 2000; Daayf et al. 2001; Peters et al. 1998; Peters et al. 1999a) also constitute a threat to potato industries because they may be indicative of the potential sexual reproduction capable of leading to more aggressive genotypes (Goodwin & Fry 1994).

2.3 Late blight resistance

2.3.1 Definitions

Resistance to late blight can be classified as either non host-specific or host-specific resistance (Kamoun 2001). Non host-specific resistance describes resistance to late blight in plant species which are not natural hosts of *P. infestans,* for

example, *Arabidopsis, Nicotiana* and parsley, while host-specific resistance is used to define resistance to late blight in its natural hosts such as potato and tomato. Within host-specific resistance, resistance types can be further divided into qualitative (monogenic) and quantitative (polygenic) resistance (Thurston 1971).

In addition, based on Flor's gene-for-gene concept, late blight resistance in potato can be also divided into non race-specific and race-specific resistance (Tonon et al. 1998).

Phytophthora infestans is a semi biotrophic microorganism. The interaction between potato and *P. infestans* is fully biotrophic in early stages of the interaction (at the tip of grown hyphae) and there is no visible defense response in this stage (Coffey & Wilson 1983).

The term "compatible interaction" is often used to describe the interaction in which no recognition between the host and the pathogen occurs and has been used to describe the interaction during the biotrophic stage of *P. infestans* infection. Accordingly, the "incompatible interaction" represents the interaction in late stages of the infection where defense responses occur at all resistance levels, including non host- and host-specific resistance, qualitative and quantitative resistance, race-specific and non race-specific resistance (van West & Vleeshouwers 2004).

2.3.2 Non host-specific resistance to late blight

Studies on the non host-specific resistance to late blight can provide valuable information about the pathogenicity of *P. infestans*, the host specialization and defense mechanisms against *P. infestans*. Three model systems have been used including

Arabidopsis (Huitema et al. 2003), *Nicotiana* (Huitema et al. 2005; Kamoun et al. 1998) and parsley (Gus-Mayer et al. 1998). The studies have been focusing mainly on two aspects: hypersensitive reactions and elicitins.

Hypersensitive reaction has been observed in all forms of non host-specific resistance to late blight in which hypersensitive reaction is induced quickly and limited to only one or two cells. The growth of *P. infestans* hyphae in the plant is often completely blocked after the occurrence of hypersensitive reaction (Kamoun 2001; Kamoun et al. 1999; Vleeshouwers et al. 2000a).

Elicitins are a family of highly conserved extracellular proteins that are secreted by all *Phytophthora* and several *Phythium* species (Kamoun et al. 1993; Nespoulous et al. 1999; Mouton-Perronnet et al. 1995). They are capable of inducing defense responses, including hypersensitive reaction, on a limited number of plant species which are not natural hosts of *P. infestans*, specifically *Nicotiana* species, radish and turnips (Kamoun et al. 1993; Kamoun et al. 1997a; Kamoun 2003; Mouton-Perronnet et al. 1995; Kamoun et al. 1998).

To date, elicitins have been proposed to be the determinant responsible for the avirulence of *P. infestans* on tobacco (Kamoun 2003). The most conclusive evidence supporting this hypothesis came from the functional analysis of *P. infestans* elicitin gene *inf1*. *P. infestans* mutant deficient in the production of INF1 elicitin showed increased virulence on tobacco (Kamoun et al. 1998).

2.3.3 Host-specific resistance to late blight

Late blight resistance in potato can be generally classified as either qualitative or quantitative resistance (Thurston 1971).

2.3.3.1 Qualitative resistance to late blight

The qualitative resistance, usually also referred to as race-specific resistance, is often conferred by a "*R*" gene that is monogenic, dominant and qualitatively inherited (Wastie 1991). It acts upon the recognition between products of the dominant resistance gene (*R*) and corresponding avirulence gene (*avr*), which is often connected to HR in the cells invaded by the pathogen and prevents any further progression of the disease (Nimchuk et al. 2003).

R genes were first introgressed from wild potato species, mainly *S. demissum.* To date, 11 race-specific *R* genes have been described (Brouwer et al. 2004) in potato.

The R gene-mediated resistance was initially successful against late blight. However it was soon discovered that these *R* genes only provided short-lived resistance in the field, as new races of *P. infestans* rapidly overcame this type of resistance (Fry & Goodwin 1997c).

Among 11 race-specific *R* genes described from *S. demissum,* locations of 5 of them have been identified on potato chromosomes. The *R1* locus is identified on potato chromosome V by the restriction fragment length polymorphism (RFLP) mapping (Leonards-Schippers et al. 1992) while *R2* locus is mapped to chromosome IV using amplified fragment length polymorphism (AFLP) markers (Li et al. 1998). *R3, R6* and *R7* were found to be organized in a cluster in the distal segment of chromosome XI

anchored by AFLP marker *TG105* (EL-Kharbotly et al. 1994; EL-Kharbotly et al. 1996).

The cloning of potato *R* genes has lagged behind due to the complexity of the potato genome. So far the *R1* and *R3a* genes have been cloned (Ballvora et al. 2002). R1 contains a conserved nucleotide binding domain (NBS), a leucine-rich repeat domain (LRR) and a leucine zipper motif. Furthermore, it was recently found that *R3* locus was comprised of two genes (*R3a* and *R3b*) with different specificities which all contributed to the original *R3*-mediated resistance (Huang et al. 2004). *R3a* has been cloned using a comparative genomic approach (Huang et al. 2005). R3a encodes a presumed cytoplasmic coiled-coil nucleotide binding sites leucine rich proteins and is constitutively expressed. R3a bears only limited similarity to R1.

2.3.3.2 Quantitative resistance to late blight

The disastrous turnout of *S. demissum*-based, *R* gene breeding led to a stagnation in the late blight resistance breeding. Therefore new breeding strategies have been investigated. One of the new strategies is the utilization of the quantitative resistance pool (non race-specific or polygenic resistance) in wild potato species (Thurston 1971).

The quantitative resistance tends to be polygenic and quantitatively inherited. It does not completely prevent the pathogen infection but slows down the development of the pathogen at infection sites (Wastie 1991).

Although the quantitative resistance is believed to be more durable than the race-specific resistance (Colon et al. 1995), this approach suffers from technical difficulties in the breeding process due to the polygenic nature of such resistance

(Wastie 1991). Moreover, assessing quantitative resistance can be difficult since environmental, developmental, and physiological factors can all influence the outcome of resistance, especially the well known correlation between late blight resistance and potato late maturity (Brouwer et al. 2004).

To date, several potato cultivars with quantitative resistance to late blight have been bred and provided a moderate level of resistance over many years of exposure to *P. infestans* in the field (Colon et al. 1995; Turkensteen 1993).

The genetic basis of quantitative resistance to late blight has been mainly studied by mapping of quantitative trait loci (QTL) based on molecular and phenotypic markers. To date, QTL for LB have been mapped in several diploid (Collins et al. 1999; Oberhagemann et al. 1999; Sandbrink et al. 2000; Visker et al. 2003) and tetraploid (Meyer et al. 1998; Bormann et al. 2004) potato populations.

Factors controlling quantitative resistance to late blight have been identified on almost every potato chromosome. Potato chromosomes III, V and VI are found to harbor some of the most promising QTL for marker-assisted breeding (Gebhardt & Valkonen 2001). The most persistent and prominent QTL for LB is located on chromosome V in a map segment tagged by random fragment length polymorphism markers GP21 and GP179 (EL-Kharbotly et al. 1994; Simko 2002; Oberhagemann et al. 1999).

2.3.3.3 Linkages between qualitative (*R*) and quantitative (QTL) gene loci

A QTL is positioned on the genetic map with less precision than a single gene. The genetic distance covered by a QTL can be influenced by the size effect. Integrations of

QTL for resistance to late blight have revealed linkages between QTL and different R genes. Several hotspots containing both R genes and QTL to different pathogens have been identified on potato chromosomes V, XI and XII (Gebhardt & Valkonen 2001).

For example, *R1* is located in a hotspot on chromosome V which also harbors a QTL for foliage late blight resistance (Leonards-Schippers et al. 1994). *R2* is positioned in a region which contains a major QTL for late blight (Meyer et al. 1998). Furthermore, the gene cluster harboring *R3, R6* and *R7* is found closely linked to a number of QTL to several pathogens (Grube et al. 2000). Beside the clustering of dominant race-specific R genes with QTL, similar linkages have been also found between QTL and a number of resistance gene-like (RGL) (Gebhardt & Valkonen 2001) and genes encoding pathogenesis-related (PR) proteins (Leonards-Schippers et al. 1994; Trognitz et al. 2002).

The linkages between QTL and race-specific *R* genes, RGL or PR genes possibly resulted from the local duplication within common ancestors, followed by functional diversification (Ellis et al. 2000; Gebhardt & Valkonen 2001). Furthermore, it might also indicate that race-specific *R* genes, RGL, and PR genes could be also candidates for quantitative resistance to LB.

2.4 Molecular aspects of the interaction between potato and *P. infestans*

2.4.1 The compatible interaction

As mentioned previously, early stages of the interaction between potato and *P. infestans* are fully biotrophic and compatible. No visible plant defense responses are activated during this stage. Currently, molecular events occurring in hyphal cells of *P.*

infestans during the colonization of potato tissues are largely unknown. Two different approaches have been taken to reveal the interaction between potato and *P. infestans* at this stage. One approach focuses on the identification of genes involved in cyst germination (Laxalt et al. 2002) and appressoria formation (van West et al. 2003) of *P. infestans*. Another approach is to study *P. infestans* genes that are differentially regulated during the colonization of the plant using differential displays or subtractive hybridizations (Pieterse et al. 1994a; Beyer et al. 2002). Furthermore, with the near completion of several small or large sequencing projects of cDNA or expressed sequence tags (ESTs) from *P. infestans*, approximately 16000 ESTs will be deposited in the *Phytophthora* Genome Consortium (PGC) database (http://www.pfgd.org/). Such information will be very valuable for the identification of genes which are expressed during early stages of *P. infestans* infection.

2.4.2 The incompatible interaction

2.4.2.1 Recognition between potato and *P. infestans*

Pathogens are armed with a diverse group of molecules to colonize their hosts. In return, plants have evolved sophisticated systems to detect signals from pathogens and induce appropriate defense responses.

Both chemical and genetic approaches have been used to study molecules produced by *P. infestans* that can be recognized by potato. To date, several *P. infestans*-derived compounds (cell wall fragments, arachidonic acid (AA) and eicosapentaenoic acid (EPA)) that are capable of inducing defense responses in potato have been identified. Meanwhile, the identification of potential *avr* genes in *P.*

infestans which interact with race-specific *R* genes in potato is currently another area of great interest.

2.4.2.1.1 *P. infestans* cell wall fragments

Hyphal cell wall (HWC) preparations of *P. infestans* with varying degrees of complexity have been reported to be effective elicitors capable of inducing defense responses in potato (Preisig & Kuc 1985). For example, *P. infestans* HWC is able to induce the expression of several defense-related genes in potato, such as glyceraldehydes-3-phosphate dehydrogenase (GAPDH)(Laxalt et al. 1996), and hydroxymethyl glutaryl coenzyme A reductase (HMGR) (Laxalt et al. 1996) and the accumulation of phytoalexins (Yao et al. 2003).

The active components responsible for the elicitation of potato defense responses have been identified as various cell wall glucans (Preisig & Kuc 1985; Bostock et al. 1986; Andreu et al. 1999). Although the detailed mechanism of the interaction between cell wall glucans from *P. infestans* and potato is largely unknown, experiments have shown that the elicitation activity of *P. infestans* HWC is not race-specific. Actually, they elicit defense responses in potato cultivars with or without R genes (Lamb 1994).

2.4.2.1.2 *P. infestans*-derived fatty acids: arachidonic acid (AA) and eicosapentaenoic acid (EPA)

AA and EPA, released from *P. infestans,* (Bostock et al. 1981), are able to elicit an array of potato defense responses such as the accumulation of phytoalexins (Bostock et al. 1981; Castoria et al. 1992; Coquoz et al. 1995) and expression of genes encoding various PR proteins (Despres et al. 1995a; Matton & Brisson 1989).

The mechanism of the AA-mediated defense response has been extensively studied in potato (Dubery et al. 2000; Gobel et al. 2002; Kolomiets et al. 2000; Veronesi et al. 1996; Rance et al. 1998). It has been proposed that the elicitation of potato defense responses by AA is mediated by oxylipin signaling compounds produced as the result of AA metabolism by lipoxygenases (LOX) (Gobel et al. 2002). This hypothesis is backed up by the claim that 5-(S)-HpETE, the 5-LOX metabolite of AA, can induce the accumulation of phytoalexins in potato tubers (Castoria et al. 1992). Furthermore, both 5-(S)-HpETE and AA can induce the DNA laddering indicative of apoptosis (Knight et al. 2001). Also, the silencing of an elicitor-induced LOX enables *P. parasitica* to infect tomato even in the presence of a resistance gene (Rance et al. 1998).

2.4.2.1.3 *P. infestans avr* products

In a typical gene-for-gene system, a resistant interaction is determined by the simultaneous expression and recognition between a plant *R* gene and a corresponding pathogen a*vr* gene (Dangl & Jones 2001; Ellis et al. 2000; Gabriel 1999; Lauge & De Wit 1998; White et al. 2000)].

In potato, *avr* genes corresponding to six race-specific *R* genes have been genetically defined (van der et al. 2001a). A*vr*3, *avr*10 and *avr*11 are likely closely linked and form a tight cluster in the linkage group VIII of an amplified fragment length polymorphism (AFLP) linkage map. A total of 18 AFLP markers have been linked to this *avr* cluster. Furthermore, bacterial artificial chromosome (BAC) contigs spanning *avr4* and *avr11* have been recently identified (Whisson et al. 2001).

Several of *P. infestans avr* genes have been targeted for positional cloning (van der et al. 2001a; van der et al. 2001b; Tyler 2002; Whisson et al. 2001). However, none of them has been successfully cloned so far due to technical difficulties such as high levels of repetitive DNA sequences in *P. infestans* genome and aberrant segregations at target loci (Kamoun 2003). However, with the help of *pex*, an algorithm designed to predict the extracellular proteins produced by *Phytophthora (Bos et al. 2003)*, *avr3a* has been recently cloned (Armstrong et al. 2005). *Avr3a* was found to encode a cytoplasmic protein and capable of triggering R3a-dependent cell death.

2.4.2.2 Defense-related signaling pathways in potato

Upon the recognition of pathogens, plants activate a broad spectrum of defensive responses (locally or systemically). In locally-induced defense responses, R-gene mediated resistance is the most effective form of defense responses (Belkhadir et al. 2004; Nimchuk et al. 2003). Meanwhile, plants have also evolved their systemic immunity in which local defenses promote a state of heightened resistance throughout the plant which prevents subsequent pathogen attacks. This phenomenon is also known as systemic acquired resistance (SAR) (Sticher et al. 1997; Klessig & Malamy 1994; Ryals et al. 1996).

The activation of plant defense responses relies on a series of signaling networks. Different signaling networks operate in various forms of plant defense responses. Meanwhile, they are also inter-connected (Katagiri 2004; kunkel & Brooks 2002; Parker et al. 2001; Feys & Parker 2000).

2.4.2.2.1 *R* gene-mediated signaling pathway

In R gene-mediated resistance, *R* gene product not only confers the specific recognition of the product of corresponding *avr* gene, but also serves as a signal transducer to elicit downstream plant defense responses (Nimchuk et al. 2003).

2.4.2.2.1.1 Recognition between *R/Avr*

Among 11 race-specific *R* genes described in potato, only R1 has been cloned. Meanwhile, the corresponding *avr* gene has not yet been found in *P. infestans*. Therefore, the interaction between potato *R* gene – *P. infestans avr* gene as well as signaling cascades downstream are largely unknown. However, the extensive mutational and functional studies on *R* gene-meditated resistance in plant species such as tomato, barley and *Arabidopsis* have provided an initial framework for the utilization of such a pathway in potato against *P. infestans*.

The most common feature of *R* genes cloned from tomato, barley and *Arabidopsis* is the presence of a so-called nucleotide binding site - leucine rich repeat (NBS-LRR) domain (Belkhadir et al. 2004)). A similar NBS-LRR domain has also been identified in the recently cloned potato *R1* gene (Ballvora et al. 2002).

Studies of proteins containing the NBS-LRR domain have revealed potential functions of different components within NBS-LRR domain. For example, each NBS-LRR domain contains: 1) a conserved NBS for ATP binding and hydrolysis (Tameling et al. 2002); 2) a amino-terminal of LRR modulating the activation of plant defense responses; and 3) specific residues located in the carboxy-terminal of the LRR responsible for the recognition between the host and the pathogen (Moffett et al. 2002; Tanabe et al. 2002).

2.4.2.2.1.2 Signaling cascades after the recognition between *R/Avr*

It has been suggested that R proteins can not act alone to recognize and transduce *avr*-dependent signals (Nimchuk et al. 2003). Therefore other factors may also participate in the recognition between the *R-avr* complex or act as early signaling partners (Rathjen & Moffett 2003; Nimchuk et al. 2003).

In other plant systems, accumulating evidence has suggested that various kinases could be signaling partners downstream of the *R-avr* interaction (Nimchuk et al. 2003; Belkhadir et al. 2004; Dangl & Jones 2001). For example, certain serine-threonine kinases are found to be essential for functions of two R genes: *Rps5* in *Arabidopsis*, which recognizes *AvrPphB* from *P. syringae* (Swiderski & Innes 2001) and *Prf1* in tomato, which interacts with *AvrPto* from *P. syringae* (Tang et al. 1996). Furthermore, some mitogen-activated protein kinases (MAPKs) have been shown to be activated by several *R-avr* interactions (Ligterink et al. 1997; Romeis et al. 1999).

2.4.2.2.2 Salicylic acid-mediated signaling pathway

2.4.2.2.2.1 Salicylic acid, the signal molecule

The involvement of salicylic acid as the signal molecule in plant defense responses, especially in systemic acquire resistance (SAR), has been extensively studied and well reviewed (Feys & Parker 2000; Morris et al. 1998; Shirasu et al. 1996; Welling 2001; Dempsey et al. 2001; shah 2004).

The most compelling evidence that demonstrates the role of salicylic acid in SAR came from transgenic tobacco plants over-expressing bacterial salicylate hydroxylase (*NahG*) which degrades salicylic acid to catechol. The over-expression of *NahG* in

tobacco effectively blocks the activation of defense genes related to the salicylic acid -mediated signaling pathway in transgenic plants (Gaffney et al. 1993). Furthermore, this hypothesis is backed up by the evidence that the exogenous application of salicylic acid or its synthetic functional analog, benzo thiadiazole-7-carbothioic acid S-methyl ester, can enhance disease resistance and lead to the activation of PR gene expression in various plant species (Dempsey et al. 2001; shah 2004).

It is a common belief that salicylic acid is one of the possible long distance signaling molecules in SAR which is supported by the evidence that 1) the onset of SAR is usually accompanied by local and systemic increases in the endogenous level of salicylic acid; 2) salicylic acid moves through out the plant (Sticher et al. 1997; Gaffney et al. 1993). However, recent studies on the salicylic acid -mediated signaling pathways in *Arabidopsis* have provided some new insights on the mechanism of salicylic acid -mediated signaling. Maldonado *et al.* (Maldonado et al. 2002) reported that certain lipid-derived molecules, which are released from the interaction between salicylic acid and DIR1 (a lipid-transfer protein required for the salicylic acid mediated SAR), are essential components of long distance signaling in SAR. Therefore, it is suggested that lipid–derived molecules could be responsible for the long distance signaling. Furthermore, this hypothesis is also supported by the following observations: 1) the activity of a lipase (SABP2), which functions as a receptor for salicylic acid in tobacco mosaic virus infected tobacco, is stimulated by the binding to salicylic acid (Kumar & Klessig 2003); 2) SFD1, a suppressor of fatty acid desaturase deficiency, is required for the activation of SAR and also involved in the lipid metabolism (Nandi et al. 2004).

2.4.2.2.2.2 Salicylic acid-mediated pathway in potato

In potato, SAR has been described in many occasions and could be induced by treatments with hyphal cell wall components, unsaturated fatty acids and jasmonic acids, resulting in enhanced resistance against LB (Cohen et al. 1991a; Cohen et al. 1993; Doke et al. 1987). The importance of salicylic acid in potato SAR has been demonstrated with transgenic potato plants expressing bacterial *nahG* (Yu et al. 1997) in which arachidonic acid failed to induce SAR in *nahG* transgenic potato plants.

Although the role of salicylic acid in SAR is well established in tobacco and *Arabidopsis*, its involvement in the potato defense mechanism is not well understood in general. However some evidence has suggested that SA might function differently in potato than in tobacco or *Arabidopsis*.

First of all, the basal level of salicylic acid in potato is 40- to 100 fold higher than that found in tobacco and *Arabidopsis* (Coquoz et al. 1995). Healthy potato plants respond poorly to the exogenous application of salicylic acid for the activation of defense responses (Yu et al. 1997).

Second, the initiation of SAR is usually correlated with the local and systemic increases in the endogenous level of salicylic acid. It is the case in *Arabidopsis* and tobacco (Dempsey et al. 2001; Klessig & Malamy 1994; Ryals et al. 1996). On the contrary, no increase in the endogenous level of salicylic acid has been observed in potato leaves treated with various elicitors (Coquoz et al. 1995).

Third, unlike the result from the *nahG* transformation experiment performed in *Arabidopsis* mentioned previously, no significant increase in the disease severity was

observed in the transgenic potato plant expressing *nahG* when infected by *P. infestans* (Yu et al. 1997).

All these differences suggest that the mechanism of the salicylic acid mediated SAR in potato might be different than those in *Arabidopsis* and tobacco. One model is that there is a regulatory component in the salicylic acid mediated signaling pathway in potato. The activation of this unknown regulatory component, which is responsive to the elicitor treatment, renders potato responsive to the high basal level of SA (Yu et al. 1997).

2.4.2.2.3 Jasmonate-mediated signaling pathway

2.4.2.2.3.1 Jasmonate, the signaling molecule

Jasmonate mediated signaling pathway is involved in various biological processes such as: the plant development, plant responses to wounding or abiotic stress and defenses against insects and pathogens (Creelman & Mullet 1997). Studies on JA-mediated signaling pathway in response to plant disease has been mainly focusing on the rhizobacteria-mediated induced systemic resistance (ISR) or induced disease resistance against insects.

The jasmonate meidated signaling pathway involves several major signal transduction events: the perception of the stimulus, the transduction of the signal, the local and systemic induction of jasmonate biosynthesis, the perception of jasmonate and the induction of downstream defense-related genes.

The jasmonate mediated signaling pathway can be triggered by a range of external signals (abiotic or biotic), such as wounding, drought, exposures to elicitors, rhizobacteria and insects (biotic) (Turner et al. 2002).

It is not known yet whether jasmonate moves between cells and tissues acting as the signaling molecule itself, or whether the local and systemic signal transduction involves an as-yet undiscovered signaling molecule. However evidence has shown that the transcriptional activation of genes related to jasmonate biosynthesis occurs locally, which suggests the presence of the signaling pathway connecting the perception of stimulus and the systemic induction of jasmonate biosynthesis (Mussig et al. 2000; Ishiguro et al. 2001; Turner et al. 2002). Two possible candidates have been suggested: mitogen-activated protein kinases (*WIPK* in tobacco (Seo et al. 1999) and *MPK4* in Arabidopsis (Ichimura et al. 2000)) and the systemin (a 18-amino-acid polypeptide (Ryan & Pearce 1998)).

The pathway for the biosynthesis of jasmonate is best characterized in *Arabidopsis* and has been extensively reviewed (Creelman & Mullet 1997; Turner et al. 2002). Some of key enzymes in the pathway are lipoxygenase (LOX), allene oxide synthase (AOS), allene oxide cyclase (AOC) and 12-oxo-phytodienoic acid (OPDA) cyclase.

The last major event in the jasmonate mediated signaling pathway is the perception of jasmonate and the induction of defense-related genes. The perception of jasmonate signal is possibly achieved through a specific receptor, which binds to jasmonate molecules. However no such receptor has been identified so far.

2.4.2.2.3.2 Jasmonate-mediated pathway in potato

The jasmonate mediated signaling pathway in potato is not well characterized in general. However, experiments on the role of jasmonate in the potato defense mechanism have provided some intriguing results.

Originally it was demonstrated that exogenous applications of jasmonic acid and jasmonic methyl ester induce the local and systemic protection against *P. infestans* in potato. Therefore it was suggested that jasmonate plays a role in potato defenses against LB (Cohen et al. 1993). However, it was later shown that jasmonate and arachidonic acid (elicitor) activate the wounding and pathogen responsive isoprenoid pathways, respectively. It was then suggested that jasmonate might function as the signaling molecule in certain wounding responsive signaling pathways (Choi et al. 1994).

Although the reason why exogenous application of jasmonate induces potato resistance against *P. infestans* is still unknown, it has been hypothesized that induction of potato defense responses by jasmonate might be due to the cross-talking between JA and AA responsive pathways(Choi et al. 1994).

2.4.2.2.4 Nitric oxide-mediated pathway in potato

Nitric oxide (NO) was first identified as a unique diffusible messenger in animals. Lately it was found that nitric oxide also functions as an important signaling molecule in certain plant defense signaling pathways responsive to microbial pathogens (Delledonne et al. 1998; Durner et al. 1998; Chandok et al. 2003).

In potato, evidence has indicated that the nitric oxide mediated signaling pathway might be also operative. For example, Noritake *et al.* (1996) reported that nitric oxide radicals can induce the accumulation of phytoalexins in potato. Furthermore, a nitrate

reductase (a nitric oxide-producing enzyme) was induced in potato tuber infected by *P. infestans* sporangia suspension or crude cell wall preparations (Yamamoto et al. 2003).

2.4.2.3 Defense responses in potato

In potato, activation of a broad spectrum of defense responses could be observed following either treatments with elicitors (either biotic or abiotic), hyphal wall components prepared from *P. infestans* or infection by *P. infestans*. Typically, they include accumulation of structural compounds (lignin), HR, defense-related secondary metabolites (SA, sesquiterpenoid phytoalexins (rishitin)) and various PR proteins.

2.4.2.3.1 Structural compounds

It is well known that plant cell walls can act as a physical barrier preventing pathogen invasions. The involvement of lignin and lignification of plant cell walls in the disease resistance has been extensively studied and well reviewed (Nicholson & Hammerschmid 1992; Vance & Sherwood 1980). In general, they could 1) reinforce the plant cell wall which makes it more resistant to mechanical penetration; 2) modify the plant cell wall by cross-linking or esterifying cell wall polysaccharides, which makes the cell wall more tolerant to fungal cell wall-degrading enzymes; 3) change the permeability of the cell wall which restricts the diffusion of enzymes and toxins from pathogens to hosts.

Hammerschmidt (1984) examined insoluble phenolic compounds in potato tuber discs and concluded that potato cell wall contained lignin. Increase in the lignin content has been observed in potato infected by various pathogens (Henderson & Fiend 1979; Ray & Hammerschmid 1998). Potato tubers with reduced levels of lignin had higher

susceptibility to *P. infestans* (Yao et al. 1995), suggesting that lignin plays a role in potato resistance to late blight.

2.4.2.3.2 Hypersensitive reaction

Hypersensitive reaction (HR) occurs as a rapid, localized necrosis near the infection site and is commonly associated with all forms of resistance in potato to *P. infestans*, such as in the non host-specific and host-specific resistance, in the race-specific resistance and sometimes, non race-specific resistance (Huitema et al. 2003). In the non host-specific resistance, HR is highly localized to a single epidermal cell while it involves a group of cells surrounding the penetrating hyphae in the race-specific resistance. In both cases, HR successfully stops further development of the pathogen. In the case of the non race-specific resistance, it appears to be ineffective in blocking the development of *P. infestans* hyphae which remain ahead of the lesion caused by HR (trailing HR phenotype) (Kamoun et al. 1999).

HR involves several major events such as oxidative bursts, changes in ion fluxes and protein phosphorylations. The production of active oxygen species at the cell surface is one of the earliest events detected in a race-specific interaction (Lamb & Dixon 1997). The rapid production of active oxygen species could inhibit the growth of pathogens through either the direct microbial toxicity or by restricting pathogen penetrations via cross-linking cell wall glycoproteins (Brisson et al. 1994). Meanwhile active oxygen species, such as H_2O_2, could act as a secondary signal which in turn activates: 1) the accumulation of phytoalexins and 2) the biosynthesis of salicylic acid which leads to the induction of downstream defense-related genes (Yang et al. 1997).

In potato tubers, the oxidative burst could be induced by treatments with hyphal cell wall (HWC) elicitors prepared from *P. infestans*. It has been shown that the oxidative burst in potato is related to the activation of an NADPH oxidase (*gp91 phox*) which is up-regulated during the phase II oxidative burst in potato tubers challenged with an cell wall elicitor and closely resembles the NADPH oxidase operating in activated neutrophils (Doke & Miura 1995).

The HWC-stimulated oxidative burst in potato tuber is found to be dependent on a calcium dependent protein kinase (Yoshioka et al. 2001). Application of protein kinase inhibitors and Ca^{2+} channel blockers can suppress the expression of *gp91 phox* homologs as well as the production of AOS in HWC treated tubers. This evidence indicates that the change in Ca^{2+} flux is important for the production of AOS in potato.

2.4.2.3.3 Secondary metabolites

The functions of phenylpropanoid compounds in disease resistance can be summarized into three basic aspects. First, they are involved in the formation of the preformed or inducible physical barrier; second, they provide precursors for the biosynthesis of antimicrobial phytoalexins; third, they can act as signaling molecules which are involved in local and systemic signaling networks of plant defense responses (Dixon et al. 2003).

Phenylpropanoid biosynthesis has been extensively studied (Weisshaar & Jenkins 1998; Hanson & Havir 1977; Dixon et al. 2003). All natural phenylpropanoids, such as lignins, coumarins, benzoic acids, etc., are derived from the deamination of phenlyalanine by phenylalanine ammonia lyase (PAL). Furthermore, phenylalanine

ammonia lyase has been proposed to be the direct rate limiting step for the production of chlorogenic acid in tobacco (Howe et al. 1996) which has been implicated in the resistance to both microbes and insects (Yao et al. 1995).

Therefore, the up regulation of *pal* has been commonly regarded as one of the indicators for the induced plant resistance and used in many studies (Wegener 2002; Walters et al. 2002; Takakura et al. 2004; Kruger et al. 2003; Galis et al. 2004; Mazeyrat et al. 1999) including in potato versus *P. infestans* (Trognitz et al. 2002; Laxalt et al. 1998), potato versus *Erwinia carotovora* (Wegener 2002), and potato versus *Fusarium sambucinum* (Ray & Hammerschmid 1998).

Potato contains approximately 40-50 *pal* genes per haploid genome. Two subfamilies of *pal* genes (*pal-1* and *pal-2*) have been identified. Both of them are induced during the pathogen infection (Joos & Hahlbrock 1992).

Defense-related phenylpropanoid compounds are not only restricted to a certain class of compounds, but also range from simple molecules, such as hydrocinnamic acids, to complex compounds, for example flavonoids and isoflavonoids (Dixon et al. 2003).

2.4.2.3.3.1 Antimicrobial compounds

Many phenylpropanoids exhibit a broad-spectrum of antimicrobial activities and have been classified as preformed "phytoanticipins" or inducible "phytoalexins" (Hammerschmid 1999). The best characterized phenylpropanoid-derived phytoalexins include pterocarpans and isoflavans (Dixon et al. 2003). Their roles in plant defense responses have been well reviewed (Hammerschmid 1999; Smith 1996; Nicholson &

Hammerschmid 1992). However, in potato, the best-known phytoalexins are terpenoids and the best known sesquiterpene phytoalexin found in potato is rishitin which was first isolated from potato cultivar Rishiri (R1) challenged by race 0 of *P. infestans* (Ishizka et al. 1969). Other sesquiterpenoid phytoalexins commonly found in potato include rishitinol, lubimin and phytuberin (Kuc & Rush 1985).

The main fungitoxic activity of rishitin appears to be membranolytic. Upon the treatment with rishitin, there is lyses of motile zoospores of *P. infestans* within a few minutes (Harris & Dennis 1977). Later, it is also shown that rishitin interacts with the liposome and affects the permeability of pathogen cell walls (Lyon 1980). Rishitin can be induced in potato by various plant pathogens (Yao et al. 2003; Ray & Hammerschmid 1998; Abenthum et al. 1995) and elicitors (biotic or abiotic) (Yoshioka et al. 2001; Lulai 2000; Noritake et al. 1996; Hoshino et al. 1994).

To date, several genes encoding key enzymes involved in the terpenoid pathways in potato have been cloned, such as 3-hydroxy-3-methyglutaryl CoA reductase (HMGR) (Choi et al. 1992). More recently, sesquiterpene cyclase and squalene synthase, two key branch point enzymes in the synthesis of sesquiterpenoid phytoalexins and sterols/steroid glycoalkaloids have also been cloned (Yoshioka et al. 1999).

2.4.2.3.3.2 Signal molecules

Some of plant secondary metabolites such as salicylic acid, jasmonic acid and ethylene can also functions as signaling molecules in the plant defense responses. Salicylic acid is mostly studied signaling molecule in potato response to *P. infestans*. In potato, SA biosynthesis occurs through the shikimate-phenylpropanoid pathway. Two

routes from coumaric acid, which is converted from phenlyalanine by phenylalanine ammonia lyase (PAL), to salicylic have been identified in potato (Coquoz et al. 1998). The first route involves the hydroxylation of *orth-* courmaric acid followed by the oxidation of the side chain (Chadha & Brwon 1974). In the second route, the side chain of courmaric acid is initially oxidized to give benzoic acid, which is then hydroxylated in the *ortho* position (Ellis & Amrhein 1971).

2.4.2.3.4 Pathogenesis-related proteins

2.4.2.3.4.1 Overview

Potato responds to pathogen attacks by the activation of an array of defense responses. Pathogenesis-related (PR) proteins have been classified as a major group of defense-related proteins, which were originally identified in tobacco plants infected by tobacco mosaic virus (TMV), but subsequently discovered in many other plant species (van Loon & van Strien 1999).

Currently, 14 different families of PR proteins are known to be induced in plants (van Loon & van Strien 1999). Some families contain proteins with known biochemical functions, such as β-1,3-glucanases (PR-2), chitinases (PR-3,PR-4,PR-8,PR-11), osmotin-like proteins (PR-5), proteinase inhibitors (PR-6), endoproteinases (PR-7), peroxidases (PR-9) and ribonuclease-like proteins (PR-10), which support their roles in the disease resistance. However, for other PR proteins including PR1, PR12, PR13 and PR14, their functions remain unclear.

2.4.2.3.4.2 PR proteins, an induced defense mechanism

In potato, several families of PR proteins are induced when potato tissues are infected by various pathogens including *P. infestans* (Table 2.4). The induction of PR proteins is not only observed when potato is infected by pathogens but also when treated with different elicitors including 1) *P. infestans* derived compounds, such as arachidonic acid (AA) (Coquoz et al. 1995) and eicosapentaenoic acid (EA) (Matton & Brisson 1989); 2) potential signaling molecules, like jasmonic acid (JA) (Kreft et al. 1997) ; 3) chemical elicitors, for example benzothiadiazoles (BTH) and β-aminobutyric acid (BABA) (Oostendorp et al. 2001; Si-Ammour et al. 2003). Furthermore some PR proteins are also induced in response to physical wounding or other abiotic stresses (Despres et al. 1995b; Zhu et al. 1995).

Table 2.4 Pathogenesis-related proteins identified in potato

Family	Functions	Patho-system	References
PR-1	Unknown	Potato – *P. infestans*	(Hoegen et al. 2002)
	Unknown	Potato - Potato virus Y	(Naderi & Berger 1997)
PR-2	Glucanase	Potato – *P. infestans*	(Tonon et al. 2002)
	Glucanase	Potato - *Erwinia carotovora*	(Linke et al. 2002)
	Glucanase	Potato – *Globodera pallida*	(Rahimi et al. 1996)
PR-3,4,8,11	Chitinase	Potato – *P. infestans*	(Schroder et al. 1992)
	Chitinase	Potato - *Erwinia carotovora*	(Linke et al. 2002)
	Chitinase	Potato - *Globodera pallida*	(Rahimi et al. 1996)
PR-5	Osmotin-like protein	Potato – *P. infestans*	(Zhu et al. 1995)
PR-9	Peroxidase	Potato – *P. infestans*	(Collinge & Boller 2001)
	Peroxidase	Potato – *F. sambucinum*	(Ray & Hammerschmid 1998)

2.4.2.3.4.3 PR genes, activated by different signaling pathways

More than one class of PR proteins are induced when potato is infected with *P. infestans*. This suggests two possibilities; 1) the expression of *pr* genes is regulated by

the same signaling pathway 2) The coordinated induction of *pr* genes results from the cross-talk among different pathways.

In *Arabidopsis* mutant *sid1* and *sid2* (SA induction deficient mutants), the induction of *pr-1* is blocked while *pr-2* and *pr-5* are expressed at the wild type level. This suggests that *pr-1*, *pr-2* and *pr-5* are regulated in different pathways in *Arabidopsis* (Nawrath & Metraux 1999).

In potato, the presence of multiple defense signaling pathways has been previously discussed. Furthermore, potato plants are protected from *P. infestans* by BABA, a compound that can activate multiple signaling pathways while salicylic acid analogue benzothiadiazoles, an inducer for SA-dependent signaling pathway, alone could not (Si-Ammour et al. 2003), which also suggests that the activation of multiple defense signaling pathways might be required for disease resistance. Therefore, the coordinated activation of different *pr* genes in potato likely results from cross-talk between different pathways.

2.4.2.3.4.4 PR proteins, potential targets for resistance breeding

Along with known antifungal activities of some PR proteins, the information above suggests that genes encoding PR proteins could be good targets for late blight resistance breeding.

To date, preliminary evidence has shown the potential of such approach. For example, over-expression of PR-1 in tobacco can enhance resistance to *P. parasitica* and *Peronospora tabacina* (Alexander et al. 1993). Increase in late blight resistance is found in transgenic potato transformed with a PR5 (Liu et al. 1994). Furthermore, the

close linkages between several *pr* genes and QTLs related to late blight resistance have suggested that PR genes might actually be components of certain QTL to late blight.

3.0 US-1 and US-8 genotypes of *Phytophthora infestans* differentially affect local, proximal and distal gene expression of phenylalanine ammonia-lyase and 3-hydroxy, 3-methylglutaryl CoA reductase in potato leaves

3.1 Abstract

Differential expression of *pal1* and *hmgr2* was investigated using northern blot analysis in two potato cultivars (Russet Burbank, susceptible and Kennebec, moderately tolerant) after inoculation with two *Phytophthora infestans* isolates from the formerly (US-1) and currently predominant genotypes (US-8). The accumulation of *pal1* transcripts was weaker in response to US-8 as compared to US-1 and occurred earlier in Kennebec than in Russet burbank. The stronger expression of *pal1* in response to US-1, as compared to US-8, is suggested to be due to defense gene suppression by the latter. No apparent strong accumulation of *hmgr2* transcripts was recorded in Russet burbank as compared to Kennebec inoculated with either US-1 or US-8. The induction of *pal1* and *hmgr2* was first observed in un-inoculated (proximal) leaflets close to the inoculated leaflets, then in un-inoculated (distal) leaflets of leaves adjacent to the inoculated leaf, and finally in local inoculated leaflets. The stronger expression of the two genes in proximal and distal leaflets, as compared to the local site of inoculation suggests the translocation of signal(s) from this site to healthy parts of the plant.

3.2 Introduction

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is one of the most devastating diseases on potato worldwide (Fry & Goodwin 1997c). It is famous for the

epidemics that destroyed potato crops in Europe in the 1840s and led to the Irish potato famine (Fry & Goodwin 1997c; Andrivon 1995) and it caused losses in both potato and tomato crops worldwide. However, it is over the last 10-15 years that late blight has re-emerged as one of the most important diseases on potatoes (Fry & Goodwin 1997b). Concurrently to this re-emergence, populations of *P. infestans* have considerably changed. Surveys conducted in Canada during 1994-2000 showed that the previously predominant *P. infestans* US-1 genotype (A1 mating type) has been gradually replaced by the US-8 genotype (A2 mating type) (Chycoski & Punja 1996; Daayf & Platt 2000; Daayf & Platt 2001a; Peters et al. 1999b; Peters et al. 2001).

An extensive literature, including epidemiological and genetic analyses, is available, describing the shifts in the *P. infestans* populations in North America and worldwide (Goodwin & Fry 1994; Goodwin et al. 1995; Goodwin et al. 1998; Fry et al. 1993; Fry & Goodwin 1997c; Daayf et al. 2000). However, relatively few studies thoroughly investigated differential potato interactions with isolates from different groups such as A1 and A2 mating types, or specifically with US-1 and US-8 genotypes, the formerly and currently predominant genotypes of the pathogen, respectively (Daayf & Platt 2001b; Fry & Goodwin 1997b; Lambert & Currier 1997; Miller & Johnson 2000). Isolates of the US-8 genotype were reported to have shorter latent periods and to produce larger lesions and more sporangia on detached potato leaflets (Kato et al. 1997) and to rotten tubers substantially faster (Lambert & Currier 1997) than their US-1 counterparts. The mechanisms, by which such differences occur, either from the pathogen or the plant side, are still not fully understood. Biochemical and molecular

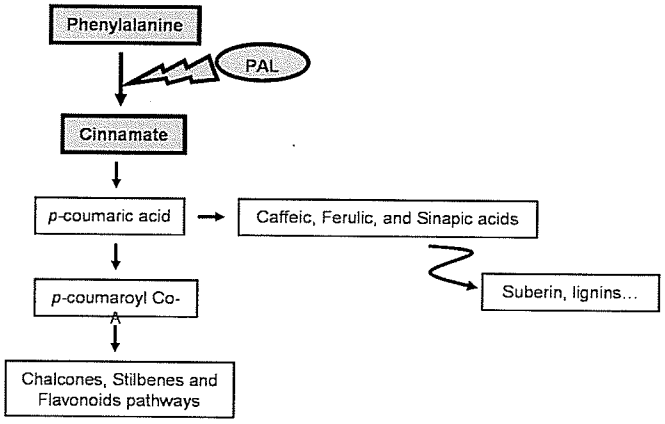
bases for the much higher pathogenic success of the new *P. infestans* genotype in potato could be searched either on the pathogen side (Avrova et al. 2003; Bos et al. 2003), because that is where the most apparent changes recently occurred, but also on the plant side (Boyd et al. 1994; Kamoun 2001; Ros et al. 2004), where consequent differential responses might have been developed. In fact, several of the studies published on host-pathogen interactions in the potato-late blight system were probably carried out using isolates from the old *P. infestans* population and only a few of them have included isolates from the new lineage (Kolomiets et al. 2000). Strains from different *P. infestans* genotypes (i.e. US-1 and US-8) constitute a material of choice toward characterizing the mechanisms that differentially regulate the activation of potato defense genes in response to the infection by *P. infestans*, and thus understanding potato defense limitations toward this oomycete. To date, however, no studies have been dedicated to the comparison of biochemical and/or molecular aspects of potato reaction specifically to US-1 and US-8.

Phenylalanine ammonia lyase (PAL) and 3-hydroxy, 3-methylglutaryl CoA reductase (HMGR) are the key enzymes in the phenylpropanoid and terpenoid pathways, respectively (Figure 3.1). They were both reported to play a role in the resistance mechanisms of many plants (Kervinen et al. 1998; Kim et al. 2004; Paiva 2000; Tanaka & Uritani 1976). PAL was shown to be associated with the early induction of resistance in potato-*P. infestans* system during an incompatible interaction but not during a compatible interaction (Cuypers & Hahlbrock 1988). Similarly, HMGR catalyzes the first step in the sesquiterpenoid phytoalexins production (Kleinig

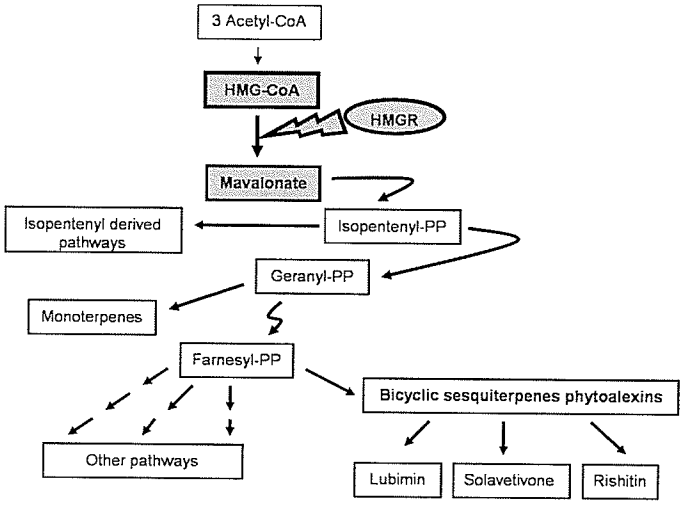
1989), and has been reported to play an important role in induced resistance in potatoes (Ito et al. 1984; Oba et al. 1985; Kondo et al. 2003).

Figure 3.1 Phenylpropanoid and Isoprenoid biosynthesis pathways

Phenylpropanoid biosynthesis pathway



Isoprenoid biosynthesis pathway



PAL: phenylalanine ammonia-lyase
HMGR: 3-hydroxy, 3-methylglutaryl CoA reductase

The objectives of this study were: (i) to assess the accumulation of mRNA transcripts of both *pal1* and *hmgr2* in potato leaves in response to inoculation with US-1 and US-8 genotypes of *P. infestans*, (ii) to follow such gene expression over time at three levels of the plant tissues: (a) leaflets inoculated with either genotype (local),

(b) healthy leaflets from the inoculated leaf (proximal), and (c) leaflets from healthy leaves adjacent to the inoculated leaves (distal), and (iii) to conduct these studies on two potato cultivars with two levels of response to *P. infestans*: Russet Burbank (susceptible), and Kennebec (moderately tolerant).

3.3 Materials and Methods

Plant materials

Potato plants (*Solanum tuberosum* L.) were produced from high quality seed tubers planted in clay pots containing soil-sand-peat-perlite mixture (4:4:4:1) and kept in a growth room at 20±2°C and 16h photoperiod. Six week-old plants from Russet burbank and Kennebec were used for inoculations. Russet burbank is a widely used commercial cultivar and is considered to be susceptible to late blight while Kennbec is known to be a moderately tolerant cultivar. Leaves were collected from three different parts of healthy or inoculated potato plants; local leaflets, which represent the primary leaflets inoculated with *P. infestans*; proximal leaflets, which are the non-inoculated secondary leaflets of the inoculated primary leaf; and distal leaflets in reference to those from the leaf adjacent to the inoculated leaf. All leaf samples were frozen in liquid nitrogen after harvest and stored immediately at -80°C until used. Additional inoculated and non-inoculated plants were kept as controls for the whole periods of experiments.

Pathogens and inoculations

Two *P. infestans* isolates were used in this study. The isolate FA1 (US-1) was provided by Dr. P. Audy (AAFC, Fredericton, NB, Canada) while the isolate D1901

(US-8) was from our lab collections. Genotyping of these and other isolates was performed previously (Unpublished results). Both *P. infestans* isolates were grown on rye B medium (Fry et al. 1993) at 20±2°C. Virulence of the two isolates was maintained by inoculating them on potato leaves and re-isolating them every 3-4 months.

For the inoculation, 10-14 days old *P. infestans* cultures grown on rye B in Petri dishes (US-1 and US-8) were smashed down using sterile distilled water and sporangia were harvested the following day by flooding the plates with sterile distilled water. Inoculations of whole plants of Russet burbank (RB) and Kennebec (KB) were performed by spraying on 100 µl of sporangia suspension adjusted at 5×10^6 sporangia ml^{-1} on the primary leaflet of the 3rd or 4th leaf. The inoculum was deposited as multiple tiny droplets using a micropipette to prevent inoculum run off. To maintain the humidity required for infection, the inoculated potato plants were kept for 48 hours in a misting chamber at 100% relative humidity. Three pots, containing two potato stems each, were considered per treatment and per time point (0, 6, 12, 24, 48, 72, 96 and 120 hours after inoculation (h.a.i.) in a randomized complete block design and the whole experiment was repeated twice overtime.

Physiologic race determination

The physiological races of the two *P. infestans* isolates (FA1 and D1901) were determined by their performance on a differential set of potato cultivars carrying late blight single resistance genes *R-1* to *R-11* (R0- Bintje, R1- Craig's Royal, R2- 1512 C16, R3- Pentland Ace, R4- 1563 C14, R5- 3053-18, R6- 2424a5, R7- 2182ef7, R8- 2424a5, R9- 2573(2), R10- 36581ad1, R11- 5008ab6; Platt, AAFC, PEI, Canada). For

the inoculation, detached leaflets were used as described above. Physiological races were determined based on the presence or the absence of the hypersensitive reaction, and the extension of the infection lesion with or without sporulation on the leaf of each differential cultivar (Daayf et al. 2001).

Differential pathogenicity of the two isolates on detached leaves and on whole plant

In parallel to the inoculated whole plants described above, leaflets collected from the 3rd or the 4th leaf were incubated *in vitro* and inoculated with the two *P. infestans* isolates as an additional control for the pathogenicity testing. The leaflets were surface-sterilized using 70% ethanol, placed in Petri plates containing a humidified filter paper, and inoculated with 20 µl of each sporangia suspension adjusted at 5×10^6 sporangia ml^{-1}. After inoculation, the leaflets were incubated in a culture chamber maintained at 20±2°C and 16h photoperiod. Five detached leaflets were considered per treatment and the experiment was repeated independently two times. Infected leaflets of cultivars Russet burbank and Kennebec from both types of inoculation (whole plant and detached leaflets) were harvested over time (0, 6, 12, 24, 48, 72, 96 and 120h.p.i.). They were then scanned using a Microtek digital scanner Model 4800 (Microtek Inc., Belleville, ON, Canada) and the disease severity was assessed as percentage of diseased area using the image analysis software ASSESS (Lamari 2002).

Preparation of total RNA

RNA was extracted only from the leaflets that were growing on the whole plant. These leaflets represent three strata: inoculated leaflets (inoculation site, local), un-inoculated leaflets from the same leaf where the main leaflet was inoculated

(proximal), and un-inoculated leaflets from leaves adjacent to the inoculated leaves (distal). The samples were collected at 0, 6, 12, 24, 48, 72, 96 and 120 h.a.i.. The harvested leaves were ground to a fine powder in a mortar pre-cooled with liquid nitrogen. Total RNA was isolated from 100 mg fresh weight following the method of Verwoerd *et al.* (1989) and the absorbance at 260 nm and the ratio A_{260}/A_{280} were used to determine RNA concentration and purity. Leaf samples harvested from the two separate sets of inoculations were used separately for RNA extractions, gel blot or dot blot. However, within each experiment, leaflets collected from two stems grown in the same pot were pooled to ensure sufficient material for RNA extraction.

RNA dot blot and gel blot analysis

RNA analyses were carried out using both dot and gel blots. RNA dot blot hybridization, performed after loading the RNA on the membranes directly, without size separation, is a commonly used technique for gene expression assays to quantify the amount of transcripts. Northern gel blotting, performed by agarose gel electrophoresis of RNA, followed by its transfer onto a porous solid support (nylon or nitrocellulose membranes), is more suitable for size determination of transcripts. RNA dot blot analysis was carried out by depositing 10 μg of denatured total RNA of each sample on a Hybond-N^+ membrane (Hoffmann-La Roche Ltd., Mississauga, ON, Canada). RNA gel blot analysis was performed on the same amount of RNA samples separated by electrophoresis under denaturing conditions. RNA was then transferred on a Hybond-N^+ membrane (Hoffmann-La Roche Ltd., Mississauga, ON, Canada) and a pre-hybridization was performed for 3-4 hours in DIG Easy high solution at 50°C. The

hybridization was conducted in the same solution at 50°C for 18 hours. Following hybridization, the membranes were washed in 2X SSC containing 0.1% SDS for 15 minutes at room temperature then in 0.5% SSC added with 0.1% SDS for 15 minutes at 68°C.

Probes for hybridization

For probes synthesis, a 1.2 Kb fragment of potato *pal1* genomic clone (Genebank no. X63103), representing the 3' conserved region of *pal* (Joos & Hahlbrock 1992), a 1.5 Kb fragment of *hmgr2* genomic DNA clone (GeneBank no. AB041031) that contains the highly conserved active site of the enzyme (Choi et al. 1992), and a 686 bp fragment of *18s r*DNA (Genebank no. 67238) were used as DNA templates for PCR amplification. The probes were synthesized and labeled using a PCR DIG labeling kit (Hoffmann-La Roche Ltd., Mississauga, ON, Canada) following the manufacturer's recommendations. The forward and reverse primers used for *pal1*, *hmgr2*, and *18s r*DNA probe synthesis were designed using OligoPerfect™ Designer software (Invitrogen™ Life Science Software, Invitrogen Inc., ON, Canada). The primer sequences were

pal1-F 5'-GCGATTTTCGCTGAAGTG-3';

pal1-R 5'-TGTGCTTCGGCACTCTGA-3';

hmgr2-F 5'-TGACGCAATGGGAATGAA-3';

hmgr2-R 5'-ATGATGGCAAGGACCTCC-3';

18s-F TAGATAAAAGGTCGACGCGG-3';

18s-R 5'-TCATTACTCCGATCCCGAAG-3'.

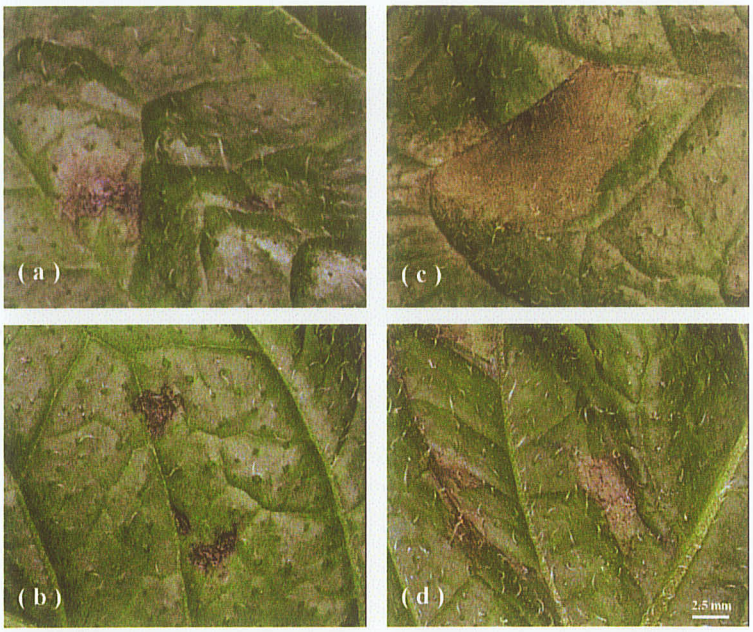
The expected size of probes for *pal1*, *hmgr2* and *18s r*DNA were 596, 530, and 686 bp, respectively. For each PCR reaction, a labeling mixture of 25µl was preheated at 95°C for 2 min followed by 25 cycles of 30s at 95°C, 30s at 55°C and 45s at 72°C using a programmed thermocycler (Techne Flexigene Inc., Canada).

Image analysis

The data of *pal1* and *hmgr2* expression were based on three independent replicates of dot blots per set of experiment. Five ηg of DIG labeled control DNA (Hoffmann-La Roche Ltd., Mississauga, ON, Canada) was loaded on the right corner of each membrane and used as a control for image development to avoid the possible variation that may be caused by the slightly different exposure duration. The films were recorded using a digital camera at 1200 dpi resolution (Sony Ltd., Toronto, ON, Canada) and the dots on the films were analyzed using ImageJ 1.32 software for Windows (http://rsb.info.nih.gov/ ij/download.html). Background hybridization was measured by sampling membrane areas outside of the loaded lanes and the radiation counts were subtracted using ImageJ 1.32 software. After background subtraction the net signal from each lane was normalized for the amount of total leaf RNA in each lane. An initial normalization was performed with two sets of RNA blots, containing 15 and 1.5 µg of total 18s RNA in order to obtain a highly reproducible hybridization intensities among experiments. Corrections of slight differences in loading were made for each gel by normalizing the results against the constitutively expressed *18s r*DNA gene. The intensity of hybridization signal represented by the integrated density ($x10^3$) was

calculated from each dot. The average of three replicates (±SE) from each dot was considered to construct the final expression profiles of *pal1* and *hmgr2* transcripts.

Figure 3.2 Late blight symptom observed 72 hours after inoculation



Symptoms of late blight observed 72 hours after inoculation on whole plants. Russet burbank (high susceptible to late blight) (a) and Kennebec (moderately resistant to late blight (b) leaves inoculated with *P. infestans* US-1 (less aggressive); Russet burbank (c) and Kennebec (d) leaves inoculated with *P. infestans* US-8 (more aggressive). Similar types of lesions were observed on inoculated detached leaves *in vitro*. The pictures show only a section of the leaflets with the typical lesions observed.

3.4 Results

Differential pathogenicity of the two US-1 and US-8 isolates on potato detached leaflets (in vitro)

Pathogenicity of *P. infestans* US-1 and US-8 isolates was assessed on both Kennebec (moderately tolerant) and Russet burbank (susceptible). Small lesion spots were observed on most inoculated leaflets 36 h.a.i.. On Kennebec leaflets inoculated with US-1, lesions were of a dark black color, initially localized and developed later into a larger diseased area. By contrast, Russet burbank leaflets inoculated with either US-1 or US-8 isolate, and Kennebec leaflets inoculated with US-8 isolate showed more

typical late blight lesions with a brown dark necrotic spot surrounded by a chlorotic ring (Figure 3.2).

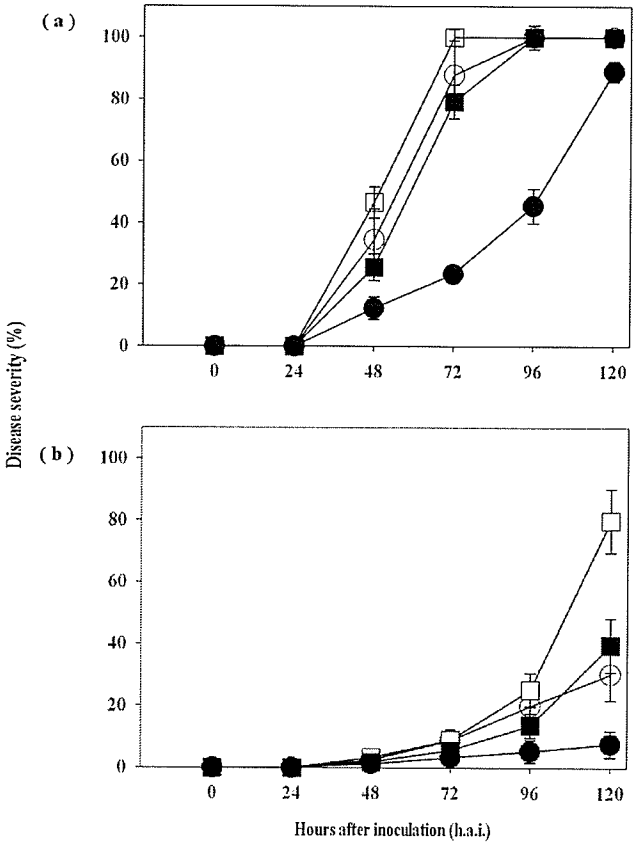
Figure 3.3a shows the disease progression on detached leaflets during a period of 5 days after inoculation (d.a.i.). No lesions were visible during the first 24 h.a.i. on either cultivar. However, after the first symptom appeared approximately 36 h.a.i., lesions then developed quickly on both cultivars. Entire Russet burbank leaflets inoculated with US-8 were destroyed 72 h.a.i.. Comparatively, on Kennebec detached leaflets, in spite of their apparent tolerance to both *P. infestans* genotypes, over 90% of the leaf area had late blight symptoms 5 days after inoculation with US-1 or US-8.

Differential pathogenicity of the two US-1 and US-8 isolates on whole potato plants

No symptoms were visible within the first 48 h.a.i. on either cultivar and only small lesions became apparent 48-72 h.a.i.. Leaflets of Russet burbank inoculated with either US-1 or US-8 and Kennebec inoculated with US-8 all had a chlorotic ring surrounding the necrotic lesion. A distinct localized necrotic lesion was only observed on Kennebec inoculated with US-1, but later developed into a larger lesion.

Disease progression on inoculated whole plants is shown in Figure 3.3b. Russet burbank inoculated with US-8 had the highest disease severity (83%) 5 d.p.i. whereas Kennebec inoculated with the US-1 isolate exhibited the lowest percent of diseased leaf area (8%). In general, disease severity was higher on Russet burbank than on Kennebec inoculated with either US-1 or US-8. The US-8 isolate was more aggressive than US-1 on both cultivars.

Figure 3.3 Disease severity of late blight on whole plants



Disease severity (% of necrotic leaf-surface area) on detached (a) or whole plant (b) leaves. Russet burbank (-○-) or Kennebec (-●-) inoculated with *P. infestans* US-1 (less aggressive); Russet burbank (-□-) or Kennebec (-■-) inoculated with *P. infestans* US-8 (more aggressive).

Physiologic race determination

Physiologic races of the two isolates were different but both isolates had virulence factors to overcome at least 5 out of the 11 known *R* genes (Table 3.1). The physiologic race of the US-1 isolate was less complex than that of the US-8 isolate. The US-1 race was 1,2,4,7,9 while the US-8 isolate one was 1,2,3,4,6,7,8,9,10.

Table 3. 1 Physiological races of *P. infestans* isolates FA-1 (US-1) and D1901 (US-8).

		Potato *R* gene										
Isolate	**d.a.i.**	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
FA-1 (US-1)	**5**	+	+	-	+	-	-	+	-	-	-	-
	10	+	+	-	+	-	-	+	-	+	-	-
D1901 (US-8)	**5**	+	+	+	+	-	+	+	+	+	+	-
	10	+	+	+	+	-	+	+	+	+	+	-

d.a.i.: days after inoculation. +/-: Presence/absence of visible lesion area on the inoculated leaves. The race composition was determined by inoculation of potato differential cultivars carrying 11 known dominant R genes (R1-R11) to *P. infestans* with isolates FA-1 and D1901. The occurrence of lesion on the inoculated potato plant (+) represents that *P. infestans* isolate carry virulence factor which can overcome the R gene presented in that potato differential cultivar.

Differential accumulation of pal1 transcripts in potato leaflets inoculated with P. infestans US-1 or US-8 (Figures 3.4, 3.5, 3.6)

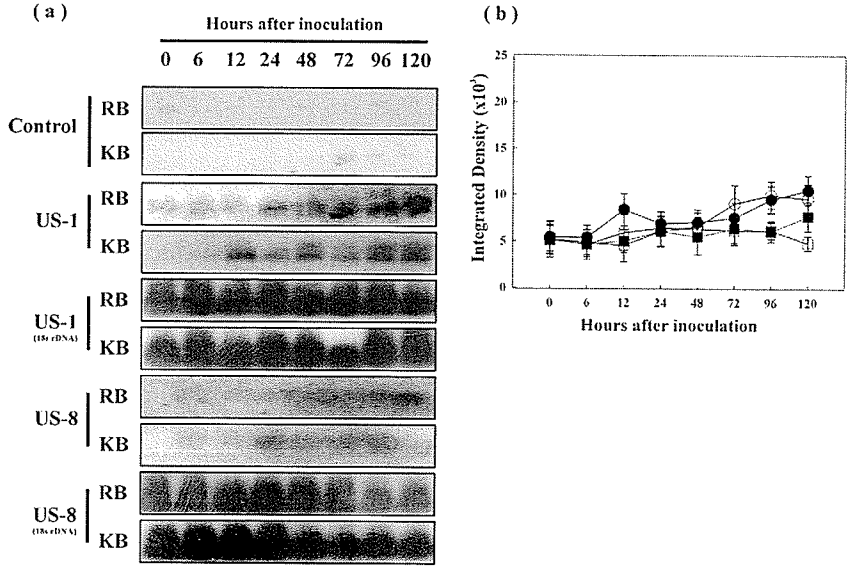
No noticeable difference in the induction of *pal1* was recorded in healthy leaflets (local, proximal and distal) of both Russet burbank and Kennebec sampled over the experimental period of 5 days (Figure 3.4). In inoculated samples, *pal1* was detected before the lesions became visible.

Local leaflets (Figure 3.4)

In Russet burbank leaflets inoculated with US-1 (local), the accumulation of *pal1* transcripts was observed from 24 - 48 h.a.i.. In contrast, the induction of *pal1* started 12 h.a.i. in Kennebec leaflets inoculated with US-1 (local). Thereafter, the level of *pal1*

transcripts was maintained at just about the detectable level in both cases until 120 h.a.i.. In Russet burbank and Kennebec leaflets inoculated with US-8 (local), mainly a baseline accumulation of *pal1* transcripts was detected with a slight increase in Kennebec around 24 h.a.i. and in Russet burbank around 120 h.a.i..

Figure 3.4 Northern blots of *pal1* expression on local leaflets



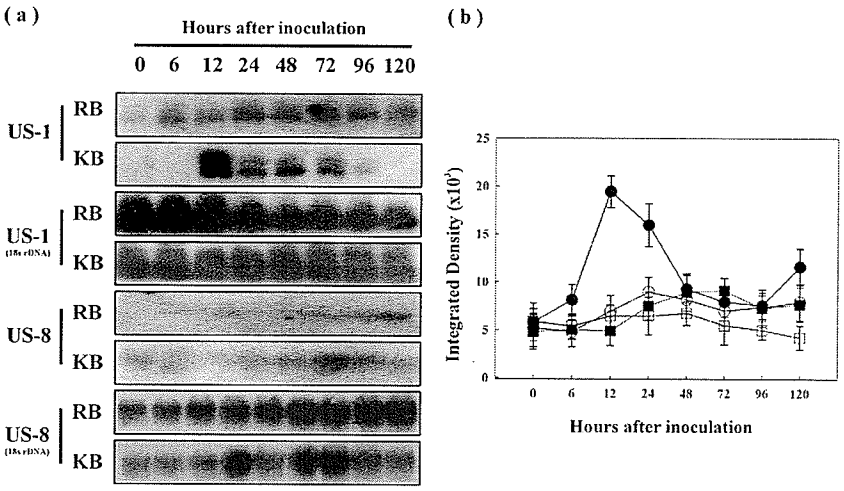
Northern gel blots showing *pal1* transcripts (a) and their relative abundance (b) on dot blots (means ± SE) in local leaflets of Russet burbank (RB) and Kennebec (KB) inoculated with isolates of *P. infestans* from two genotypes: US-1 (less aggressive) and US-8 (more aggressive). *18s r*DNA probe was used as internal control in the northern blot (a) to normalize the expression of *pal1*. No significant difference was observed in the accumulation of *18s r*DNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (-○-) RB x US-1; (-●-) KB x US1; (-□-) RB x US-8; (-■-) KB x US-8.

Proximal leaflets (Figure 3.5)

In un-inoculated Russet burbank leaflets, adjacent to the ones inoculated with US-1 (proximal), a baseline of *pal1* expression was observed starting at 6 h.a.i. with its highest level recorded at 24-72 h.a.i.. By contrast, in proximal Kennebec leaflets from leaves inoculated with US-1, a rapid increase in the accumulation of *pal1* transcripts was observed and the highest induction was recorded 12 h.a.i.. No accumulation of *pal1*

transcripts above the basal level was observed in proximal leaflets of Russet burbank plants inoculated with US-8 while a slight accumulation was detectable in their Kennebec counterparts around 72 h.a.i..

Figure 3.5 Northern blots of *pal1* expression on proximal leaflets



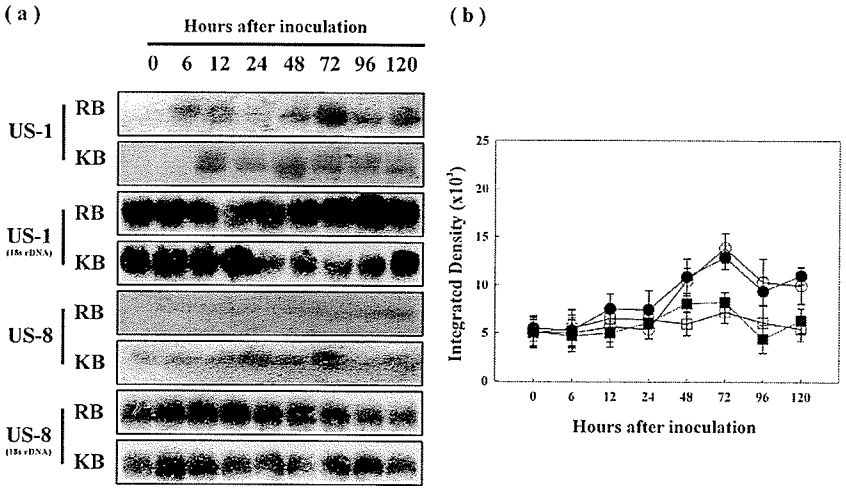
Northern gel blots showing *pal1* transcripts (a) and their relative abundance (b) on dot blots (means ± SE) in proximal leaflets of Russet burbank (RB) and Kennebec (KB) inoculated with isolates of *P. infestans* from two genotypes: US-1 (less aggressive) and US-8 (more aggressive). *18s r*DNA probe was used as internal control in the northern blot (a) to normalize the expression of *pal1*. No significant difference was observed in the accumulation of *18s r*DNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (-○-) RB x US-1; (-●-) KB x US1; (-□-) RB x US-8; (-■-) KB x US-8.

Distal leaflets (Figure 3.6)

In un-inoculated (distal) leaflets of Russet burbank from leaves adjacent to the ones inoculated with US-1, a baseline of *pal1* induction was perceptible at 6 h.a.i., but a great accumulation was recorded 72 h.a.i.. Comparatively, in distal leaflets of Kennebec plants inoculated with US-1, *pal1* transcripts accumulated to a detectable level since 12 h.a.i. with a maximum recorded 48 h.a.i.. There was no detectable accumulation of *pal1* transcripts in distal leaflets of Russet burbank plants inoculated with US-8, whereas an

accumulation was detected at 24 h.a.i in their Kennbec counterparts with a maximum recorded 72 h.a.i..

Figure 3.6 Northern blots of *pal1* expression on distal leaflets



Northern gel blots showing *pal1* transcripts (a) and their relative abundance (b) on dot blots (means ± SE) in distal leaflets of Russet burbank (RB) and Kennebec (KB) plants inoculated with isolates of *P. infestans* from two genotypes: US-1 (less aggressive) and US-8 (more aggressive). *18s r*DNA probe was used as internal control in the northern blot (a) to normalize the expression of *pal1*. No significant difference was observed in the accumulation of *18s r*DNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (-○-) RB x US-1; (-●-) KB x US1; (-□-) RB x US-8; (-■-) KB x US-8.

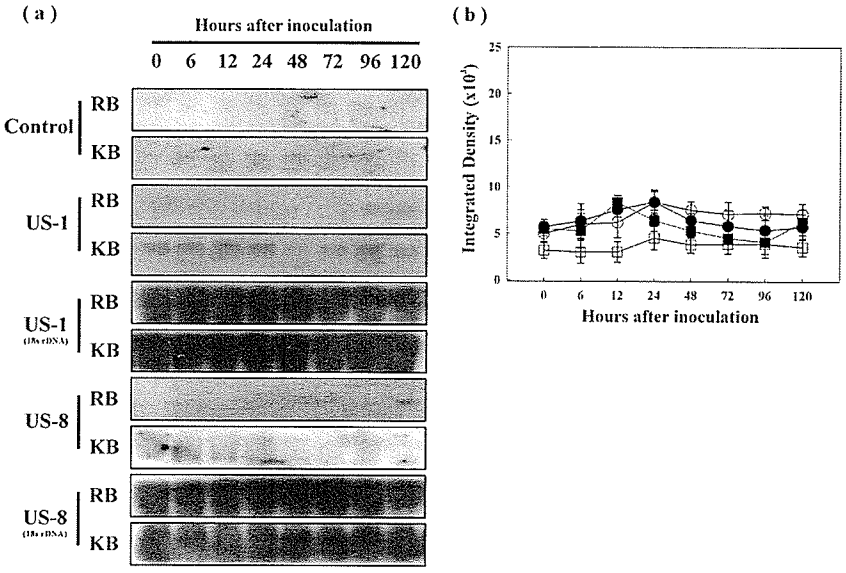
Spatio-temporal accumulation of pal1 transcripts (Figures 3.4, 3.5, 3.6)

The earliest accumulation of *pal1* transcripts was observed in proximal leaflets followed by local and distal leaflets. The induction of *pal1* in proximal leaflets was stronger than in local and distal leaflets. The pattern of *pal1* transcripts accumulation was also different in the two cultivars tested. Furthermore, the two isolates of *P. infestans* had different effects on the timing of *pal1* transcripts accumulation, which occurred earlier in response to the US-1 isolate than to the US-8 one.

Differential accumulation of hmgr2 transcripts in potato leaflets inoculated with P. infestans US-1 or US-8 (Figures 3.7, 3.8, 3.9)

No accumulation of *hmgr2* transcripts was observed overtime in healthy leaflets (local, proximal and distal) from Russet burbank, whereas a baseline accumulation was recorded in Kennebec (Figure 3.7). In inoculated plants, the accumulation of *hmgr2* transcripts preceded the development of visible lesions. In general, the pattern of accumulation was different from that of *pal1*.

Figure 3.7 Northern blots of *hmgr2* expression on local leaflets



Northern gel blots showing *hmgr2* transcripts (a) and their relative abundance (b) on dot blots (means ± SE) in local leaflets of Russet burbank (RB) and Kennebec (KB) inoculated with isolates of *P. infestans* from two genotypes: US-1 (less aggressive) and US-8 (more aggressive). *18s r*DNA probe was used as internal control in the northern blot (a) to normalize the expression of *hmgr2*. No significant difference was observed in the accumulation of *18s r*DNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (-○-) RB x US-1; (-●-) KB x US1; (-□-) RB x US-8; (-■-) KB x US-8.

Local leaflets (Figure 3.7)

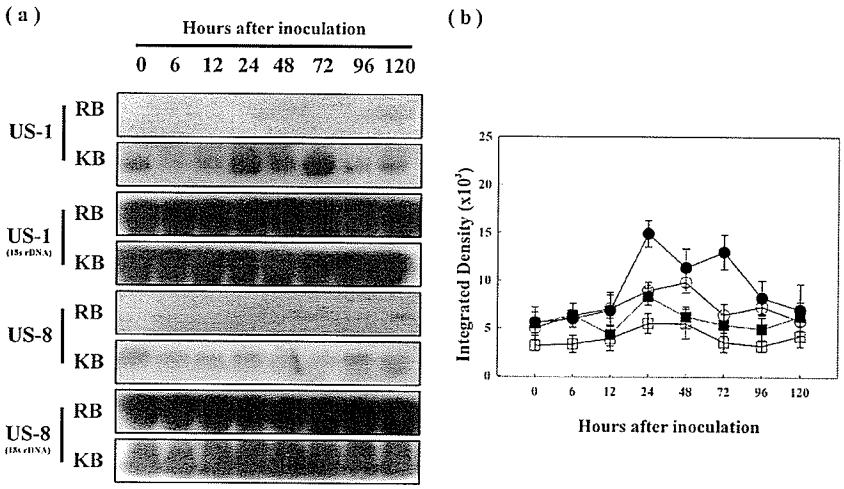
There was no accumulation of *hmgr2* mRNA transcripts in local leaflets of Russet burbank inoculated with US-1. In local leaflets inoculated with US-8, a slight induction

was recorded 120 h.a.i.. In Kennbec leaflets only a baseline accumulation was observed overtime in both cases.

Proximal leaflets (Figure 3.8)

A strong transient accumulation of *hmgr2* transcripts was recorded 24 to 72 h.a.i. in proximal leaflets of Kennebec plants inoculated with US-1. In contrast, no noticeable accumulation was observed in their Russet burbank counterparts. No noticeable accumulation of *hmgr2* mRNA transcripts was observed in proximal leaflets of either Russet burbank or Kennebec plants inoculated with US-8, where only a baseline was perceptible.

Figure 3.8 Northern blots of *hmgr2* expression on proximal leaflets

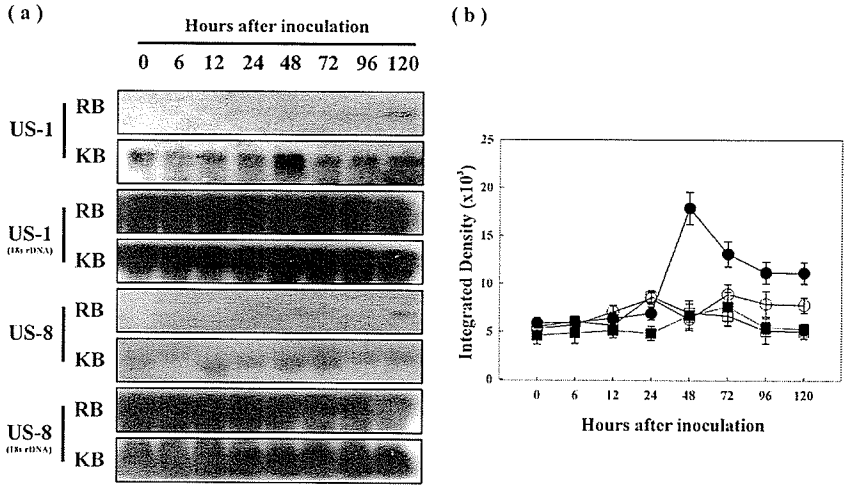


Northern gel blots showing *hmgr2* transcripts (a) and their relative abundance (b) on dot blots (means ± SE) in proximal leaflets of two potato cultivars Russet burbank (RB) and Kennebec (KB) inoculated with isolates of *P. infestans* from two genotypes: US-1 (less aggressive) and US-8 (more aggressive). *18s r*DNA probe was used as internal control in the northern blot (a) to normalize the expression of *hmgr2*. No significant difference was observed in the accumulation of *18s r*DNA transcripts in most samples. (-○-) RB x US-1; (-●-) KB x US1; (-□-) RB x US-8; Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (-■-) KB x US-8.

Distal leaflets (Figure 3.9)

No visible accumulation of *hmgr2* transcripts was observed in distal leaflets of Russet burbank plants inoculated with either US-1 or US-8 except for a weak accumulation 120 h.a.i. in response to US-1. However, a strong accumulation of *hmgr2* transcripts was observed 48 h.a.i. in distal leaflets of Kennebec plants inoculated with US-1. The accumulation level remained high until 120 h.a.i.. A transient accumulation of *hmgr2* transcripts was observed also in distal leaflets of Kennebec plants inoculated with US-8 48 to 72 h.a.i., but not as strong as with US-1.

Figure 3.9 Northern blots of *hmgr2* expression on distal leaflet



Northern gel blots showing *hmgr2* transcripts (a) and their relative abundance (b) on dot blots (means ± SE) in distal leaflets of Russet burbank (RB) and Kennebec (KB) inoculated with isolates of *P. infestans* from two genotypes: US-1 (less aggressive) and US-8 (more aggressive). *18s r*DNA probe was used as internal control in the northern blot (a) to normalize the expression of *hmgr2*. No significant difference was observed in the accumulation of *18s r*DNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (-○-) RB x US-1; (-●-) KB x US1; (-□-) RB x US-8; (-■-) KB x US-8.

Spatio-temporal accumulation of hmgr2 transcripts (Figure 3.7, 3.8, 3.9)

The *hmgr2* transcripts accumulated early in proximal leaflets, then in distal leaflets of Kennebec plants inoculated with US-1, whereas there was no induction of *hmgr2* in local leaflets of Russet burbank or Kennebec, in response to US-1 or US-8, over the period of 120 h.a.i.. The level of *hmgr2* transcripts observed in distal leaflets was

relatively stronger than in proximal leaflets. Comparison between the two tested cultivars showed differences in the patterns of accumulation of *hmgr2* transcripts with no perceptible spatio-temporal accumulation in Russet burbank leaflets either in response to US-1 or US-8 and a strong transient accumulation in Kennebec leaflets especially in response to US-1. Induction of *hmgr2* was greater in response to US-1 than to US-8, especially in Kennebec, a moderately tolerant cultivar to *P. infestans*.

3.5 Discussion

The US-8 isolate of *P. infestans* used in this study was more aggressive than the US-1 isolate on both cultivar Russet burbank and Kennebec. The necrotic lesions observed on Kennebec leaflets infected with US-1 initially looked like hypersensitive reaction but their progression expanded overtime to the rest of the leaflet surface. The differential pathogenicity of the two pathogen isolates was observed in both detached leaflets and whole plants, with a slower progression of US-1 (less aggressive) in the latter case. The faster disease progression on detached leaflets may be due, at least in part, to their "surviving" state as nutrients supply from the original plant is removed.

Recent advances in genetic, biochemical and cytological characterization of disease resistance have shown constant relation between hypersensitive response and all known forms of resistance to *Phytophthora* species (Kamoun 2001; Kamoun et al. 1999; Vleeshouwers et al. 2000a). Hypersensitive response follows recognition between specific plant receptors encoded by resistance (*R*) genes and pathogen signal molecules encoded by avirulence genes (*avr*) (Dangl & Jones 2001; Hammond-Kosack & Jones 1997; Lamb 1994; Staskawicz et al. 1995). Cultivar Kennebec harbors resistance gene

R-1 and was shown to be resistant to some US-1 isolates (Kolomiets et al. 2000). However, in the present study, the US-1 isolate was shown to be virulent on both Kennebec and the potato differential line carrying *R-1* resistance gene as did the US-8 isolate. Therefore, the resistance of Kennebec or Russetburbank to two *P. infestans* isolates used in this study should be considered under the category of non race-specific resistance.

Evidence suggesting that *pal* and *hmgr* genes are related to potato resistance against *P. infestans* has been reported by many authors. Cuypers & Hahlbrock (1988) early reported that the accumulation of *pal* mRNA and proteins was faster during an incompatible potato x *P. infestans* interaction than during a compatible interaction. Similarly, Yoshioka *et al.* (1996) has found that levels of *hmgr* mRNA and enzyme activity were higher during an incompatible than during a compatible interaction. Plant defense related phenolic compounds and sesquiterpenoid phytoalexins have been also shown to accumulate more rapidly in incompatible than in compatible interactions (Cuypers & Hahlbrock 1988). In the present study, we compare data in two compatible interactions, in both cultivars, where the two isolates have different levels of aggressiveness. Transcripts of *pal1* and *hmgr2* increased in both Russet burbank and Kennebec leaflets inoculated with US-1. In response to this isolate, the accumulation of either gene's transcripts occurred at an earlier stage of infection than in response to US-8, and remained at a level above the expression baseline. In Kennebec in particular, the increase in *pal1* and *hmgr2* transcripts in the presence of US-8 (more aggressive) isolate was slower and much weaker, as compared to the relatively strong and early

induction of these transcripts after inoculation with US-1 (less aggressive). This was in line with the differential disease responses of cultivars Kennebec (moderately tolerant) and Russet burbank (highly susceptible) to the two isolates.

We also investigated the accumulation of *pal1* and *hmgr2* transcripts in leaflets from different parts of potato plants. In Kennbec leaflets, there was a 12-24 hours delay in induction of *pal1* and *hmgr2* mRNA in distal than in proximal leaflets. Similarly, Beligni *et al.* (1999) showed that a GRAPDHc (glyceraldehydes-3-phosphate dehydrogenase) was induced 12 hours earlier in un-inoculated short distance leaves (proximal) than in long distance leaves (distal). It might be reasonable to assume that the delay in *pal1* and *hmgr2* induction between proximal and distal leaflets is related to the time required for a signal(s) movement from inoculated leaflets to distant leaflets. However, more information is needed before we speculate on the nature of the translocated signal(s) during this interaction. Systemic plant responses have been described for several decades (Hutcheson 1998), and salicylic acid, one of the potential signal molecules, has been previously shown to move from its production site in the inoculated leaves to remote leaves by the phloem (Shulaev et al. 1995).

There was no or a weak and late induction of *pal1* and *hmgr2* in local leaflets inoculated with either *P. infestans* US-1 or US-8 genotype isolates while a noticeable early induction was observed in proximal and distal leaflets. This result is in agreement with previous findings by Choi *et al.* (1992) and Yoshioka *et al.* (1996) who reported the suppression of *hmgr* and *pal* mRNA accumulation in potato leaves infected by *P. infestans*, respectively, and with other findings reporting different suppressors of potato

defense response against *P. infestans* (Andreu et al. 1999; Ozeretskovskaya et al. 2001). Inhibition of *pal* expression was also found in other plant-pathogen interactions. Lee *et al.* (Lee et al. 1992) have reported that there was a substantial suppression, in the presence of the pathogen, of mRNA levels in susceptible tomato lines as compared to resistant ones. All these findings suggest that the differential expression and inhibition of *pal* and *hmgr* in response to US-1 and US-8 may be directly related to the potato defense suppressors, previously reported in *P. Infestans* (Boyd et al. 1994; Yoshioka et al. 1996). The nature and the mode of action of these suppressors are still unclear. Andreu *et al.* (1999) showed an inhibition of the accumulation of sesquiterpene phytoalexins in potato tubers by glucans produced by virulent isolates of *P. infestans* as compared to less virulent ones. Ozeretskovskaya *et al.* (2001) observed that β-glucans isolated from *P. infestans* cell walls caused a local and race-specific suppression of the plant defense response. Under race-specific interaction, Ordoñez *et al.* (1997) reported the presence of dominant specific suppressors of *R* gene function that were elicited by specific isolates of the fungus and that segregated in the host populations independently. One original finding in the present study is that despite the potential suppression at the site of inoculation, especially with the US-8 isolate, *pal1* and *hmgr2* expression were still detected in tissues remote from the inoculation site. This suggests the possible translocation of signal(s) escaping the initial suppression by the pathogen to healthy parts of the plant. Another hypothesis that conforms with our finding is that the effect of some *P. infestans* suppressors is only local, as suggested for β-glucans by Ozereskovskaya *et al.* (2001).

The level of expression of *pal1* and *hmgr2* was different in potato plants inoculated with US-1 and US-8 isolates. This differential expression of the two genes is possibly regulated at the transcription level as shown for *pal* on French bean cell suspension (Bolwell 1992) and for *hmgr* in eukaryotes in general (Goldstein & Brwon 1990; Istvan et al. 2000). Since we did not examine the enzymatic activity of PAL and HMGR in the present study, a post-transcriptional regulation mechanism of these two proteins could not be excluded. With this regard, it has been reported that HMGR activity is controlled both at the translation and the transcription levels in aged potato tubers (Yoshioka et al. 1996). Few studies have suggested also that the binding activities of promoters *trans*-binding factors are responsible for the activation of genes involved in the phenlypropanoid pathway (Arias et al. 1993; Kato et al. 1995). Although no information is available on the mechanism responsible for the initiation of *pal1* and *hmgr2* genes' transcription, they seemed to be coordinately induced and/or slightly suppressed by US-1 and highly suppressed in the presence of the US-8 isolate. This suggestion is in agreement with the findings reported by Andreu *et al.* (1999) who showed that the accumulation of sequiterpene phytoalexins, initially controlled by the activity of HMGR, was not affected by glucans produced by less virulent isolates of *P. infestans* while it was highly suppressed by those produced by highly virulent isolates.

Clearly, many more studies are necessary to dissect and understand the complex mechanisms involved in the gene regulation of potato defense responses to pathogens like *P. infestans*. However, in the light of the present findings, it may be suggested that the differential responses of Kennebec to US-1 and US-8 isolates might be partially due

to the timing and the level of induction of potato defense genes such as *pal1* and *hmgr2*. A suppression of induction of plant defense genes by the US-8 isolate at the local site of infection and a translocation of a signal to other healthy parts of the plant might be also hypothesized.

4.0 Local and distal gene expression of *pr-1* and *pr-5* in potato leaves inoculated with isolates from the previously (US-1) and currently predominant (US-8) genotypes of *Phytophthora infestans* (Mont.) de Bary

4.1 Abstract

The time-course and the spatial accumulation of PR-proteins *pr-1* and *pr-5* gene transcripts were investigated in two potato cultivars differing in their levels of susceptibility to late blight, caused by *Phytophthora infestans* (mont.) de Bary. Cultivars Russet Burbank (susceptible) and Kennebec (moderately tolerant) were inoculated with either *P. infestans* genotype US-1 (previously predominant) or US-8 (currently predominant). A strong induction of both genes was detected in both cultivars inoculated with either *P. infestans* genotype, as compared to the healthy-controls. The accumulation of transcripts from both genes occurred earlier in Kennebec than in Russet burbank leaflets. By comparing the two *P. infestans* isolates tested, a stronger and earlier induction of both PR genes was recorded in response to P. infestans US-1 as compared to US-8 genotype. The spatio-temporal profiling of *pr-1* and *pr-5* genes expression showed a strong and early accumulation of transcripts at the local infection site, a late and intermediate level of induction at the proximal site, and no or very weak induction at a distal site remote from the infection site. These results show that *pr-1* and *pr-5* genes both are related to the defense mechanisms of potato to late blight, and that the higher infection success of *P. infestans* with US-8 as compared to US-1 might be due to the late and/or the weak induction of these defense genes.

4.2 Introduction

During a potato-pathogen interaction, compatibility is constitutively expressed as a disease gradient among potato plants depending on their level of susceptibility to the pathogen. Incompatibility occurs through an active hypersensitive-like reaction, resulting from a specific recognition between a receptor of the host and a corresponding elicitor of the pathogen (Michelmore & Meyers 1998; Kawchuk et al. 2001), followed by the activation of defense genes and the production of antimicrobial substances (Yoshioka et al. 1999). This leads to an early localized cell death around the infection site that often limits the pathogen progression. During this process, up- and down-regulations of many genes, including those coding pathogenesis-related (PR) proteins are observed (Choi et al. 1994; van Loon & van Strien 1999).

Pathogenesis-related proteins accumulate rapidly at the intra- or extra-cellular level under various biotic and abiotic stimuli, including fungal, elicitor, and physical or chemical treatments (van Kan et al. 2005; van Loon & van Strien 1999; van Loon 1999; Kim & Hwang 2000; Graham et al. 2003; Przymusinski et al. 2004). In case of induction under pathological conditions, the importance of these proteins to plant defense has been related to (i) their rapid and early accumulation (van Loon & van Strien 1999), often associated with incompatibility (Linthorst 1991), (ii) their antimicrobial activity (Woloshuk et al. 1991; Ponstein et al. 1994; Beerhues & Kombrink 1994; Fung et al. 2002; Velazhahan & Muthukrishnan 2003), and (iii) their ability to reduce symptom development (Niderman et al., 1995; Liu et al., 1994). Many studies have reported a correlation between the timing of *pr* gene expression and the initiation and the duration of systemic acquired resistance (SAR) (McGee et al. 2001;

Vleeshouwers et al. 2000b; Vleeshouwers et al. 2000a), although the causal-effect relationship between the expression of PR-proteins and SAR has never been thoroughly proven. PR proteins were initially isolated from tobacco showing a hypersensitive reaction against tobacco mosaic virus (van Loon & van Kammen 1970). They have thereafter been found in a wide range of both mono- and dicotyledonous plant species (van Loon 1999). These proteins are determined by the type of interaction between the host and the pathogen even though their induction is not specific to one particular pathogen (Cuypers & Hahlbrock 1988). In potato leaves challenged by *Phytophthora infestans*, induced PR proteins differ from those isolated from tobacco by their numbers and by their function during the infection process (Friends 1991). In many cases, this function is related to their antifungal activity. For example, members of the PR-2 family are known for their β-1,3-glucanase activity (Beerhues & Kombrink 1994; Ji & Kuc 1996), PR-3 for their chitinolytic activity (van Loon & van Strien 1999; van Loon 1999), and PR-5 for their osmo-permeabilization of fungal plasma membranes (van Loon et al. 1994; Abad et al. 1996). The mode of action of members of the PR-1 family (van Loon & van Strien 1999) remains unclear, except that in tobacco and tomato such proteins inhibit zoospore germination and mycelial growth of *P. infestans* (Niderman et al. 1995).

P. infestans is the oomycete causing late blight, and is a major constraint to potato production around the world (Duncan 1999; Fry et al. 1993). Disease management has mostly relied on integrated management strategies based on disease monitoring, some cultural practices, and heavy use of chemicals (Daayf & Platt 2000;

Duncan 1999). The population structure of this pathogen has changed during the last two decades, including an increase in aggressiveness and insensitivity to chemical fungicides (Daayf & Platt 2001a; Daayf & Platt 2001b). In North America, the old US-1 genotype has been displaced by US-8, which is more aggressive on most potato cultivars. This genotype causes larger lesions, produces higher numbers of sporangia, and has shorter latent periods (Kato et al. 1997).

In the potato - *P. infestans* system, many aspects of this interaction have been studied (Cuypers & Hahlbrock 1988; Vleeshouwers et al. 2000b; Vleeshouwers et al. 2000a). However, no data is available about the differential responses of potato to the previously (US-1) *versus* currently predominant genotype (US-8) of this pathogen. The objectives of the present study were: (i) to assess the differential accumulation of *pr-1* and *pr-5* transcripts in two potato cultivars Russet Burbank (susceptible) and Kennebec (moderately tolerant), in response to inoculations with US-1 and US-8 genotypes, and (ii) to follow such gene expression both overtime: time after inoculation, and space: local inoculation site, proximal (leaflets adjacent to the inoculated leaflet) and distal leaflets (from adjacent leaves) remote from the inoculation site.

4.3 Materials and Methods

Plant materials

Tuber seeds of potato cultivars Russet Burbank and Kennebec were used to generate plants under controlled conditions. The choice of these two cultivars was justified by the fact that Russet burbank is susceptible to late blight and widely used as a commercial cultivar while Kennebec is known to be moderately tolerant to this

disease. Plants were grown in clay pots containing soil-sand-peat-perlite mixture (4:4:4:1) in a growth room maintained at 20±2°C with 16h photoperiod.

Pathogens

One isolate of *P. infestans* (FA1) belonging to the previously predominant US-1 genotype (more aggressive) and one (D1901) belonging to the currently predominant US-8 (more aggressive) were used in this study. The physiological races of the two isolates were previously determined by their reactions on a differential set of potato cultivars harboring specific resistance genes *R-1* to *R-11*. The physiologic race of FA1 isolate was 1,2,4,7,9 while that of the D1901 isolate was found to be 1,2,3,4,6,7,8,9,10 (Wang et al. 2005b). Both isolates were maintained on rye B medium at 20±2°C as described by Goodwin (1995) with repetitive inoculations followed by re-isolations from potato leaves every 3 to 4 months in order to maintain their virulence.

Inoculation procedure

For inoculations, 5 to 6 week old potato plants were used. Inoculum was prepared from both US-1 and US-8 isolates by smashing down 10 to 14-days old *P. infestans* cultures using distilled water. Sporangia were then harvested 24h later in sterile distilled water and the suspensions were adjusted at 5×10^6 sporangia per ml. Inoculations were performed on the primary leaflet of the 3rd or 4th leaves of potato plants by placing tiny droplets for a total of 100 µl on their surface. Inoculated potato plants were kept for 48 hours in a moisture chamber with 100% relative humidity. All the experimental design schemes were randomized complete blocks with three blocks

and three replicates per each treatment. The whole experiment was repeated independently twice.

Leaf harvesting and total RNA preparation

Three types of leaves were collected: local leaves represented by leaflets of the primary inoculated leaves; proximal leaves, which are the secondary non-inoculated leaflets of the primary leaves; and distal leaves in reference to the leaflets from other non-inoculated leaves. The time-course of harvest was 0, 3, 6, 12, 24, 48, 72, 96, and 120 h.a.i.. All the harvested leaf samples were frozen in liquid nitrogen and immediately stored at -80°C until further use. For total RNA extraction, 100 mg FW generated from 6 leaf samples of each type (local, proximal, and distal) were used. They were then ground to a fine powder within a pre-cooled mortar in liquid nitrogen. Total RNA was isolated following the method of Verwoerd (1989) and quantified at A_{260}. Two separate RNA extractions were performed from each independent experiment.

RNA dot and gel blot analysis

Dot blots were performed by depositing 10 μg of denatured total RNA of each sample on the Hybond-N^+ membrane (Hoffmann-La Roche Ltd., Mississauga, ON, Canada) while gel blots were carried out using 10 μg of RNA pre-separated by electrophoresis under denaturing conditions and transferred onto Hybond-N^+ membrane. The pre-hybridizations were performed in DIG Easy high solution at 50°C for 3 to 4 hours. The same solution was used for the hybridization at 50°C during 18 h and the membranes were washed at room temperature using 2X SSC containing 0.1%

SDS for 15 min. A second washing of the membranes was performed using 0.5% SSC containing 0.1% SDS at 68°C for 15 min.

Probe for hybridization

For probes generation from DNA templates, the following fragments were used: 589bp of *pr-1* gene, 380bp of *pr-5* gene and 686 bp fragment of 18s RNA (Genebank no. 67238). The probes were then labeled using a PCR DIG labeling kit (Hoffmann-La Roche Ltd., Mississauga, ON, Canada) following the manufacturer's recommendations. The authenticity of the amplicfication product was verified by electorphoresis and restriction enzyme digestion. The primers were designed using OligoPerfect™ Designer software (Invitrogen™ Life Science Software, Invitrogen Inc., ON, Canada) and the primers' sequences used for *pr-1*, *pr-5*, and 18s RNA probe synthesis were :

pr-1-F 5'-TCACTCTTGTGATGCCCAAA-3'

pr-1-R 5'-AGTGGAAACAAGAAGATGCA-3'

pr-5-F 5'-TAAAGCTTCCGGCGTATTTG-3'

pr-5-R 5'-AATCGGTAGGACCACATGGA-3'

18S-F TAGATAAAAGGTCGACGCGG-3'

18S-R 5'-TCATTACTCCGATCCCGAAG-3'.

Image analysis

Dot blots of the *pr-1* and *pr-5* transcripts were repeated three times. In order to avoid possible variation in image development due to slightly different exposure times, 5 ng of DIG labeled control DNA (Hoffmann-La Roche Ltd., Mississauga, ON,

Canada) was loaded on the right corner of each membrane and used as a control. The dots on films were photographed using a digital camera at 1200 dpi resolution (Sony Ltd., Toronto, ON, Canada) and analyzed using ImageJ 1.32 (http://rsb.info.nih.gov/ij/download.html). The expression profiles of the *pr-1* and *pr-5* transcripts were calculated by integration of the density of each dot and an average of three replicates was considered in the final construction. Corrections of slight differences in loading were made by normalizing the results against the constitutively expressed 18s RNA gene.

4.4 Results

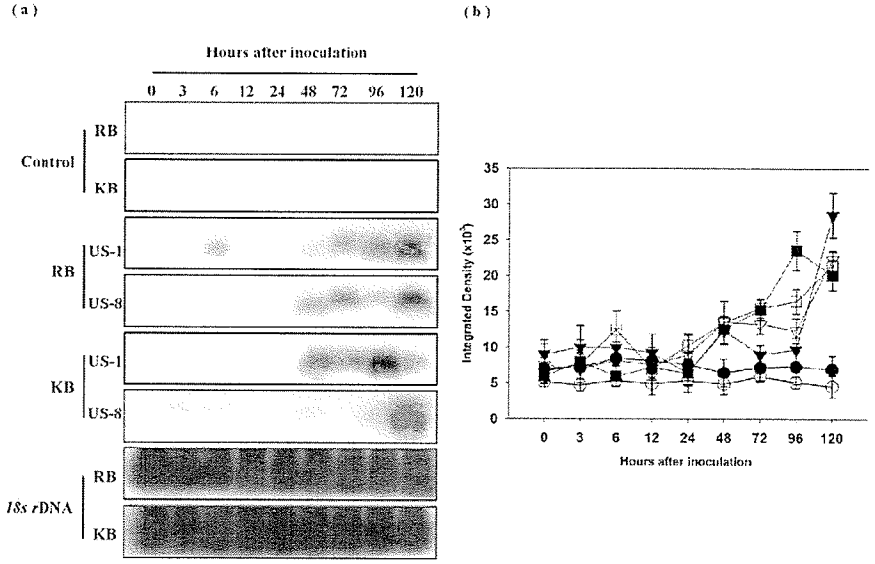
Spatio-temporal accumulation of pr-1 transcripts

Local leaflets (Figure 4.1)

No accumulation of *pr-1* transcripts was observed in healthy-control local leaflets of both tested cultivars over the experimental period of 5 days. However, a transient strong accumulation of *pr-1* mRNA transcripts was observed 48 h.a.i. in Russet burbank and Kennebec leaflets inoculated with either US-1 or US-8. This accumulation was recorded prior to the appearance of lesions (72 h.a.i., date not shown) and occurred at a higher level in Kennebec than in Russet burbank leaflets. No difference in accumulation of *pr-1* transcripts was observed in susceptible Russet burbank leaflets inoculated with either US-1 or US-8. The strongest accumulation was recorded 120 h.a.i. in both cases. A weak and early accumulation of *pr-1* mRNA was observed 6 h.a.i. in response to US-1 and not to US-8. In Kennebec leaflets (moderately tolerant), *pr-1* transcripts accumulated in response to US-1 (less aggressive) earlier than to US-8

(more aggressive). The highest level of *pr-1* transcripts accumulation was recorded at 96 h.p.i. with US-1 and 120 h.a.i. with US-8.

Figure 4. 1 Northern blots of *pr-1* expression on local leaflets

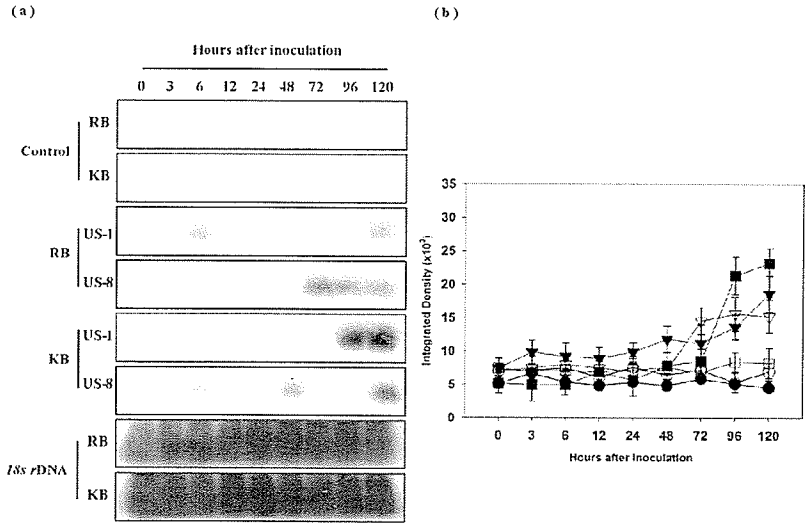


Northern gel blots showing *pr-1* transcripts accumulation (a) and densitometric analysis of the abundance of *pr-1* mRNA (b) on dot blots (means ± SE) in local leaflets of two potato cultivars, Russet burbank (highly susceptible, RB) and Kennebec (moderately tolerant, KB) inoculated with isolates of *P. infestans* from genotypes: US-1 (less aggressive) and US-8 (more aggressive). *18s r*DNA probe was used as internal control in the northern blot (a) to normalize the expression of *pr-1*. No significant difference was observed in the accumulation of *18s r*DNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (-○-) RB control; (-●-) KB control; (-□-) RB x US-1; (-■-) KB x US-1; (-▽-) RB x US-8; (-▼-) KB x US-8.

Proximal leaflets (Figure 4.2)

As observed in local leaflets, there was no accumulation of *pr-1* transcripts in Russet burbank and Kennebec healthy-control proximal leaflets. The accumulation of *pr-1* mRNA in Russet burbank leaflets inoculated with either US-1 or US-8 was weak except at 72 h.a.i. in response to US-8. A weak and early accumulation of *pr-1* mRNA was observed 6 h.a.i. in response to US-1 and not to US-8. In Kennebec leaflets, *pr-1* transcripts accumulated strongly in response to US-1 as compared to US-8. This accumulation occurred also earlier with US-1 (96 h.a.i) *versus* US-8 (120 h.a.i.).

Figure 4. 2 Northern blots of *pr-1* expression on proximal leaflets

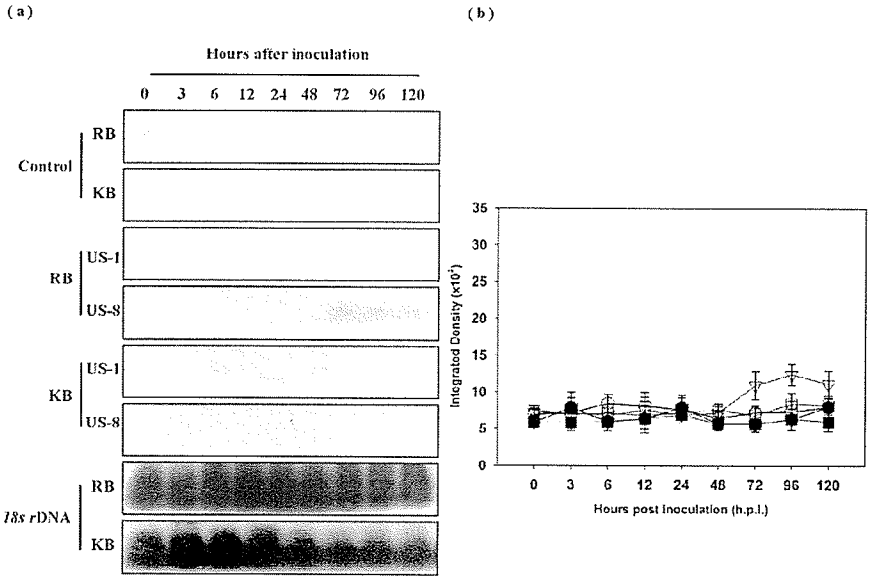


Northern gel blots showing *pr-1* transcripts accumulation (a) and densitometric analysis of the abundance of *pr-1* mRNA (b) on dot blots (means ± SE) in proximal leaflets of two potato cultivars Russet burbank (highly susceptible, RB) and Kennebec (moderately tolerant, KB) inoculated with isolates of *P. infestans* from genotypes: US-1 (less aggressive) and US-8 (more aggressive). *18s r*DNA probe was used as internal control in the northern blot (a) to normalize the expression of *pr-1*. No significant difference was observed in the accumulation of *18s r*DNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (-○-) RB control; (-●-) KB control; (-□-) RB x US-1; (-■-) KB x US-1; (-▽-) RB x US-8; (-▼-) KB x US-8.

Distal leaflets (Figure 4.3)

There was no accumulation of *pr-1* mRNA transcripts in healthy-control distal leaflets originating from Russet burbank or Kennbec. No accumulation of these transcripts was observed also in both inoculated cultivars with either US-1 or US-8 except a very weak accumulation in Russet burbank leaflets in response to US-8 starting at 72 h.a.i..

Figure 4. 3 Northern blots of *pr-1* expression on distal leaflets



Northern gel blots showing *pr-1* transcripts accumulation (a) and densitometric analysis of the abundance of *pr-1* mRNA (b) on dot blots (means ± SE) in distal leaflets of two potato cultivars Russet burbank (highly susceptible, RB) and Kennebec (moderately tolerant, KB) inoculated with two isolates of *P. infestans* from two genotypes: US-1 (less aggressive) and US-8 (more aggressive). *18s r*DNA probe was used as internal control in the northern blot (a) to normalize the expression of *pr-1*. No significant difference was observed in the accumulation of *18s r*DNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (-○-) RB control; (-●-) KB control; (-□-) RB x US-1; (-■-) KB x US-1; (-∇-) RB x US-8; (-▼-) KB x US-8.

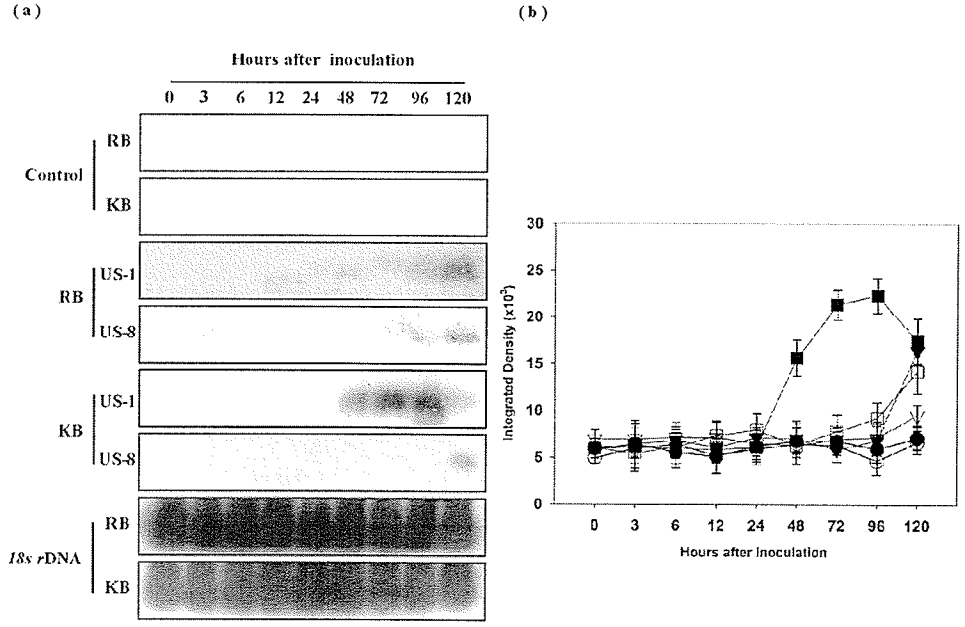
Spatio-temporal accumulation of pr-5 transcripts

Local leaflets (Figure 4.4)

No accumulation of *pr-5* mRNA transcripts was observed in Russet burbank or Kennebec healthy-control local leaflets. However, these transcripts accumulated differentially in both Russet burbank and Kennebec leaflets inoculated with either US-1 or US-8. The earliest strong accumulation of *pr-5* transcripts was observed in Kennebec leaflets inoculated with US-1 48 h.a.i.. No noticeable accumulation of *pr-5* transcripts

was observed before 120 h.a.i. in Russet burbank leaflets inoculated with either US-1 or US-8 and in Kennebec leaflets inoculated with US-8.

Figure 4. 4 Northern blots of *pr-5* expression on local leaflets

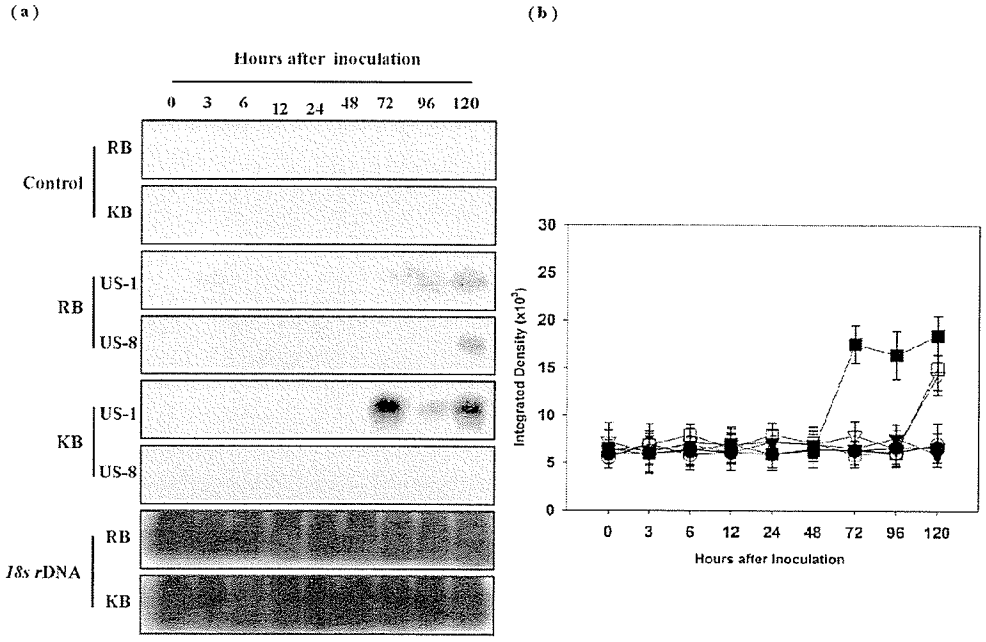


Northern gel blots showing *pr-5* transcripts accumulation (a) and densitometric analysis the abundance of *pr-5* mRNA (b) on dot blots (means ± SE) in local leaflets of two potato cultivars Russet burbank (highly susceptible, RB) and Kennebec (moderately tolerant, KB) inoculated with isolates of *P. infestans* from genotypes: US-1 (less aggressive) and US-8 (more aggressive. *18s r*DNA probe was used as internal control in the northern blot (a) to normalize the expression of *pr-5*. No significant difference was observed in the accumulation of *18s r*DNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (-○-) RB control; (-●-) KB control; (-□-) RB x US-1; (-■-) KB x US-1; (-∇-) RB x US-8; (-▼-) KB x US-8.

Proximal leaflets (Figure 4.5)

There was no accumulation of *pr-5* transcripts in Russet burbank and Kennebec healthy-control proximal leaflets. The strongest and earliest accumulation of *pr-5* transcripts occurred at 72 h.a.i. in Kennebec leaflets in response to US-1. A weak accumulation of *pr-5* transcripts was also perceptible 120 h.a.i. in Russet burbank leaflets in response to both US-1 and US-8, whereas no accumulation was recorded in Kennebec leaflets in response to US-8.

Figure 4. 5 Northern blots of *pr-5* expression on proximal leaflets

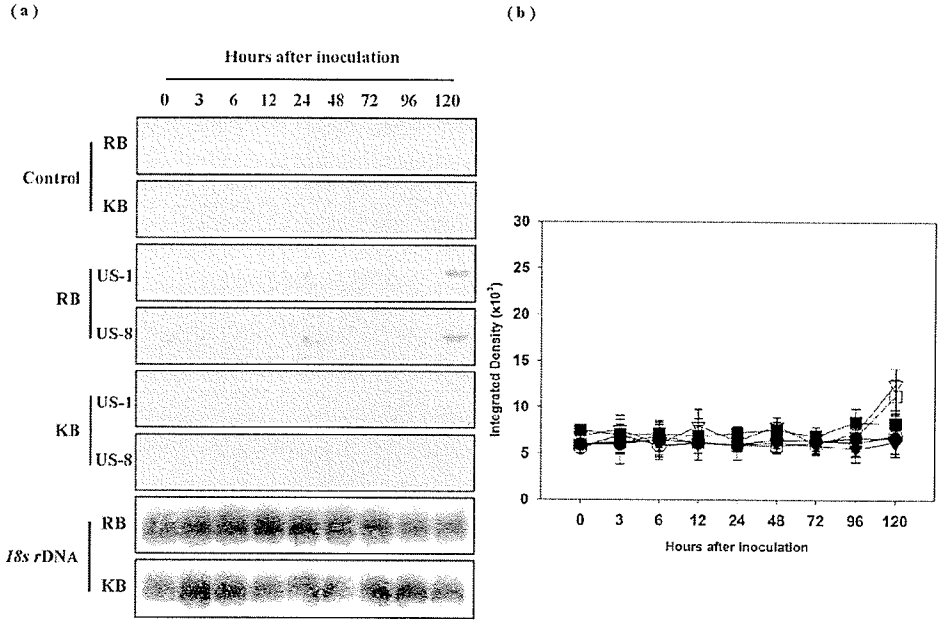


Northern gel blots showing *pr-5* transcripts accumulation (a) and densitometric analysis of the abundance of *pr-5* mRNA (b) on dot blots (means ± SE) in proximal leaflets of two potato cultivars Russet burbank (highly susceptible, RB) and Kennebec (moderately, KB) inoculated with isolates of *P. infestans* from genotypes: US-1 (less aggressive) and US-8 (more aggressive). *18s r*DNA probe was used as internal control in the northern blot (a) to normalize the expression of *pr-5*. No significant difference was observed in the accumulation of *18s r*DNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (-○-) RB control; (-●-) KB control; (-□-) RB x US-1; (-■-) KB x US-1; (-∇-) RB x US-8; (-▼-) KB x US-8.

***Distal leaflets* (*Figure 4.6*)**

There was no accumulation of *pr-5* transcripts in Russet burbank and Kennebec healthy-control distal leaflets. As well, no accumulation of *pr-5* mRNA was observed in Russet burbank or Kennebec leaflets in response to US-1 or US-8 except for a very weak perceptible accumulation 120 h.a.i. in Russet burbank challenged by either US-1 or US-8.

Figure 4. 6 Northern blots of *pr-5* expression on distal leaflets



Northern gel blots showing *pr-5* transcripts accumulation (a) and densitometric analysis of the abundance of *pr-5* mRNA (b) on dot blots (means ± SE) in distal leaflets of two potato cultivars Russet burbank (highly susceptible, RB) and Kennebec (moderately tolerant, KB) inoculated with two isolates of *P. infestans* from genotypes: US-1 and US-8. *18s r*DNA probe was used as internal control in the northern blot (a) to normalize the expression of *pr-5*. No significant difference was observed in the accumulation of *18s r*DNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (-○-) RB control; (-●-) KB control; (-□-) RB x US-1; (-■-) KB x US-1; (-▽-) RB x US-8; (-▼-) KB x US-8.

Comparison between the spatio-temporal patterns of pr-1 and pr-5

The comparison of the accumulation of *pr-1* and *pr-5* transcripts over all the treatments analyzed showed an earlier and stronger induction of *pr-1* and *pr-5* genes in both tested cultivars in response to US-1 (less aggressive) than to US-8 (more aggressive). Accumulation of both genes' transcripts occurred strongly and earlier in Kennebec (moderately tolerant) than in Russet burbank (susceptible) leaflets. The spatial profiling of *pr-1* and *pr-5* genes showed a strong and early accumulation of transcripts at the local infection site, then an intermediate level of induction at the proximal site, and no or very weak induction at the distal site.

4.5 Discussion

The potato-*P. infestans* system has been used to study various biochemical aspects of host-pathogen interactions (Andreu et al. 1999; Bos et al. 2003; Niderman et al. 1995). Although a number of studies have been dedicated to profile *pr* genes during the infectious process (Niderman et al. 1995; Vleeshouwers et al. 2000b; Hoegen et al. 2002), no one has compared potato defense responses to the previously (US-1) *versus* currently predominant genotype of *P. infestans* (US-8).

In the present study, a strong and rapid accumulation of *pr-1* transcripts was observed *prior* to the appearance of the lesions on both susceptible Russet burbank and moderately tolerant Kennebec inoculated with US-1 (less aggressive). In comparison, such accumulation was rather late in response to US-8 (more aggressive). This result shows that *pr-1* gene can be quickly induced after the infection and concur with other findings showing an accumulation of *pr-1* transcripts 12 h.a.i. in detached potato leaflets inoculated with *P. infestans*, and in pepper leaves challenged with an incompatible pathogen (Hoegen et al. 2002; Kim & Hwang 2000). Interestingly, an early induction of *pr-1* was observed on RB inoculated with US-1 both in local and proximal leaflets. However, no hypothesis is available to explain such an early expression, except that it might be related to the successive alterations in *P. infestans* structure during early stages of infection as shown by Birch et al. (1999) and Avrova et al. (2003). Indeed, these authors have shown that *P. infestans* produces several cell types *prior* to and during the early stages of infection (formation and encytement of zoospores, production of germ tube, and development of appresoria, hyphae,

haustoria, and sporangiophores). Each of these developmental stages is under the control of specific genes that might generate a specific interaction with those of the host plant. Transcripts of *pr-5* accumulated also strongly and earlier in Kennebec (moderately tolerant) inoculated with US-1 (less aggressive) as compared to US-8 (more aggressive). These findings suggest that the level and the time-course accumulation of *pr-1* and *pr-5* transcripts might be related to the level of tolerance of Kennebec to *P. infestans* genotype US-1. The non-sufficient and/or the late accumulation of these transcripts might also be related to the higher infection success of US-8 on both cultivars as compared to US-1. Given the low sensitivity of the Northern blot technique in detecting differences between low levels of transcripts, these findings should be confirmed using a more accurate and sensitive method such as the real-time quantitative PCR.

Recent studies on *pr-1* and *pr-5* genes, have revealed that these genes were strongly expressed following the infection of various plant species by various pathogens (Hoegen et al. 2002; Park et al. 2005; Ponstein et al. 1994) and that they have antifungal activity (Abad et al. 1996; Hoegen et al. 2002; Niderman et al. 1995; Park et al. 2005; Ponstein et al. 1994). The induction of *pr-1* and *pr-5* observed here in potato cultivars Russet burbank and Kennebec inoculated with either *P. infestans* US-1 or US-8 isolates corroborated the possible involvement of these genes in the differential disease responses of the two cultivars to each genotype, and of each cultivar to the two genotypes.

The growth of *P. infestans* US-1 seems to be inhibited to some extent on Kennebec leaflets where the reaction appears to be a 'true' incompatible interaction (Lazarovits & Higgins 1976). The connection between the pathogen growth inhibition and the induced expression of PR genes suggests the involvement of one or several of these proteins in successful defense reactions against the pathogen. Indeed, many PR-proteins are known for their demonstrated anti-microbial activity *in vitro* or after overexpression in transgenic plants (Beerhues & Kombrink 1994; Niderman et al. 1995; Vleeshouwers et al. 2000b; Woloshuk et al. 1991). The overexpression of osmotin (PR-5) derived from tobacco has led to a reduction of *P. infestans* in potato (Liu et al. 1994), most likely *via* a permeabilization of the fungal plasma membrane (Abad et al. 1996). Elsewhere, the over-expression of PR-1a or PR-1b in tobacco to control Tobacco Mosaic Virus (Cutt et al. 1989; Linthorst et al. 1989) has been shown to enhance significantly the resistance levels to two oomycetes (Alexander et al. 1993). The antifungal effect of potato PR-1 on *P. infestans* through inhibition of both zoospore germination and mycelial growth has also been pointed out (Niderman et al. 1995) even though the mechanism of such effect is still unknown.

The local and distal accumulation of PR-1 and PR-5 mRNA transcripts observed in the present study were different depending on the potato cultivar, the pathogen genotype and the stratum of leaf examined. The transcripts accumulated earlier in local, then in proximal leaves with a delay of 24 to 72 hours. However, a very weak or no accumulation at all was recorded in distal leaflets, suggesting the activation of *pr* genes mostly in tissues close to the infection site. This concurs with other findings suggesting

the antifungal activity of PR-proteins acting at the local and to some extent at a close proximity of the infection site (Graham et al. 2003; Park et al. 2005). Furthermore, it has been reported also that PR-proteins targeted to the vacuole or outside of the cell are less involved as components of the front line defense action but probably have major effects after the decompartimentalization has occurred, particularly against biotrophic pathogens (Hammond-Kosack & Jones 1997). However, PR-proteins localized in the cytoplasm such as PR-1 and PR-5 rapidly accumulate after elicitor treatments or pathogen inoculations and might be involved in the front line of defense response (Somssich et al. 1989; Hahlbrock et al. 1995). The present findings, showing a delay in PR-1 and PR-5 accumulations by 24 to 72 hours in proximal leaflets as compared to locals, corroborate the suggestion that these two proteins are involved in the front line defense mechanisms of potato against *P. infestans*.

Plants exhibit generally localized and/or systemic acquired resistances (LAR and SAR) against invading pathogens. LAR takes place in a ring of living cells surrounding the lesion and exhibiting an activation of host defense-related genes including the PRs (Costet et al. 2002). LAR provides a locally highly uncongenial setting for the invading pathogen (Heath 2000). The importance of PRs in the plant defense mechanisms in this case has been related, among other reasons, to their ability to reduce the development of symptoms in the area surrounding the lesions (Liu et al. 1994; Niderman et al. 1995). SAR develops, however, beyond tissues exhibiting LAR (Ross 1961) and provides a low, though significant, level of resistance against a subsequent infection (Ryals et al. 1996; Sticher et al. 1997). It occurs in the primary infected parts of the plant and

throughout the host. A strong expression of a wide range of defense responses occurs in LAR including the accumulation of antimicrobial proteins such as PRs (Fritig et al. 1998). SAR messenger RNA coding for acidic and basic PR-1, PR-2, PR-3, and PR-5 proteins were shown, in many pathosystems, to accumulate in high amounts (Brederode et al. 1991; Ward et al. 1991). The timing of their expression has been related to the initiation and the duration of systemic acquired resistance without any causal-effect relationship thoroughly proven (McGee et al. 2001; Vleeshouwers et al. 2000b). Our results show that *pr-1*and *pr-5* were expressed early in local leaves and that they might be related to the LAR. However, an expression of the same genes in proximal and distal leaves is in accordance with a SAR-related induction.

In light of the present findings, it may be concluded that PR-proteins, particularly PR-1 and PR-5 are a component of the defense mechanism 'puzzle' of potato against *P. infestans*. Their precise function is still unknown in the limitation of the infectious process in some cultivars such as Kennebec, and further studies are still needed to confirm whether they are components of the front or the back lines of defense. Additional studies will be carried out using real-time quantitative PCR as a more sensitive method for detection and quantification of gene expressions.

5.0 Genes encoding pathogenesis-related proteins, PR-2, PR-3 and PR-9, are differentially regulated in potato leaves inoculated with isolates from US-1 (old) and US-8 (new) genotypes of *Phytopthora infestans (Mont.)* de Bary

5.1 Abstract

Late blight caused by *Phytophthora infestans* (Mont.) de Bary is a major constraint to potato production worldwide. In North America, this disease has re-emerged as a major concern to potato producers due to a significant shift in its population structure. To develop sustainable control strategies, it is necessary to have a better understanding of differential interactions that potato has developed with the previously dominant, less aggressive (US-1) versus the currently pre-dominant, more aggressive (US-8) genotypes of the pathogen. In our study, two potato cultivars, Russet Burbank (susceptible) and Kennebec (moderately tolerant), and two *P. infestans* genotypes, US-1 and US-8, were used. Induction patterns of genes encoding three PR proteins (*pr*-2, *pr-3* and *pr-9*) in response to isolate from *P. infestans* genotype US-1 and US-8 were studied using northern blot analysis in three leaf strata and different times after inoculation. Our results indicated that these *pr* genes were activated earlier in both cultivars when inoculated with *P. infestans* US-1 as compared to US-8 genotype. In addition, the induction of these genes occurred earlier in Kennebec than in Russet Burbank in response to both genotypes. Furthermore, *pr-2* and *pr-3* were found to be induced both locally and systemically while *pr-9* was induced only locally at the site of inoculation in Kennebec inoculated with *P. infestans* US-1.

5.2 Introduction

Phytophthora infestans (Mont.) de Bary is the casual agent of potato late blight, one of the most devastating diseases on potatoes (Fry & Goodwin 1997b). In the last two decades, dramatic changes have been recorded in the structure of *P. infestans* populations in North America (Goodwin et al. 1998; Peters et al. 1998). The so-called US-8 genotype, which is more aggressive and resistant to chemical fungicides, has displaced the US-1 genotype and became the predominant genotype in the United States and Canada (Daayf et al. 2000; Daayf et al. 2001; Daayf & Platt 2001a).

Potatoes respond to pathogen attacks by the activation of various defense-related genes which can be divided into different groups based on their biological functions and patterns of induction. Pathogenesis-related (PR) proteins, which have been classified as a major group of defense-related proteins, were originally identified in tobacco after the infection by the tobacco mosaic virus (TMV), but they were also subsequently found in a large variety of plant species, including potatoes (Van Loon & Van Strien 1999; Buchter et al. 1997). Up to date, PR proteins have been classified into 14 structurally and functionally distinct families (Van Loon & Van Strien 1999), some of which are comprised of proteins with known biological functions.

PR-2 represents a group of β-1,3-glucanases (Van Loon & Van Strien 1999), whose role in disease resistance is often related to their glucanase activities. PR-2 can either directly impair the growth of a fungus by hydrolyzing β-1,3/1,6-glucans within fungal cell walls (Mauch et al. 1988) or releasing short glucan fragments from pathogen cell walls, which can be also recognized by plants and further induce plant defense responses (Ebel & Cosio 1994).

PR-3 represents chitinases, another group of fungal cell wall-degrading enzymes capable of degrading chitins within fungal cell walls (Van Loon & Van Strien 1999). It has been shown that chitinase, either alone or in combination with β-1,3-glucanase, can inhibit the growth of different fungi *in vitro* (Mauch et al. 1988).

PR-9 is classified as a group of peroxidases (Van Loon & Van Strien 1999) and their involvement in plant disease resistance has been demonstrated in many occasions, including in potatoes (Young et al. 1995; Yoshida et al. 2003; Collinge & Boller 2001). They can contribute to plant disease resistance in several ways including i) the strengthening of plant cell walls through the deposition of lignin which is thought to be a general defense mechanism against a broad spectrum of pathogens (Hammerschmidt & Kuc 1980; Vance & Sherwood 1980); ii) the production of toxic radicals such as hydrogen peroxides (Peng & Kuc 1992; Way et al. 2000; Mayer 1987).

The involvement of *pr-2*, *pr-3* and *pr-9* in the interaction between potato and *P. infestans* has been previously demonstrated (Beerhues & Kombrink 1994; Kim et al. 2000; Collinge & Boller 2001; Buchter et al. 1997; Schroder et al. 1992). However no data is available about the level of their induction in the context of non race-specific resistance. Specifically, no such data is available in potato cultivars with different levels of field resistance, when infected with different groups of *P. infestans* such as the previously (US-1) versus currently predominant genotype (US-8).

We previously demonstrated that the expression of *pal* and *hmgr*, genes encoding key enzymes of phenylpropanoid and terpenoid pathways, respectively, were differentially regulated in two potato cultivars inoculated with isolates of *P. infestans*

from US-1 and US-8 genotypes (Wang et al. 2005b). More recently, we described differential induction of *pr-1* and *pr-5* using the same model (Wang et al. 2005a). In these studies, we have shown that US-8 inhibited *pal* and *hmgr* expression at the local site of infection, while such inhibition was much less important with US-1 and not apparent on *pr-1* and *pr-5* with both genotypes. In the present study, we extended our investigation to *pr-2*, *pr-3* and *pr-9* in order to assess whether a pattern(s) of induction/inhibition of PR proteins was maintained throughout this system, including at the local site of infection and at proximal and distal tissues remote from the infection site. For this purpose, the objectives of this study were 1) to compare induction patterns of *pr-2*, *pr-3* and *pr-9* in two potato cultivars, Russet Burbank (RB, susceptible) and Kennebec (KB, moderately tolerant), in response to the inoculation with *P. infestans* isolates from the US-1 or US-8 genotype; 2) to compare their systemic induction profiles in time (after inoculation) and space: local (inoculated leaflets), proximal (leaflets adjacent to the inoculated leaflets) and distal leaflets (leaflets from leaf adjacent to the inoculated leaf).

5.3 Materials and Methods

Plant materials and P. infestans

Two potato cultivars (*Solanum tuberosum L.*) were used in our study. Russet Burbank (RB) is a widely used commercial cultivar and highly susceptible to *P. infestans* while Kennebec (KB) has moderate foliage resistance to *P. infestans*. Potato plants were generated from high quality potato seed tubers and grown in clay pots containing soil mixtures of soil:sand:peat:perlite at the ratio of 4:4:4:1. Potato plants

were kept in a growth chamber (20±2°C, 16 hour photo period) for 6 weeks prior the inoculation.

The virulence of two *P. infestans* isolates used in this study are summarized in Table 1. Both *P. infestans* isolates were kept on rye B medium (Goodwin et al. 1995) at 20±2°C and the virulence of isolates was maintained by repetitive inoculations on RB leaves and re-isolations every 3-4 months.

Table 5. 1 Fungal isolates used in this study

Fungal isolate	Mating type	Genotype	Race composition	Origin
FA-1 (US-1)	A1	US-1	1.2.4.7.9.	New Brunswick, Canada
D-1091 (US-8)	A2	US-8	1.2.3.4.6.7.8.9.10	Manitoba, Canada

The inoculation of P. infestans

Sporangia suspensions were collected from 10-14 days old *P. infestans* cultures by first washing *P. infestans* plates with 10 ml autoclaved distilled water and then harvesting sporangia the second day by flooding the plates with 10 ml autoclaved distilled water.

For the inoculation, 100 µl of sporangia suspension ($5x10^6$ sporangia/ml) was inoculated on the primary leaflet of third or fourth full grown potato leaf. *P. infestans* sporangia suspension was deposited on the potato leaflet as multiple tiny droplets using a micropipette to prevent the inoculum from running off.

After the inoculation, potato plants were kept at 100% humidity for 48 hours to maintain the humidity required for the infection. Three pots, with 2 stems in each pot, were considered as the replicate per treatment (healthy Russet burbank and Kennebec controls; Russet burbank and Kennebec inoculated with *P. infestans* US-1 or US-88)

and per sampling time point (0, 6, 12, 24, 48, 72, 96, and 120 hours after inoculation (h.a.i.)). The inoculation was repeated twice overtime.

Leaf sampling procedures

Potato leaflets were collected from 3 leaf strata including inoculated leaflets (local (L)), un-inoculated leaflets in the same leaf where *P. infestans* was inoculated (proximal (P)) and un-inoculated leaflets from the leaf adjacent to the inoculated leaf (distal (D)). The samples were collected at 8 different time points. For the control, the terminal leaflet of the third full grown leaf from individual healthy potato plant was collected at each time point. The harvested leaf samples were ground to a fine power immediately after the sampling and stored in -80°C until the total RNA extraction.

Preparations of total RNA

Total RNA was extracted with the method described by Verwoerd *et al.* (1989). The absorbance at 260 nm and A_{260}/A_{230} ratio were used to determine the concentration and purity of RNA samples. The integrity of total RNA was further confirmed by formaldehyde-agarose electrophoresis. Leaf samples collected from the two separate sets of inoculations were used separately for RNA extractions. However, within each treatment, RNA extracted from two stems within in same pot was pooled to ensure enough amount of RNA for the hybridization.

Probe synthesis and Northern blot

Probes for the northern hybridization were labelled with a PCR DIG labelling kit (Hoffmann-La Roche Lta, Mississauga, Ontario, Canada) following manufacturer's recommendations. The PCR primers (listed in table 2) used for the amplification of

pr-2, *pr-3* and *pr-9* were designed based on sequences stored in Genbank using OligoPerfect™ designer software (Invitrogen™ Life Science Software, Invitrogen, Inc., Ont., Canada).

Table 5. 2 Probes for Northern blot analysis

PR family	Properties	Genbank Assession No.	Origin	Primers for probe synthesis	Tm (°C)	Size of amplicon (bp)
PR-2	Glucanase	U01901	cDNA, potato	PR2F:tgatccgaatcaaggagctt PR2R: tgtcttgtgtggcaccaaat	60	662
PR-3	Chitinase	AF024537	cDNA, potato	PR3F:gatgataccgcccgtaagaa PR3R:tgggaacattcagcatacca	60	493
PR-9	Peroxidase	AJ401150	cDNA, potato	PR9F:aagaaacaacaccagggcac PR9R:tgccctcaagctgaagaaat	60	485

For each PCR reaction , a reaction mixture (25 μl in volume) was preheated at 95°C for 2 minutes followed by 25 cycles of 30s at 95°C, 30s at 60°C and 45s at 72°C using a programmed thermocyler (Techne Flexigene, Inc., Canada). The authenticity of amplicons was further confirmed by restriction enzyme digestion analysis based on the sequence information presented in GenBank.

Denatured total RNA samples (10 μg) were separated with 1.5% agarose gel containing 1.2 % formaldehyde. Equal loadings of RNA samples were confirmed by the ethidium bromide staining. Separated total RNA samples were then transferred from the agarose gel to a Hybond-N^+ membrane (Hoffmann-La Roche Lta, Mississauga, Ontario, Canada). Transferred RNA samples were fixed on the membrane by baking it at 80°C for two hours.

For the hybridization, membranes were pre-hybridized in 15 ml Dig Easy High solution ((Hoffmann-La Roche Lta, Mississauga, Ontario, Canada) for 4 hours at 50°C

followed by the hybridization in the same solution with the corresponding probe at 25 ng/ml concentration for 18 hours at 50°C .

Following the hybridization, membranes were washed in 2×SSC containing 0.1% SDS (w/v) for 15 minutes each at room temperature followed by another two washes in 0.5XSSC containing 0.1% SDS (w/v) for 15 minutes each at 68°C. The hybridization was performed on samples collected from two inoculations and only representatives were shown.

Signal detection and image recording

Hybridization signals were developed using a DIG luminescent detection kit ((Hoffmann-La Roche Lta, Mississauga, Ontario, Canada) and exposed to a Kodak BioMax MS film with intensifying screening (Sigma-Aldrich) for 5 hours in dark. Developed films were recorded with a digital camera at the resolution of 1,200 dpi (Sony, Ltd, Toronto, Ontario, Canada).

5.4 Results

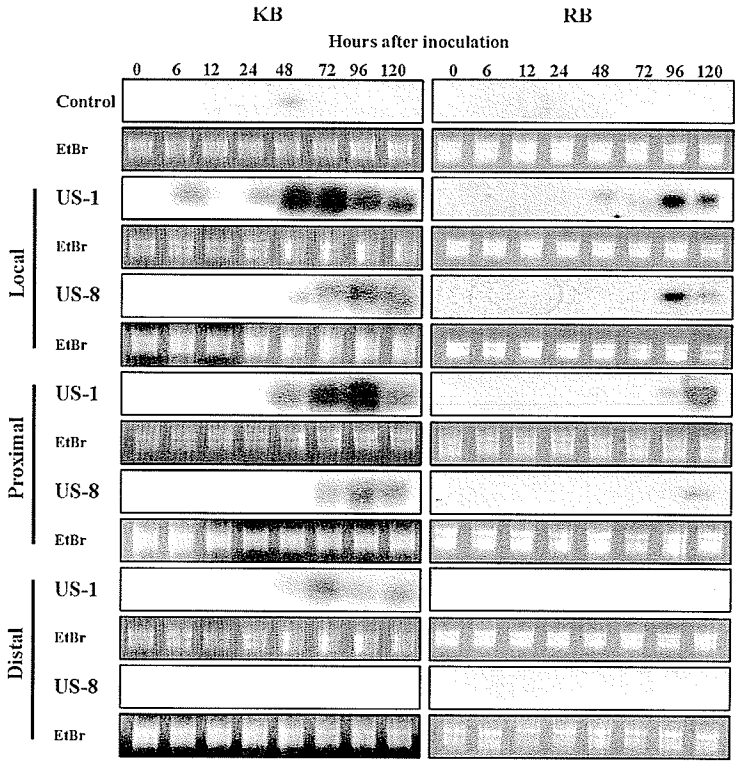
Differential accumulation of pr-2 transcripts in leaflets inoculated with P. infestans US-1 or US-8 (Figure 5.1)

In Kennebec (KB, Figure 5.1)

No induction of *pr-2* was observed in most control leaflets collected from healthy Kennebec plants. A faint band indicating a slight induction was occasionally observed 48 h.a.i.. After inoculation with either US-1 or US-8, *pr-2* was induced in Kennebec and, in general, the induction of *pr-2* occurred earlier in Kennebec×US-1 than in KB×US-8. In local leaflets, *pr-2* was weakly induced at 6 h.a.i. in Kennebec×US-1 with

the highest level of induction found from 48 to 72 h.a.i., whereas in Kennebec×US-8, *pr-2* was not induced until 48 h.a.i. with the highest level of induction found at 96 h.a.i.. In proximal leaflets, *pr-2* was induced at 48 h.a.i. in Kennebec×US-1 while in Kennebec×US-8, it was not induced until 72 h.a.i.. In distal leaflets, *pr-2* was induced at 72 h.a.i. in Kennebec×US-1, whereas no induction was found in Kennebec×US-8.

Figure 5. 1 Northern gel blots of *pr-2* expression



Northern gel blots showing the expression of *pr-2* in Kennebec (KB, left) and Russet Burbank (RB, right) inoculated with *P. infestans* US-1 or US-8 genotype isolates. EtBr represents agarose electrophoresis of total RNA stained with ethidium bromide and it indicates the equal loading of total RNA loaded in every lane.

In Russet Burbank (RB, Figure 5.1)

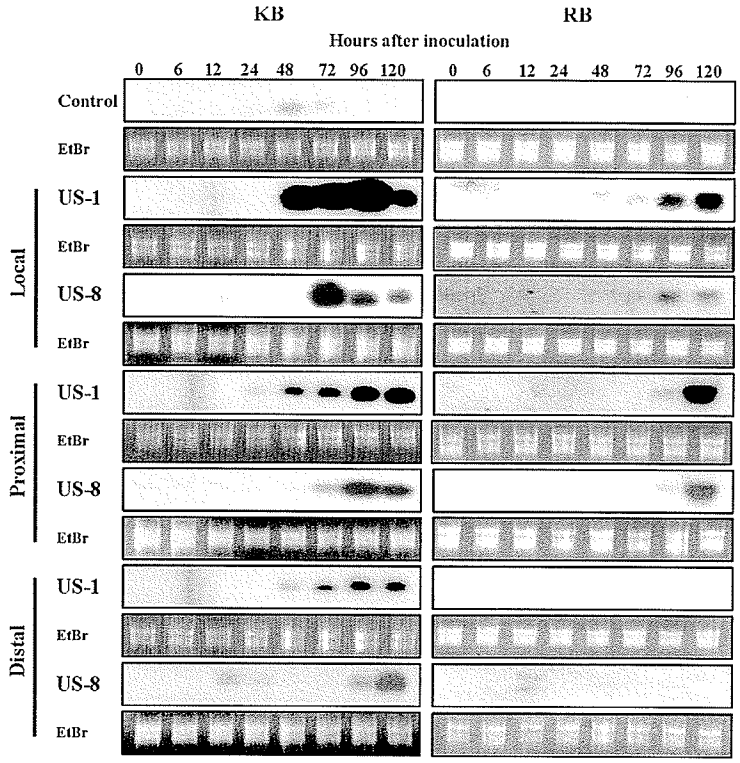
No induction of *pr-2* was found in any of healthy control leaflets. Nevertheless it was induced when Russet burbank was inoculated with either US-1 or US-8. In local leaflets, *pr-2* was weakly induced in Russet burbank×US-1 at 48 and 72 h.a.i. with the

highest level found at 96 h.a.i., whereas no induction of *pr-2* was observed in Russet burbank×US-8 until 96 h.a.i.. In proximal leaflets, *pr-2* was weakly induced in Russet burbank×US-1 at 96 h.a.i. while no induction of *pr-2* was observed in Russet burbank×US-8 until 120 h.a.i.. In distal leaflets, no induction of *pr-2* was found in Russet burbank×US-1 and Russet burbank×US-8. It was interesting to observe again that when *pr-2* accumulated, it did first in local then in proximal leaflets

Overall, a stronger and earlier induction of *pr-2* was observed in Kennebec as compared to Russet burbank inoculated with two *P. infestans* isolates. *Pr-2* was induced earlier and stronger in two cultivars inoculated with US-1 than with US-8. This difference is more apparent in Kennebec than in Russet burbank. Furthermore, the induction of *pr-2* extended to distal leaflets only in Kennebec×US-1, whereas no induction was observed in the corresponding leaflets in Kennebec×US-8, Russet burbank×US-1, or Russet burbank×US-8. It was interesting to observe that the strongest induction of *pr-2* appeared first in local followed by proximal then distal leaflets. It was also remarkable that such intensity of induction was decreasing from local to distal leaflets.

Differential accumulation of pr-3 transcripts in leaflets inoculated with P. infestans US-1 or US-8 (Figure 5.2)

Figure 5. 2 Northern gel blots of *pr-3* expression



Northern gel blots showing the expression of *pr-3* in Kennebec (KB, left) and Russet Burbank (RB, right) inoculated with *P. infestans* US-1 or US-8 genotype isolates. EtBr represents agarose electrophoresis of total RNA stained with ethidium bromide and it indicates the equal loading of total RNA loaded in every lane.

In Kennebec (RB, Figure 5.2)

No induction of *pr-3* was observed in most healthy control leaflets. However a weak hybridization signal was occasionally detected in leaflet collected at 48 h.a.i.. Similar to *pr-2*, *pr-3* was found to be induced in Kennebec after inoculation with either US-1 or US-8 and, in general, the induction of *pr-3* occurred earlier in KennebecxUS-1 then in KennebecxUS-8. In local leaflets, a stronger induction of *pr-3* was observed in KennbecxUS-1 starting at 48 h.a.i., whereas it was not induced in KennebecxUS-8 until 72 h.a.i.. In proximal leaflets, *pr-3* was first induced at 48 h.a.i. in KennebecxUS-1, whereas no induction of *pr-3* was detected in KennebecxUS-8 until 72 h.a.i.. In distal

leaflets, *pr-3* was also found to be induced earlier in KennebecxUS-1 than in KennebecxUS-8.

In Russet Burbank (RB, Figure 5.2)

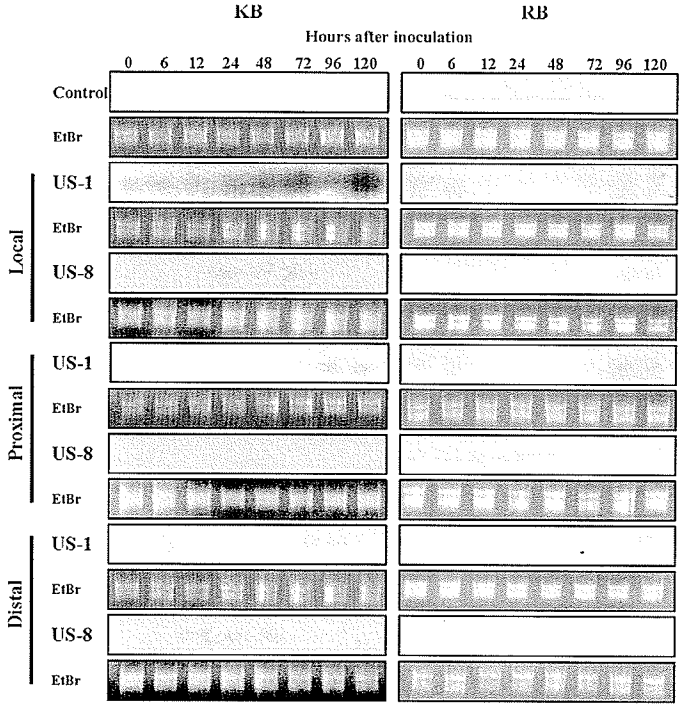
No induction of *pr-3* was detected in any healthy Russet burbank leaflets collected and it was up regulated in Russet burbank after inoculated with either US-1 or US-8. In general, the induction of *pr-3* seems to be stronger in Russet burbank×US-1 although the timing of its induction was found to be similar between Russet burbank×US-1 and Russet burbank×US-8. In local leaflets, the induction of *pr-3* appeared to be stronger in Russet burbank×US-1 than in Russet burbank×US-8 (96 and 120 h.a.i.). No difference in terms of the timing of its induction was observed between two treatments. In proximal leaflets, *pr-3* was induced at the same time (120 h.a.i.) in Russet burbank×US-1 and Russet burbank×US-8 and, again, the level of *pr-3* induction was higher in Russet burbank×US-1. In distal leaflets, no detectable induction of *pr-3* was observed in either Russet burbank×US-1 or Russet burbank×US-8.

Overall, induction of *pr-3* was stronger and occurred earlier in Kennebec than in Russet burbank in response to US-1 or US-8. *Pr-2* was induced earlier and stronger in potato cultivars inoculated with US-1 than with US-8. The systemic induction of *pr-3* was observed in both Kennebec and Russet burbank especially in Kennebec. The induction of *pr-3* extended to distal leaflets of Kennebec while no such induction was found in distal leaflets of Russet burbank. The earliest and strongest induction of *pr-3* was found first in local followed by proximal and then distal leaflets.

Differential accumulation of pr-9 transcripts in potato leaflets inoculated with P. infestans US-1 or US-8 (Figure 5.3)

No induction of *pr-9* was observed in healthy control leaflets of either Kennebec or Russet burbank collected during the whole sampling period. On the other hand, *pr-9* was induced in local leaflets collected from Kennebec×US-1. However, its level of the induction was much weaker compared to those of *pr-2* and *pr-3* in general. No induction of *pr-9* was observed in local leaflets of Russet burbank×US-1, proximal or distal leaflets of Kennebec×US-1, Kennebec×US-8, Russet burbank×US-1 and Russet burbank×US-8.

Figure 5. 3 Northern gel blots of *pr-9* expression



Northern gel blots showing the expression of *pr-9* in Kennebec (KB, left) and Russet Burbank (RB, right) inoculated with *P. infestans* US-1 or US-8 genotype isolates. EtBr represents agarose electrophoresis of total RNA stained with ethidium bromide and it indicates the equal loading of total RNA loaded in every lane.

5.5 Discussion

In this study, we showed that *pr-2*, *pr-3* and *pr-9* were differentially regulated in potato leaves inoculated with isolates from *P. infestans* US-1 and US-8 genotypes. *Pr-2* and *pr-3* were locally and systemically induced in both cultivars whereas *pr-9* was found to be induced locally and only in Kennebec.

It was not surprising that *pr-2* was induced in both Kennebec and Russet burbank upon inoculations with *P. infestans,* since *pr-2* is commonly induced in plants in response to pathogen infections (Zareie et al. 2002; Kang & Buchenauer 2002; Park et al. 2005) including in potatoes (Linke et al. 2002; Rahimi et al. 1996). However, it was somehow interesting to observe that *pr-2* and *pr-3* shared a comparable pattern of spatio–temporal induction. It is a common belief that oomycetes lack chitin in their cell walls and are not expected to be affected by chitinases (Vleeshouwers et al. 2000b). Nevertheless, the presence of chitin synthase genes in several species of oomycetes (Werner et al. 2002; Mort-Bontemps et al. 1997) and the inhibitory effect of polyoxin D, a chitin synthase inhibitor, on the growth of *Saprolegnia* (Bulone et al. 1992) have suggested otherwise. Furthermore, the actin-binding study by Takemoto *et al.* (Takemoto et al. 1997) has shown that a basic chitinase might be involved in the cytoplasmic aggregation of potato cells, which is an important event in the cellular defense of potato. Our results, along with the result of Schroder *et al.* (Schroder et al. 1992), are in line with these findings and suggest that chitinases might also play a role in the defense mechanism of potato against *P. infestans.* However we can not rule out

the possibility that up regulation of *pr-2* and *pr-3* are not specifically triggered by chitins but are part of a more general response.

The systemic induction of *pr-2* and *pr-3* in proximal and distal leaflets in our study is most likely a part of systemic acquired resistance in potatoes in response to the local infection. The systemic accumulation of PR proteins has been associated with systemic acquired resistance in many plant species (Pozo et al. 2002; Madi & Katan 1998) including potatoes (Schroder et al. 1992; Vleeshouwers et al. 2000b).

Furthermore, *pr-2* and *pr*-3 displayed different induction kinetics in terms of the timing and magnitude of their induction. *Pr-2* was induced earlier than *pr-3*. On the other hand, the accumulation of *pr-3* appeared to be greater than that of *pr-2*. The reasons behind the earlier induction of *pr-2* and the greater accumulation of *pr-3* were not investigated at this point. However, the earlier induction of *pr-2* in our study is in agreement with the dual function of the corresponding protein (β-1,3-glucanase) in plant defense responses. Such an early induction of *pr-2* could be related to the early activity of the corresponding protein in releasing short β-glucan elicitors from pathogens (Ebel & Cosio 1994) which are capable of further enhancing the induction of defense-related genes.

Also, it was interesting to observe that the timing and magnitude of *pr-2* and *pr-3* inductions were affected not only by the site of infection (local, proximal and distal), but also by cultivars (highly susceptible and moderately tolerant) and pathogen genotypes (US-1 and US-8).

Pr-2 and *pr-3* were induced earlier and stronger in Kennebec than in Russet burbank after inoculation. This may suggest the presence of mechanisms in Kennebec that ensure the earlier triggering of defense responses to *P. infestans* as compared to Russet burbank. Kennebec is known to possess resistance gene R1 while Russet burbank has none of the 11 known race-specific R genes (Wang et al. 2005b). The observation that induction of *pr-2* and *pr-3* occurred earlier in Kennebec (R1) than in Russet burbank suggests a potential role of R1 in the resistance of Kennebec to *P. infestans*. Similarly, Vleeshouwers *et al.* (Vleeshouwers et al. 2000b; Vleeshouwers et al. 2000a) observed that potato cultivar Ehud carrying a strong R1 gene responded differentially to two *P. infestans* isolates in terms of infection efficiency and lesion growth rate. Since R1 gene had been cloned, its silencing in Kennebec could further verify whether or not it is involved in the differential response of Kennebec to *P. infestans*.

The two *P. infestans* isolates (FA-1 and D1901) used in this study are both capable of overcoming resistance conferred by the R1 gene (table 1). Therefore, based on the 11 known race-specific R genes, reactions observed in Kennebec/Russet burbank in response to these two *P. infestans* isolates should all be considered under the category of non race-specific resistance, also known as partial resistance. In most cases, partial resistance to late blight in potato is related to the presence of qualitative trait loci (QTL) (Avrova et al. 2004b). To our knowledge no such QTL has been described in Kennebec. However, a number of QTLs related to late blight resistance have been identified in several other potato cultivars (Avrova et al. 2004a). Therefore it is possible that

Kennebec also possesses QTLs responsible for an earlier induction of defense-related genes upon *P. infestans* infection. Furthermore, some potato QTLs contributing to late blight resistance were found to be clustered with known *R* genes or *R* gene homologs (Leister et al. 1996; Young et al. 1995), which may suggest a possible link between the presence of R1 and such QTLs.

We previously reported that HR-like necrotic lesions with the typical trailing hypersensitive reaction phenotype were only observed in Kennebec×US-1 while not in Kennebec×US-8 (Wang et al. 2005b). The trailing hypersensitive reaction has been previously suggested to be related with weak *R-Avr* gene interactions or gene-dosage effects and often leads to partial resistance (Kamoun et al. 1999). Although more evidence is required to prove it is the case in our study, the earlier induction of *pr-2* and *pr-3* in Kennebec, combined with the appearance of the trailing hypersensitive reaction observed visually previously in Kennebec×US-1 but not in Kennebec×US-8, Russet burbank×US-1 or Russet burbank×US-8 (Wang et al. 2005b), may suggest such a possibility.

In this study, *pr-9* was strongly induced only in local leaflets from Kennebec×US-1. Although the exact role of this *pr-9* (peroxidases) in potato related to late blight resistance is unknown, defense-related peroxidases are often involved in plant disease resistance through the lignification of plant cell walls or the production of toxic radicals (Vance & Sherwood 1980). Furthermore, the induction of certain peroxidases is sometime specifically observed in incompatible interactions which

suggests that peroxidases may be involved in the R-gene mediated resistance (Van Pelt-Heerschap & Smit-Bakker 1999; Young et al. 1995).

Based on the 11 known race-specific R genes described in potato, all the interactions in our study should be classified under the category of compatible interactions based on our race determination tests (Vance & Sherwood 1980). However, if our previous assumption is correct about the presence of weak *R-avr* interactions or gene dosage effects between Kennebec and US-1, it may explain the local induction of *pr-9* in Kennebec×US-1 to some extent, as well as the much weaker and delayed accumulation compared to a typical incompatible interaction. Furthermore, the induction pattern of *pr-9* observed in our study is very similar to those of *stprx2* (Collinge & Boller 2001) and *gst1* (Martini et al. 1993), 2 potato peroxidases which have been shown to be locally induced by avirulence isolates of *P. infestans*. On the other hand, no induction of *pr-9* was observed in Kennebec×US-8 which could indicate that no such weak *R-avr* interaction exits in this case. It is also possible that the difference in the accumulation of *pr-9* transcripts is related to different potato cultivars used or to potato possessing multiple genes encoding putative peroxidases (Yoshida et al. 2003; Kim et al. 2000).

Due to the increase in late blight severity in current potato cultivars, it has been suggested that changes in the potato susceptibility to late blight could result from changes in the virulence of the pathogen at least through the suppression of defense responses by suppressors produced by *P. infestans* (Andreu et al. 1999; Wang et al. 2005b; Lebreton et al. 1999). In this study, we showed that the timing and magnitude of

the induction of three *pr* genes were correlated with the level of late blight resistance as well as the virulence of *P. infestans*. The strongest and earliest induction of these *pr* genes were found in the moderately tolerant cultivar (Kennebec) inoculated with the less virulent strain (US-1), comparatively, which suggested that changes in the activation of plant defense mechanism could be one of the factors affecting the susceptibility of potato to late blight. However, as compared to *pal* and *hmgr*, which were shown previously to be clearly inhibited by US-8 at the site of infection (Wang et al. 2005b), no such inhibition was observed with *pr-2*, *pr-3* or *pr-9*.

Pr-9 was only locally induced in Kennebec and its induction pattern in this study was very similar to those of peroxidases involved in the R gene-mediated resistance (Collinge & Boller 2001; Martini et al. 1993; Young et al. 1995). On the other hand, *pr-2* and *pr-3* were locally and systemically induced in both potato cultivars in response to *P. infestans*. It has been previously reported that *pr-2/3* expressions are often related to systemic acquired resistance (Graham et al. 2003; Gaudet et al. 2000; Bertini et al. 2003; Feys & Parker 2000) and induced by various external stimuli (Graham et al. 2003; Doke et al. 1987; Cohen et al. 1993; Tonon et al. 2002). Therefore co-induction of *pr-9* and *pr-2/3,* along with the differential induction of *pal* and *hmgr* (Wang et al. 2005b) and of *pr-1* and *pr-5* (Wang et al. 2005a), indicates that multiple signalling pathways are involved in the activation of defense-related genes in potato against *P. infestans*.

6.0 Potato foliar defense responses to *Phytophthora infestans* genotypes US-1 and US-8 in two cultivars with different levels of resistance

6.1 Abstract

Defense responses were investigated in two potato cultivars with different level of resistance to late blight, Russet Burbank (RB, susceptible) and Kennebec (KB, moderately tolerant), after inoculation with isolates from two *P. infestans* genotypes, US-1 (previously predomiant, mildly aggressive) and US-8 (currently predominant, highly aggressive). The accumulation of brown lignin-like materials and increases in the cell wall affinity to trypan blue surrounding infection sites were observed in leaf discs of Kennebec inoculated with US-1, while such reactions were not observed in Kennebec inoculated with US-8, Russet burbank inoculated with US-1 or US-8, 24 hours after inoculation. Southern blot analysis indicated that the distribution of *pal, hmgr, pr-1* and *pr-5* was reasonably conserved between the two cultivars and multi-gene families were present in both cultivars. Their expression in three leaf strata (local, proximal and distal) and at different times after inoculation were evaluated using SYBR real-time RT PCR. The results showed that the activation of these defense-related genes was affected not only by genotypes of *P. infestans,* but also by the cultivar and the proximity to the inoculation site. These genes were induced earlier in Kennebec than in Russet burbank and in response to US-1 compared to US-8. In general, the earliest and strongest induction of these genes was observed in Kennebec inoculated with US-1. Furthermore, induction of *pal* and *hmgr* was suppressed at the site of infection while such suppression was not observed in the expression of *pr-1* or

pr-5. These results indicate that changes in either the activation or suppression of defense responses were related to late blight susceptibility of tested potato cultivars.

6.2 Introduction

Late blight, caused by the oomycete *Phytophthora infestans,* is the most severe disease on potato (*Solanum tuberosum L.*) world-wide (Duncan 1999). Disease management is heavily dependent on the application of fungicides, which is expensive and has negative effects on the environment. Therefore, the utilization of host resistance, either by classical breeding methods or genetic engineering, has been considered as a potential alternative capable of replacing at least some of the chemical inputs used to control this disease.

Based on the classical gene-for-gene concept, race-specific resistance is conferred by a major resistance gene (R) which encodes a specific receptor recognizing the elicitor encoded by the corresponding *avr* gene in the pathogen (Belkhadir et al. 2004; Hammond-Kosack & Jones 1997). To date, 11 race-specific R genes have been introgressed from *S. demissum* into potato (Colon et al. 1993). The R-gene mediated resistance was initially successful. However it was soon discovered that these R genes only provide short-lived resistance in the field as new races of *P. infestans* rapidly overcame this type of resistance (Fry & Goodwin 1997b). Non race-specific resistance, also known as filed resistance, is commonly observed in wild *Solanum* species (Fry & Goodwin 1997a) and is believed to be more durable than race-specific resistance since it does not impose adequate selection pressure to allow new virulent races to dominate the pathogen populations (James & Fry 1983). Non race-specific resistance to LB could

result from plant's intrinsic properties or may be induced by non-specific elicitors produced by different races of *P. infestans* (Vleeshouwers et al. 2000a). Recently, there has been emerging evidence suggesting that R gene receptors recognized by specific pathogen elicitors might also be involved in the partial resistance to late blight (Kamoun et al. 1999).

In potato, the hypersensitive response has been observed in cultivars with either race-specific or non race-specific resistance to *P. infestans*. The occurrence of the hypersensitive reaction in potato with non race-specific resistance to late blight has been suggested to be related to the presence of weak *R-avr* interactions or gene dosage effects (Vleeshouwers et al. 2000a; Kamoun et al. 1999).

In both race-specific and non race-specific resistances, the induction of host defense responses involves differential regulation of countless defense-related genes (Nimchuk et al. 2003). For example, *pal* encodes phenylalanine ammonia lyase (PAL) which converts phenylalanine to an activated (hydroxyl) cinnamic acid, an essential step for the synthesis of a wide variety of phenolic compounds such as flavonoids, isoflavonoids, and lignin (Dixon et al. 2003). *Hmgr* encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), which is essential for the biosynthesis of sesquiterpene phytoalexins in potato (Choi et al. 1992), is another example. Both *pal* and *hmgr* have been shown previously to be involved in potato resistance to late blight (Henderson & Fiend 1979; Hoshino et al. 1994; Yao et al. 1995; Yoshioka et al. 1999). Different groups of PR proteins have been also involved in this interaction. For example, *pr-1* and *pr-5,* encoding PR-1 and PR-5 respectively, have

also been shown to be induced in potato upon infection by *P. infestans* (Wang et al. 2005a; Hoegen et al. 2002; Liu et al. 1994).

In the last two decades, the population structure of *P. infestans* in North America has changed dramatically. The previously predomiant population of *P. infestans* (US-1 genotype, A-1 mating type) has been replaced by new, more aggressive populations (*i.e.* US-8 genotype, A2 mating type) (Daayf & Platt 2000; Daayf et al. 2000; Goodwin et al., 1997). A better understanding of the mechanisms involved in the partial resistance to different strains of *P. infestans* pathogen will be beneficial for the utilization of host resistance and development of new breeding strategies. Prior to the present study, we have described the differential expression of *pal*, *hmgr*, *pr-1* and *pr-5* using northern blot analysis in Russet Burbank and Kennebec upon inoculation with isolates from *P. infestans* genotype US-1 and US-8 (Wang et al. 2005b; Wang et al. 2005a). These studies assessed the regulation of these genes both at the local site of inoculation and sites remote from it and showed the local suppression of *pal* and *hmgr,* but not *pr-1* and *pr-5,* by *P. infestans* US-8 genotype isolate. In present study, our objectives were to (i) assess the accumulation of *pal-1*, *hmgr-2*, *pr-1* and *pr-5* transcripts using a more quantitative tool, i.e. quantitative real-time RT-PCR in order to confirm the differential accumulation patterns of those genes in response to the two *P. infestans* isolates; (ii) describe histological changes at the local site of inoculation in response to *P. infestans* isolates from US-1 and US-8 genotypes; and (iii) to confirm the local suppression versus the proximal induction of *pal* and/or *hmgr* based on the assessment of their final products such as the phytoalexin rishitin.

6.3 Materials and Methods

Plant materials and growth condition

Russet Burbank (RB, highly susceptible) and Kennebec (KB, moderately resistant) were used in our study. Eye cuttings from high quality potato seed tubers were used to produce potato plants. Potato plants were grown in clay pots (soil:sand:peat:perlite at the ratio of 4:4:4:1) and kept in a growth chamber (20±2°C, 16 hour photo period).

P. infestans isolates and inoculations

Phytophthora infestans isolates were grown on rye B medium (Goodwin et al. 1995) at 20±2°C and their virulence was maintained by repetitive inoculations on RB leaves and re-isolations every 3-4 months. The virulence of the two *P. infestans* isolates used in this study were described previously (Wang et al. 2005b).

100 µl of the sporangial suspension ($5x10^6$ sporangia/ml), collected from 10-14 days old *P. infestans* cultures (Wang et al. 2005b), was applied to the primary leaflet of the fourth full grown potato leaf. The inoculum is deposited on the potato leaflet as multiple tiny droplets using a micropipette to prevent the inoculum from running off. Then potato plants are kept in 100% humidity for 48 hours to maintain the humidity required for the infection.

Microscopical observations

Phytophthora infestans inoculum was inoculated on the back of detached leaflets. Leaf discs containing one inoculum droplet were excised 24 and 48 hours after inoculation (h.a.i.). The excised leaf disc was first destained and fixed in Farmer's fluid (acetic acid (99%)/ethanol (96%)/chloroform, 1:6:3) until it had been fully cleared

(approximately 1 hour). Leaf discs were then stained with trypan blue (Colon et al. 1993) for the observation of mycelial growth and phloroglucinol-HCl (O'Brien & McCully 1981) for the detection of lignin-like materials. The preparations for the microscopic survey on the inoculated detached potato leaflets were from three separated inoculations. Twenty leaf discs of approximately 30 mm^2 were cut and subjected to the trypan blue or phloroglucinol-HCl staining.

Genomic DNA extraction and southern blot hybridization

Genomic DNA was extracted from healthy leaves of Kennebec and Russet burbank using the method described by Taylor and Powell (Taylor & Powell 1982). Five μg of DNA was digested with *AluI* or *MseI* overnight and then size-separated on 1.0% agarose gels. Following electrophoresis, DNA was blotted onto Hybond-N+ membrane (Roche, Canada) and hybridized with probes for *pal, hmgr, pr-1* and *pr-5* (Table 1). Probe labelling and the hybridization were performed as previously described (Wang et al. 2005b) except that the hybridization temperature was decreased to 42°C.

Table 6. 1 Probes for Southern blot analysis

Designation	Genbank Assession No.	Origin	Primers for probe synthesis	Tm (°C)	Size (bp)
Pal	X63103	cDNA, potato	*palF*:5'-GCGATTTTCGCTGAAGTG-3' *palR*:5'-TGTGCTTCGGCACTCTGA-3'	60	596
Hmgr	AB041031	cDNA, potato	*hmgrF*:5'-TGACGCAATGGGAATGAA-3' *hmgrR*:5'-ATGATGGCAAGGACCTCC-3'	60	530
Pr-1	AJ250136	cDNA, potato	*pr1*F:5'-TCACTCTTGTGATGCCCAAA-3' *pr1*R:5'-AGTGGAAACAAGAAGATGCA-3'	60	589
Pr-5	X67244	cDNA, potato	*pr5*F:5'-TAAAGCTTCCGGCGTATTTG-3' *pr5*R:5'-AATCGGTAGGACCACATGGA-3'	60	380

Leaf sampling procedures for real time PCR analysis

Potato leaflets were collected from three strata including inoculated leaflets (local (L)), un-inoculated leaflets in the same leaf where *P. infestans* was inoculated (proximal (P)) and un-inoculated leaflets from the leaf adjacent to the inoculated leaf (distal (D)). The terminal leaflet of the fourth full grown leaf from individual healthy potato plant was collected and used as the control at each time point. Potato leaflets were collected at 0, 6, 12, 24, 48, 72, 96, and 120 h.a.i. The harvested leaf samples were ground to a fine power immediately after the sampling and stored at -80°C until used for total RNA extraction. For every single time point, leaflets from three separate pots were collected and RNA was extracted separately and used for real time PCR analysis.

Total RNA and cDNA synthesis

Total RNA was extracted with the method described by Verwoerd *et al* (Verwoerd et al. 1989). The absorbance at 260 nm and A_{260}/A_{230} ratio was used to determine the concentration and purity of RNA samples. Leaf samples collected from three replicates were used separately for RNA extractions. Prior to cDNA synthesis, RNA samples were treated with RNAase free DNAase (Invitrogen), following the manufacturer's protocol. Reverse transcription was performed using 2 μg of total RNA, random primers and Superscript II RT (Invitrogen) in a total volume of 20 μl. The reaction was incubated at 25°C for 10 minutes followed by incubation at 42°C for 50 minutes. The reaction was terminated by incubation at 65°C for 10 minutes followed by RNAase treatment (Invitrogen).

SYBR green real time RT-PCR assays

Real time PCR was performed using SYBR@Green I technology on a Smart Cycle thermal cycler (Cepheid, Sunnyvale, CA) in the presence of 2.5 mM $MgCl_2$. For each PCR sample, 20µl reaction solution were prepared in a LightCycler capillary (Cepheid, Sunnyvale, CA) containing 2µg cDNA preparation, 0.5 µM of each primer and 10 µl of qPCR Mastermix (Invitrogen). Samples were run for 40 cycles under the following thermal cycling protocol: samples were preheated at 95°C for 15s. Then, 40 amplification cycles were run: 15s at 95°C, 5s at the annealing temperature as indicated in Table 2 and 15s at 72°C. The primers were designed using OligoPerfect™ Designer software (Invitrogen™ Life Science Software, Invitrogen Inc., ON, Canada) and the sequences of primers used for *pal, hmgr, pr-1*, *pr-5* and tubulin are listed in Table 2. The amplification efficiency of primer pairs for genes of interest was tested. Standard curves was developed for each gene by plotting the logarithm of known concentration (10-fold dilution series from 10 ng to 1pg/25 µl reaction volume) of the gene of interest against Cycle of threshold (Ct) value and used to calculate the amount of expression in samples (µg/25ul). Ct value is the threshold cycle number at which the fluorescence emission of the PCR product begins to be statistically significant from the background. Ct value is inversely related to log of initial template concentration. Potato tubulin gene was included in the real-time PCR assay and used as a constitutively expressed internal control. To minimize the possible variation from reverse transcription, the ratio of gene of interest/tubulin (µg genes of interest / µg tubulin) was used to construct the relative expression curve.

Table 6. 2 Primers for quantitative real-time RT-PCR

Designation	Genbank Assession No.	Origin	Primers sequence	Tm (°C)	Size (bp)
Pal	X63103	cDNA, potato	*palF*:5'-GCGATTTTCGCTGAAGTG-3' *palR*:5'-TGTGCTTCGGCACTCTGA-3'	62	91
Hmgr	AB041031	cDNA, potato	*hmgrF*:5'-TGACGCAATGGGAATGAA-3' *hmgrR*:5'-ATGATGGCAAGGACCTCC-3'	62	92
Pr-1	AJ250136	cDNA, potato	*pr1*F:5'-TCACTCTTGTGATGCCCAAA-3' *pr1*R:5'-AGTGGAAACAAGAAGATGCA-3'	60	86
Pr-5	X67244	cDNA, potato	*pr5*F:5'-TAAAGCTTCCGGCGTATTTG-3' *pr5*R:5'-AATCGGTAGGACCACATGGA-3'	60	121

Thin layer chromatograph (TLC) analysis of rishitin accumulation

Two hundred mg of potato leaf tissue were ground to a fine powder in liquid nitrogen and extracted with 10 ml of 80% methanol overnight in the dark at room temperature. Petroleum ether was used to remove most of the chlorophylls, waxes and lipids from the leaf extracts. The cleared leaf extract was then evaporated and the aqueous residue was extracted with ethyl acetate twice (Daayf et al. 1997). The ethyl acetate phase from two extractions was pooled and evaporated in a small flask under nitrogen. It was then re-suspended in 200 μl of 100% methanol.

For TLC analysis, a total of 50 μl of final methanol extract was loaded on a TLC plate (silica gel plate, $SHGF_{254}$). The plate was developed with 4% methanol in chloroform. Rishitin was detected using vanillin/sulphuric acid spray (Lyon 1972).

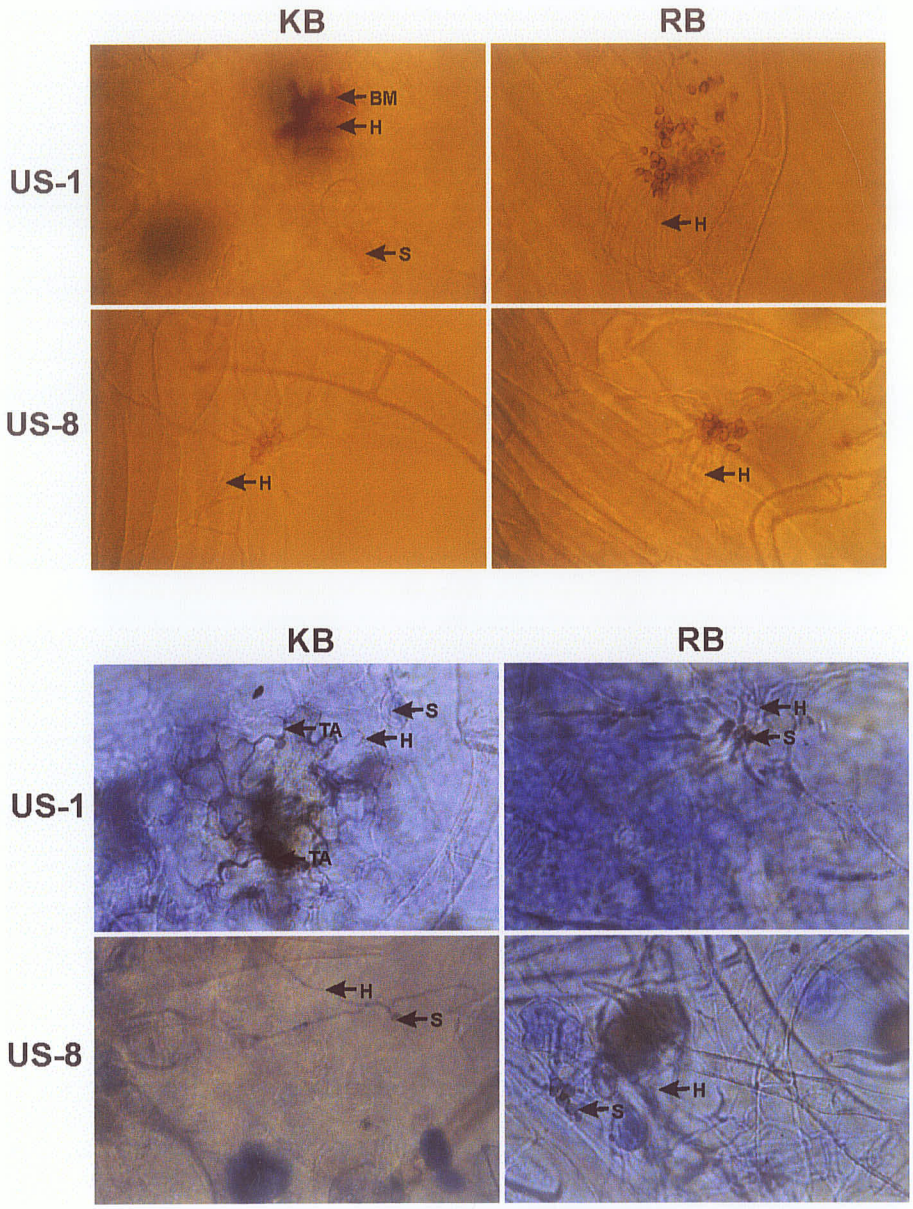
6.4 Results

Histological observations

At 24 h.a.i., the pathogen penetration took place in all treatments. In Kennebec×US-1 leaf discs stained with phloroglucinol-HCl, it was common to observe the accumulation of brown lignin-like materials (Figure 6.1a) along with an increase in the cell wall affinity for trypan blue in cells near the site of penetration (Figure 6.1b). The growth of hyphae was limited. In leaf discs of Kennebec×US-8, Russet burbank×US-1 and Russet burbank×US-8 sampled at 24 h.a.i., mesophyll cells were indistinguishable from those in non-inoculated healthy leaf tissues after staining with phloroglucinol-HCl and trypan blue. No accumulation of brown lignin-like materials or increases in the cell wall affinity for trypan blue was observed at 24 h.a.i..

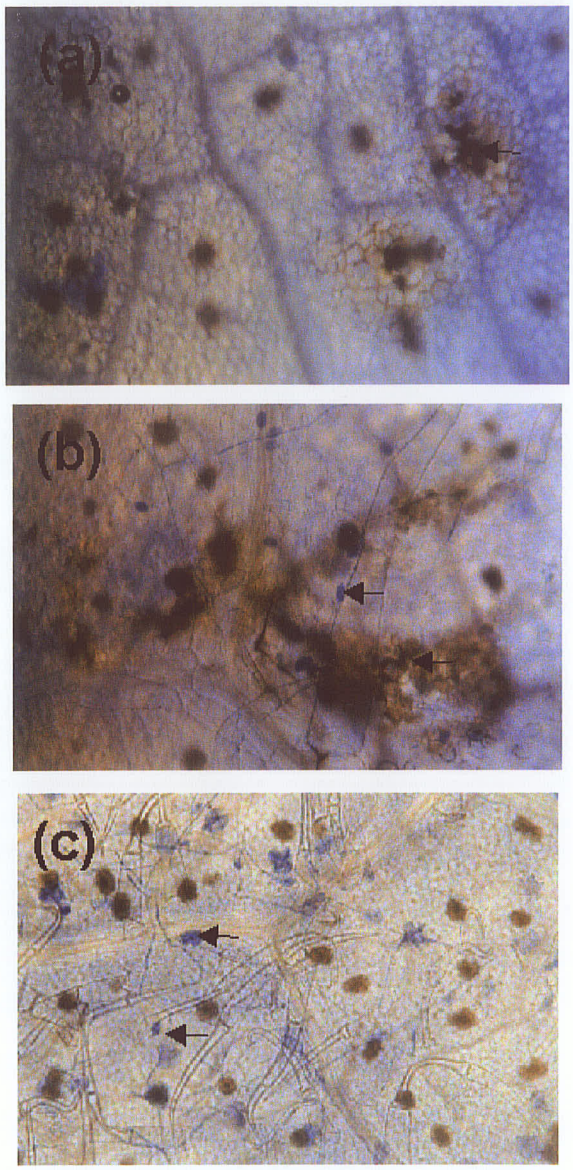
At 48 h.a.i., expanding necrotic lesions (Figure 6.2b) and biotrophic growth of mycelium (Figure 6.2c) were commonly seen in all four treatments (Kennebec×US-1, Kennebec×US-8, Russet burbank×US-1 and Russet burbank×US-8). In summary, three types of symptoms were observed around infection sites: (i) hypersensitive-like necrotic lesion, in which the development of mycelium was limited (Figure 6.2a); (ii) expanding necrotic lesions, in which the cells surrounding the infection site become necrotic and there was extensive intercellular mycelium development (Figure 6.2b); (iii) no necrotic cells found near the penetration site and extensive development of intercellular mycelium (Figure 6.2b). Hypersensitive-like necrotic lesions were commonly observed in Kennebec×US-1 and rarely in Kennebec×US-8, Russet burbank×US-1 and Russet burbank×US-8. Hypersensitive reaction-like necrotic lesions observed in our study were very similar to the trailing hypersensitive reaction described by Vleeshouwers et al. (2000), in which growing hyphae stay ahead of

Figure 6. 1 Phloroglucino-HCl (a) and trypan blue (b) staining of leaf tissues inoculated with *P. infestans* 24 h.a.i.



The presence of brown lignin-like materials (a) and increased trypan blue staining (b) in HR surrounding mesophyll cells of KB leaf inoculated with *P. infestans* US-1 24 h.a.i.. No such reactions were observed in KB×US-8, RB×US-1 or RB×US-8 24 h.a.i.. BM, brown lignin-like materials; S, sporangia; H, hyphae; TA, trypan blue aggregation.

Figure 6. 2 Trypan blue (b) staining of leaf tissues inoculated with *P. infestans* 48 h.a.i.



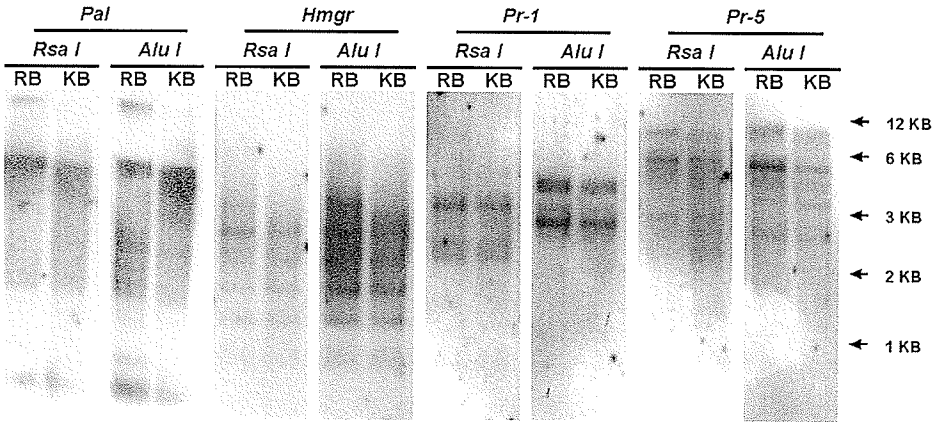
Trypan blue staining of leaf tissues after inoculation with *P. infestans* (48 h.a.i).. Three types of symptoms were observed around infection sites: (i) hypersensitive-like necrotic lesion (arrow), in which the development of mycelia was limited (a); (ii) expanding necrotic lesions (arrow), in which the cells surrounding the infection site become necrotic and there were extensive intercellular mycelium development (b); (iii) no necrotic cells found near the penetration site (arrow) and there was extensive development of intercellular mycelium (c).

hypersensitive reaction - responding cells. Hypersensitive reactions were ineffective in blocking further development of hyphae and they convert into true lesions 72 h.a.i.

Southern blot analysis

Southern blot analysis was used to study the presence of *pal-1, hmgr-2, pr-1* and *pr-5* in two cultivars. Genomic DNA of Russet burbank and Kennebec was hybridized with probes for potato *pal*, *hmgr*, *pr-1* and *pr-5* genes (Figure 6.3). For all of the four defense-related genes investigated, a similar hybridization pattern was observed between Russet burbank and Kennebec, indicating that these genes are fairly conserved among the two cultivars tested. Within each cultivar, multiple hybridization bands were observed, suggesting the presence of multi-gene families for *pal, hmgr, pr-1* and *pr-5*. There was a difference in the intensity of different hybridization bands, which could result from either the sequence diversity or variations in the number of copies among the family members (Fig. 6.3)

Figure 6. 3 Southern blot analysis of *pal, hmgr, pr-1* and *pr-5* distribution in Kennebec and Russet burbank



Southern blot analysis showing the presence of *pal* (phenylalanine ammonia lyase), *hmgr* (3-hydroxy, 3-methylglutaryl CoA reductase), *pr-1 (pathogenesis related)* and *pr-5* in Russet burbank (RB) and Kennebec (KB). Genomic DNA of Russet burbank and Kennebec was completely digested with restriction enzyme *RsaI* or *AluI*, and then hybridized against probes for potato *pal*, *hmgr*, *pr-1* and *pr-5* genes. Arrows on the right indicate the size of DNA ladder.

Expression analysis of defense-related genes (pal, hmgr, pr-1 and pr-5)

Expression of pal-1 (Figure 6.4a)

No variation in the accumulation of *pal-1* transcripts was observed in the healthy control overtime. In local leaflets, a weak induction of *pal-1 i*n Kennebec×US-1 and Kennebec×US-8 was observed at 96 and 120 h.a.i.. No induction of *pal-1* was found in Russet burbank×US-1 or Russet burbank×US-8. In proximal leaflets, *pal-1* was first induced at 12 h.a.i. in Kennebec×US-1 and the induction level was maintained in the rest of the sampling period. *Pal-1* was also induced in Russet burbank×US-1 but it was only at 72 h.a.i. No induction was observed in Kennebec×US-8 until 120 h.a.i. and none was detected in Russet burbank×US-8. In distal leaflets, *pal-1* was only weakly induced in Kennebec×US-1 (24 h.a.i.) and Russet burbank×US-1 (120 h.a.i). The induction in distal leaflets was much weaker and delayed than in proximal leaflets.

In general, the expression of *pal-1* was induced earlier in Kennebec and Russet burbank inoculated with US-1 than with US-8. The stronger and earlier induction of *pal* was found in Kennebec. It was interesting to notice that the induction of *pal-1* first occurred in proximal than local then distal leaflets.

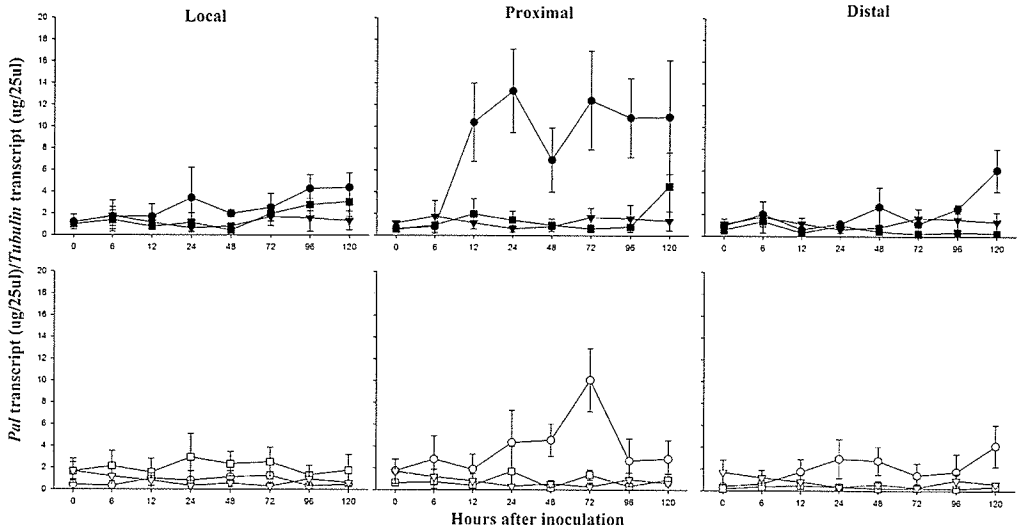
Expression of hmgr-2 (Figure 6.4b)

No changes in the level of *hmgr-2* transcript accumulation were recorded overtime in the healthy control samples. No variation in the accumulation of *hmgr-2* transcripts was found in local leaflets from Kennebec×US-1, Kennebec×US-8, Russet burbank×US-1 or Russet burbank×US-8. In proximal leaflets, *hmgr-2* was induced in proximal leaflets collected from Kennebec×US-1, in which *hmgr-2* was first induced at 12 h.a.i. and the level of induction was maintained until 96 h.a.i.. No variation in the

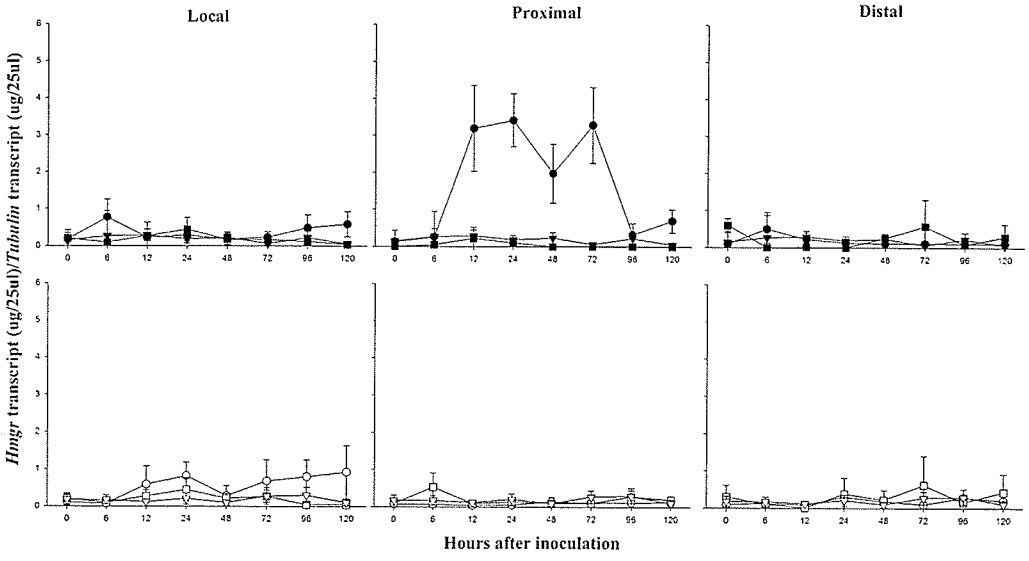
accumulation of *hmgr-2* transcripts was found in the distal leaflets collected from all four treatments.

Figure 6. 4 Quantitative real time RT-PCR analysis of defense-related gene expression

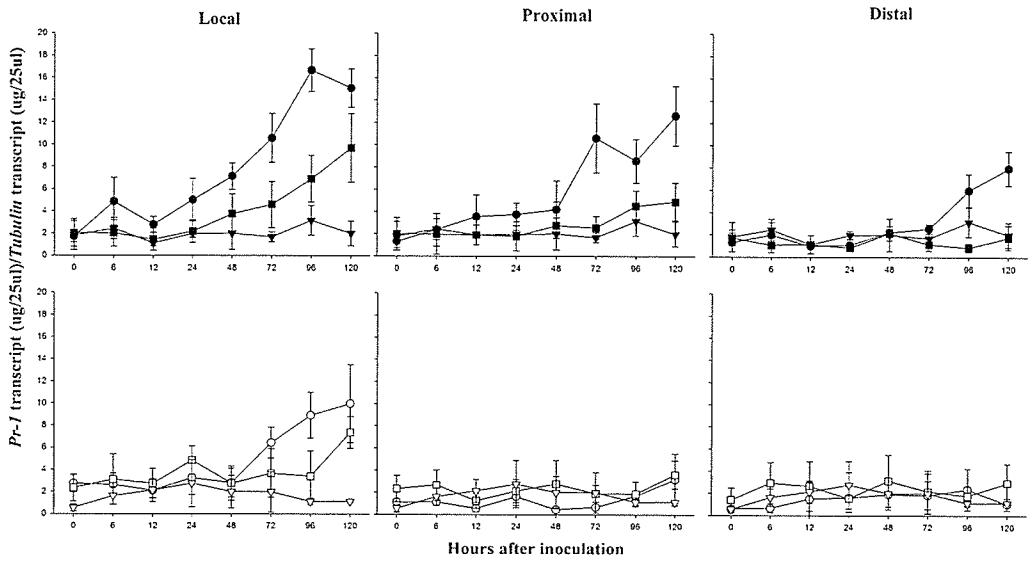
(a) *pal* (phenylalanine ammonia lyase)



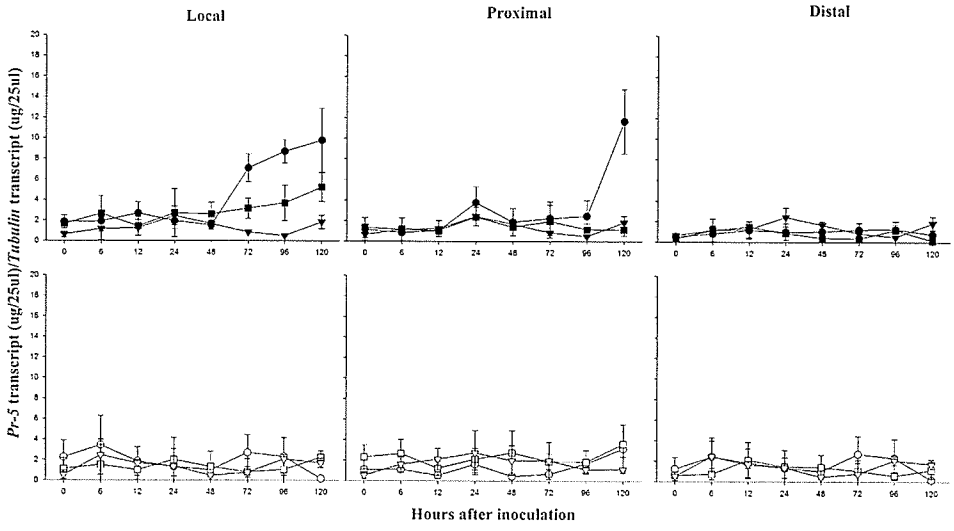
(b) *hmgr* (3-hydroxy, 3-methylglutaryl CoA reductase)



(c) *pr-1 (pathogenesis related protein)*



(d) *pr-5 (pathogenesis related protein)*



Quantitative real time RT-PCR analysis of the accumulation of (a) *pal* (phenylalanine ammonia lyase), (b) *hmgr* (3-hydroxy, 3-methylglutaryl CoA reductase), (c) *pr-1 (pathogenesis related)* and (d) *pr-5* transcripts (means ± SE) in local (L), proximal (P) and distal (D) leaflets of Kennebec and Russet burbank inoculated with two isolates of *P. infestans* from two genotypes: US-1 and US-8. (-▼-) Kennebec×control; (-●-) Kennebec×US-1; (-■-) Kennbec×US-8; (-▽-) Russet burbank×control; (-○-) Russet burbank×US-1; (-□-) Russet burbank×US-8.

Expression of pr-1 (Figure 6.4c)

No induction of *pr-1* was observed in healthy leaflets. *Pr-1* was induced after Kennebec and Russet burbank were inoculated with either US-1 or US-8. In local leaflets, *pr-1* was induced earlier in potat leaves in response to US-1 than to US-8 and in Kennebec than Russet burbank after inoculation. The strongest and earliest induction of *pr-1* was found in Kennebec×US-1. In proximal leaflets, *pr-1* was strongly induced from 72 to 120 h.a.i. in Kennebec×US-1 while no such induction was found in Kennebec×US-8, Russet burbank×US-1 or Russet burbank×US-8. In distal leaflets, *pr-1* was only induced in Kennebec×US-1 (96 and 120 h.a.i.) (Fig. 6.4c).

Expression of pr-5 (Figure 6.4d)

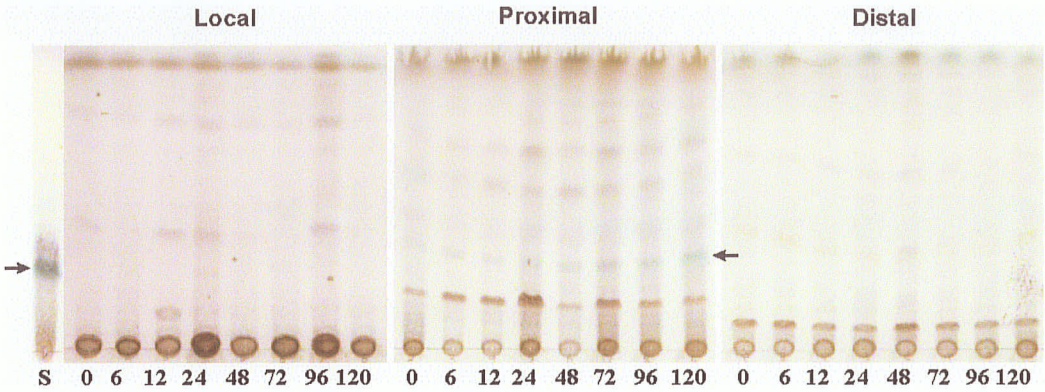
Pr-5 was not induced in healthy potato leaflets. Among the treatments, *pr-5* was only induced in the local and proximal leaflets collected from Kennebec×US-1. The induction of *pr-5* first appeared in local followed by proximal then distal leaflets. No significant variations were recorded in the induction of *pr-5* in Kennebec×US-8, Russet burbank×US-1 or Russet burbank×US-8 (local, proximal and distal).

TLC analysis of rishitin accumulation in infected potato leaves

Extracts from potato leaves inoculated with *P. infestans* were analyzed with thin layer chromatography. The accumulation of rishitin was detected using the vanillin/sulphuric acid spray (Figure 6.5). In Kennebec×US-1, no accumulation of rishitin was detected in local or distal leaflets. In proximal leaflets, rishitin was detected 24 h.a.i. and remained detectable in the rest of the sampling period. Similar experiments were also performed on samples from Kennebec×US-8, Russet burbank×US-1 and

Russet burbank×US-8. No accumulation of rishitin was detected using TLC in those samples (data not shown).

Figure 6. 5 Thin layer chromatograph analysis of rishitin accumulation in local, proximal and distal leaflets of Kennebec inoculated with *Phytophthora infestans* US-1 genotype isolate



Thin layer chromatograph analysis of rishitin accumulation in methanol extracts of Kennebec leaves inoculated with *Phytophthora infestans* US-1 genotype isolate.The accumulation of rishitin was detected using the vanillin/sulphuric acid spray (Lyon 1972). The purified rishitin was used as a standard (S) in thin layer chromatography and arrows pointed the blue band representing rishitin.

6.5 Discussion

Based on the current knowledge on plant defense responses, the recognition between the plant receptors and the corresponding pathogen elicitors is followed by a hypersensitive reaction, and the initiation of signalling cascades leading to the activation of downstream plant defense-related gene expression (Baker et al. 1997; Belkhadir et al. 2004; Gabriel 1999; Rathjen & Moffett 2003).

Russet burbank has none of the known R resistance genes while Kennebec possesses the resistance gene R1. The two *P. infestans* isolates, FA1 (US-1) and D1901 (US-8), are both capable of overcoming the R1 gene (Wang et al. 2005b). Therefore, the interactions between potato and *P. infestans* (Kennebec×US-1, Kennebec×US-8,

Russet burbank×US-1, Russet burbank×US-8) in our study should all be considered under non race-specific resistance. It was interesting to notice that hypersensitive reaction-like reaction was induced in both cultivars. The timing and frequency of the occurrence of hypersensitive reaction in the two tested cultivars was related to their relative levels of resistance to *P. infestans*. Hypersensitive reaction was induced earlier and more frequently in Kennebec (moderately tolerant) than in Russet burbank (highly susceptible) after inoculation. The hypersensitive reaction-like reaction in our study appeared to be ineffective in blocking lesions progress and was similar to trailing hypersensitive reaction phenotype described by Vleeshouwers *et al.* (2000a), in which hyphae grew ahead of hypersensitive reaction-responding cells. The induction of hypersensitive reaction in the two tested cultivars is in agreement with the finding of Vleeshouwers *et al.* (2000a) where hypersensitive reaction was associated with all forms of resistance to *P. infestans*, including both race-specific and non race-specific resistance.

In potato, non race-specific resistance to late blight, also known as partial resistance, is often related to the presence of qualitative trait loci (QTL) (Kamoun et al. 1999). Integration of QTL for resistance to late blight has revealed the close linkage between QTL and different R genes (Leonards-Schippers et al. 1994; Gebhardt & Valkonen 2001). It has been shown that some dominant R genes can also confer partial resistance in several plant systems (Lauge et al. 1998). Therefore it has been suggested that R genes could act as components of QTL and be involved in the partial resistance to late blight. Our observation that the hypersensitive reaction occurred earlier and

more frequently in Kennebec (R1) than in Russet burbank (none) supports such a potential role of R1 in the resistance of Kennebec to *P. infestans*. R1 could enhance the sensitivity of potato in terms of pathogen perception and activation of defense responses (Ferris 1955; Vleeshouwers et al. 2000a).

Beside the difference in the induction of trailing hypersensitive reaction between the two cultivars, Hypersensitive reaction was found to occur earlier and more frequently in potato plants inoculated with US-1 than with US-8. It could be explained by the weak interaction between R genes and ligands produced by different *P. infestans* isolates as previously proposed by Kamoun et al. (1999). In a weak *R-avr* interaction, a pathogen *avr* gene could encode the "weak" ligand which interacts with the corresponding resistance gene product. In our case, the qualitative difference we observed in the frequency and timing of trailing HR induction between Kennebec×US-1 and Kennebec×US-8 could also result from the ligand(s) produced by *P. infestans* US-1 or US-8 isolates. The two *P. infestans* isolates might be producing either the same ligand in different quantities or ligands with different reactivity to the corresponding plant receptor(s).

P. infestans has been shown to be capable of producing suppressors which can inhibit hypersensitive reactions. Doke *et al.* (1979b) reported that the induction of hypersensitive reaction was suppressed in potato tubers inoculated with an incompatible race of *P. infestans*. Conversely, once the tissue had been inoculated with an incompatible race of *P. infestans*, the inhibition of hypersensitive reaction did not occur, which suggested that the compatible race of *P. infestans* was capable of

producing compounds suppressing the initiation and magnitude of the hypersensitive reaction. In our study, the comparison was made between reactions of potato to 2 compatible *P. infestans* isolates. The induction of trailing hypersensitive reaction was found in Kennebec inoculated with either US-1 or US-8. Furthermore, hypersensitive reactions in our study were ineffective in blocking further development of *P. infestans* hyphae and there was less and weaker hypersensitive reactions in Kennebec×US-8 than in Kennebec×US-1. Therefore based on these observations, the possibility that *P. infestans* isolates could produce suppressors which inhibit an effective hypersensitive reaction can not be ruled out (Doke et al. 1979a; Doke et al. 1979b; Garas et al. 1979). Previously, Garas et al. (1979) reported that a suppressor from the compatible races of *P. infestans* was more active in inhibiting hypersensitive reaction and accumulation of rishitin and lubimin than the suppressor extracted from the incompatible race. In this study, we observed that less and weaker hypersensitive reaction was induced in Kennebec in response to US-1 than to US-8, which indicated that the potential suppressors from US-8 could be more effective in suppressing hypersensitive reaction. At the transcriptional level, the up regulation of *pal* and *hmgr* only found at some distance away from the inoculation site is clearly suggesting that suppression of gene expression occur at the site of the inoculation. Whether such suppression is related to the differential hypersensitive reaction development was not investigated at this time.

To monitor the activation of defense-related genes, we studied the expression profile of four defense-related genes using quantitative real time RT-PCR. Overall, we found that these genes were induced earlier in Kennebec than in Russet burbank after

inoculation, and in potato inoculated with US-1 than with US-8. This pattern is very similar to the pattern of hypersensitive reaction induction we observed. The earlier induction of hypersensitive reaction and four defense-related genes in Kennebec can be explained by the presence of certain mechanisms in Kennebec which trigger earlier activation of defense responses to *P. infestans*. Furthermore, induction of these genes appeared earlier in Kennebec×US-1 than in Kennebec×US-8. The activation of defense responses was also observed in Russet burbank inoculated with US-1. For example, *pal* and *pr-1* were also induced in Russet burbank inoculated with US-1 although they were weaker and delayed compared to their induction in Kennebec. Since Russet burbank possesses none of the 11 known R genes, the activation of *pal* and *pr-1* in this cultivar can not be related to resistance mediated by known R genes.

Our results showed that the four defense-related genes were systemically upregulated in leaflets remote from the inoculation site (proximal and distal). This result is in agreement with our previous finding with northern blot analysis in which *pal*, *hmgr*, *pr-1* and *pr-5* were found to be systemically induced in potato in response to *P. infestans* (Wang et al. 2005a; Wang et al. 2005b). The systemic induction of these defense-related genes could be explained by systemic acquired resistance which has been previously described in potato at many occasions (Doke et al. 1987; Vleeshouwers et al. 2000b; Cohen et al. 1993). The systemic induction of defense-related genes could result in an enhanced state of resistance in non-infected plant tissues. Several modes of signalling have been proposed such as through salicylic acid, lipid-derived molecules, and reactive oxygen species (Baker et al. 1997; Dempsey et al. 2001; Nimchuk et al.

2003; Shirasu et al. 1996; Van Bel & Gaupels 2004). However, the nature of the long-distance signal(s) which trigger(s) the systemic induction of defense genes in this system is still unknown.

It is remarkable that only weak or no induction of *pal* and *hmgr* occurred in the local leaflets inoculated with US-1 or US-8, respectively, while these two genes were induced in much larger amounts in proximal leaflets. In contrast, the earliest induction of *pr-1* and *pr-5* were found in local leaflets followed by proximal then distal leaflets. This result is in agreement with our previous finding with northern blot analysis in which the expression of *pal* and *hmgr* were suppressed at the site of infection (Wang et al. 2005b) and in line with the results of Choi (Choi et al. 1992) and Yoshioka (Yoshioka et al. 1996) in which the accumulation of *pal* and *hmgr* was suppressed in potato tubers inoculated with *P. infestans*. However, the current and our previous studies are the first to show that such gene down-regulation (i) occurs locally and not systemically; (ii) occurs also in potato foliage and not only in the tubers; (iii) is specific to some genes (eg. *pal-1* and *hmgr-2*) and not to others (i.e. *pr-1* and *pr-5*); and (iv) occurs differentially in response to different genotypes of *P. infestans*.

Suppression of host defense responses is thought to play an important role in plant-microbe interactions, especially those involving biotrophic pathogens that require live plant cells to establish a successful infection (Heath 2000). The nature and mode of suppression involved in our study is still not clear. However water-soluble glucans produced by different isolates of *P. infestans* have been reported to be capable of suppressing the accumulation of sesquiterpene phytoalexins (Andreu et al. 1999;

Ozeretskovskaya et al. 2001; Currier 1981) in potato tubers. Another class of suppressors produced by *P. infestans* is extracellular protease inhibitors which can directly interact with and inhibit host proteases (Tian et al. 2004).

Up to date, the mode of the action of suppressors in plant defense responses is not well characterized in general. Three possible mechanisms have been proposed. Suppressors can inhibit the interactions between the elicitor from the pathogen and the corresponding receptor from the plant by blocking the receptor site (Doke et al. 1979a; Doke et al. 1979b; Garas et al. 1979). Also, recent studies have suggested that suppressors may block the signal transduction pathway during the elicitor-mediated activation of the defense response (Yoshioka et al. 1990; Shiraishi et al. 1991; Toyada et al. 1992). Furthermore, suppressors could affect the formation of binding complexes in the promoter region and suppress the expression of specific genes such as *pal* at the transcription level (Wada et al. 1995).

In our study, the expression of *pal* and *hmgr* seems to be coordinately induced and suppressed by *P. infestans* US-1 or US-8 isolates. The accumulation of the sesquiterpene phytoalexin, rishitin, was only found in the proximal leaflets of Kennebec inoculated with US-1, which is in agreement with the pattern of *hmgr* induction revealed by quantitative real-time RT-PCR analysis. HMGR catalyzes the first step specific to isoprenoid biosynthesis which produces mevalonic acid, the basic precursor for all cellular isoprenoid and sesquiterpene phytoalexins. Our finding suggests that the suppression of sesquiterpene phytoalexins could be related to the suppression of *hmgr* induction by a suppressor from *P. infestans*. Andreu *et al.* (Wada

et al. 1995; Andreu et al. 1999) has shown that the accumulation of sesquiterpene phytoalexins was affected by β-glucans produced by highly virulent *P. infestans* strains. Nevertheless, whether the early accumulation of glucanase in this system (Schroder et al. 1992) is involved in the release of glucans with potential suppressing activity has yet to be investigated. It is not known yet whether the suppression of *pal* and *hmgr* result from the activity of the same suppressor. Studies have shown that *P. infestans* is capable of producing different types of molecules which can suppress potato defense response. For example, a kazal-like extracellular serine protease (EPI1) inhibitor has been recently identified from *P. infestans* (Tian et al. 2004). EPI1 can specifically inhibit subtilisin, a major serine protease and pathogenesis-related protein P69B (PR-7) from tomato. β-glucans with different polymerization from *P. infestans* are also capable of suppressing potato defense response (Andreu et al. 1999; Ozeretskovskaya et al. 2001).

The localized suppression of *pal* and *hmgr* in our study indicates that the possible translocation of the signal which leads to the induction of *pal* and *hmgr* in the proximal leaflets is not affected or entirely inhibited by the potential suppressors. Furthermore, *Pr-1* and *pr-5* were found to be induced both locally and systemically in our study. This suggested that the expression of the two *pr* genes may be regulated through a signalling system different from the one regulating the expression of *pal* and *hmgr*.

In summary, we studied the defense response in potato cultivars with partial resistance to *P. infestans*. The comparisons were made between two potato cultivars and two compatible *P. infestans* isolates (US-1 and US-8). Our study indicated the

quantitative nature of resistance to *P. infestans* in two tested potato cultivars and the outcome of the interaction is affected by both the activation and suppression of plant defense responses by elicitors and suppressors produced by *P. infestans*.

7.0 General Discussion

7.1. Differential activation of defense responses in potato in response to *P. infestans* genotype US-1 and US-8

Relatively little is known about the molecular mechanisms of potato defense response in responses to *P. infestans* infection (Birch & Whisson 2001). Such information could be crucial to the development of effective strategies to combat this disease. From the host perspective, resistance is often dependent on the activation of various defense mechanisms (Birch et al. 2003). Our results showed that defense responses were activated earlier in response to *P. infestans* US-1 isolate (low aggressiveness) than to US-8 isolate (high aggressiveness) in two tested potato cultivars. Furthermore, the activation of defense responses is also affected by the level of late blight resistance in a particular potato cultivar. For example, the defense-related genes were induced earlier in Kennebec, a potato cultivar with moderate tolerance to *P. infestans*, than in Russet burbank, which is highly susceptible to *P. infestans*. These results suggested that recent increase in late blight severity on current potato cultivars resulted from a difference in the activation of potato defense responses by the more aggressive *P. infestans* isolates.

7.2. Occurrence of hypersensitive reaction in potato cultivars with partial resistance to late blight in response to *P. infestans* infection

Hypersensitive reaction (HR) is a defense response commonly associated with R gene-mediated resistance in many host-pathogen systems (Nimchuk et al. 2003; Lamb & Dixon 1997) and results from the recognition between the receptor encoded by R gene and the specific elicitor encoded by the corresponding *avr* gene in the pathogen (Gabriel 1999; Hammond-Kosack & Jones 1997; Hutcheson 1998; Nimchuk et al. 2003; Vleeshouwers et al. 2000a). In our study, HR was found to be induced in both cultivars in response to either *P. infestans* US-1 or US-8 isolate. The occurrence of HR in the case of non race-specific resistance in our study is in agreement with the findings of Wilson and Coffey (1980) and Vleeshouwers (2000a) in which HR was found to be associated with both race-specific and non race-specific resistance.

It is a common belief that non race-specific resistance may be due to intrinsic properties of the plant or could be induced by non-specific elicitors produced by all races of the pathogen (Agrios 2005; Vleeshouwers et al. 2000a). However, recent studies on the occurrence of HR in potato cultivars with partial resistance to late blight in response to *P. infestans* infection supported the hypothesis that R gene receptors recognized by specific pathogen elicitors might also be involved (Kamoun et al. 1999). The correlations we observed between the timing of HR induction and the presence of R1 in Kennebec suggested that R1 could be involved in the resistance of Kennebec to *P. infestans*. R1 could enhance the sensitivity of Kennebec in terms of pathogen perception and activation of defense responses (Ferris 1955; Vleeshouwers et al. 2000a). Even though most R genes are overcome by *P. infestans*, this might mean that

R gene might still benefit the plant if they are integrated to non race-specific resistance in ether classical or biotechnology-based breeding.

7.3. Localized and systemic induction of defense-related genes

Plants react to pathogen attacks by activating a great variety of biochemical and molecular responses which could occur at two levels: localized acquired resistance (LAR) or systemic acquired resistance (SAR) (Kombrink & Somssich 1995; Schmelzer et al. 1995; Beligni et al. 1999). Localized acquired resistance occurs in the proximity of infection sites and is associated with the activation of various host defense-related responses including hypersensitive reactions and expression of genes encoding various PR proteins (Costet et al. 2002). In our study, hypersensitive reaction and seven defense-related genes were found to be induced in the local leaflets of tested potato cultivars inoculated with two isolates of *P. infestans*. The timing of their induction is related to the level of late blight resistance in tested cultivars. It suggests that local acquired resistance is probably involved in the resistance of Kennebec and Russet burbank to *P. infestans*. The timing of the activation of defense response is related to the resistance of potato to *P. infestans*.

Systemic acquired resistance (SAR), which generally follows a localized unsuccessful pathogen attack and involves an enhanced state of resistance to a broad spectrum of pathogens (Ryals et al. 1996), has been described in several plant species, including potato (Vleeshouwers et al. 2000b; Ryals et al. 1996; Cohen et al. 1991b; Cohen et al. 1993; Doke et al. 1987). SAR is commonly associated with increased expression of genes encoding various PR proteins in uninfected plant tissues

(Vleeshouwers et al. 2000b). In our study, *pr-1, pr-2, pr-3, pr-4* and *pr-5* were induced both locally and systemically. The systemic induction of these genes could be part of systemic acquired resistance in potato initiated by *P. infestans* infection. The nature of the long-distance signal(s) which initiates the systemic induction of defense-related genes is currently still unknown in potato. Several modes of signaling has been proposed for systemic acquired resistance, such as signaling through salicylic acid, lipid-derived molecules, and reactive oxygen species (Baker et al. 1997; Dempsey et al. 2001; Nimchuk et al. 2003; Shirasu et al. 1996; Van Bel & Gaupels 2004). Salicylic acid is one of the best characterized signaling molecules and seems to be capable of moving from its production site in the inoculated leaves to remote leaves through tobacco phloem (Shulaev et al. 1995). Nevertheless, determining whether salicylic acid also functions as a long-distance signaling molecule in potato systemic acquired resistance has been complicated by the fact that potato possesses high base level of salicylic acid (Yu et al. 1997). Other molecules, such as jasmonic acid and nitric oxide might, be also involved as signaling molecules in such systemic reactions, but only little has been done regarding the potential role of these molecules in this pathosystem.

7.4. Suppression of potato defense reactions by *P. infestans*

In response to the continual challenge by a broad spectrum of pathogens, plants have evolved a diverse battery of defense responses activated upon the recognition of potential invaders. Accordingly, pathogens have developed the capability of suppressing host defense responses as a form of counter-defense mechanism (Rose et al. 2002; Tian et al. 2004). To date, few pathogen molecules

capable of suppressing host defense responses have been identified, such as tomatinase, a saponin-detoxifying enzyme from *Septoria lycopersici* (Bouarab et al. 2002) and glucanase inhibitor proteins produced by *P. sojae* (Rose et al. 2002). Several studies have indicated that *P. infestans* and other *Phytophthora* species are also capable of producing suppressors such as water-soluble glucans that suppress various plant defense responses (Andreu et al. 1999; Ozeretskovskaya et al. 2001). Furthermore, a kazal-like extracellular serine protease inhibitor, targeting tomato pathogenesis-related protease P68B, has been recently identified from *P. infestans* (Andreu et al. 1999; Tian et al. 2004).

In our study, the expression of *pal* and *hmgr* as well as the synthesis of sesquiterpene phytoalexin rishitin were suppressed at the site of inoculation. Our result is in agreement with the findings of Andreu et al. (1999) in which the production of rishitin was found to be suppressed in potato tubers inoculated with different isolates of *P. infestans.* In our study, *hmgr* and rishitin were both found to be induced in proximal leaflets of Kennebec×US-1, suggesting that the suppression effect from *P. infestans* is localized. It has been previously shown that water-soluble high molecular compounds capable of inhibiting or delaying the activation of defense response in potato tubers were released from zoospores of compatible races of *P. infestans* and their germination fluid during the infection process (Doke et al. 1979a). Furthermore, the localized suppression of *pal* and *hmgr* also suggests that the possible translocation of signal(s) associated with SAR is not affected or entirely inhibited by the suppressors from *P. infestans*. It is interesting that the induction of *pr* genes is not suppressed by *P. infestans*

which suggests that the suppression effect of *P. infestans* on *pal* and *hmgr* has some level of specificity. One of the possible models is that suppressors of *P. infestans* could specifically affect the formation of binding complexes in the promoter region of specific genes, such as *pal* (Wada et al. 1995), and suppress its expression at transcription level.

7.5. Future studies

Our study indicated that a fine balance between induction/suppression of plant defense responses seems to determine the resistance or susceptibility of potato cultivars to late blight. Defense responses were found to be activated earlier in Kennebec than in Russet burbank in response to *P. infestans* and the potential role of resistance gene R1 was previously discussed. Nevertheless, the involvement of R1 in the resistance of Kennebec to *P. infestans* can be better explained using gene silencing techniques such as RNAi or antisense RNA (Ballvora et al. 2002).

Defense responses were found to be activated earlier in potato in response to US-1 than US-8. However, it is still not clear whether the similar induction pattern of defense-related genes could be observed in potato in response to other new *P. infestans* genotypes such as US-11 (A1 mating type, highly aggressive). Furthermore, techniques such as differential display or subtractive hybridization can provide a better overall understanding of differential regulation of plant defense-related genes in potato in response to different genotypes of *P. infestans.*

We demonstrated that defense response in potato was suppressed by *P. infestans*. However, the nature of the suppressors produced by the two *P. infestans* isolates was not investigated at this point and could lay ground for a new line of research.

8.0 References

Abad,L.R., Paino,U., Liu,D., Narasmhan,M.L., Reuveni,M., Zhu,J.K., Niu,S., Singh,N.K., Hasegawa,P.M., and Bressan,R.A. 1996. Antifungal activity of tobacco osmotin has specificity and involves plasma membrane permeabilization. Plant Science **118:** 11-23.

Abenthum,K., Hildenbrand,S., and Ninnemann,H. 1995. Elicitation and accumulation of phytoalexins in stems, stolons and roots of *Erwinia*-infected potato plants. Physiological and Molecular Plant Pathology **46:** 349-359.

Agrios,G. 2005. Plant pathology, 4th eds. Academic Press, San Diego.

Alexander,D., Goodman,R.M., Gut-Rella,M., Glascock,C., Weymann,K., Friedrich,L., Maddox,D., Ahl-Goy,P., Luntz,T., and Ward,E. 1993. Increased tolerance to two oomycete pathogens in transgenic tobacco expressing pathogenesis-related protein 1a. Proceedings of the National Academy of Sciences of the United States of America. **90:** 7327-7331.

Andreu,A., Tonon,C., Damme,M.V., Huarte,M., and Daleo,G. 1999. Effect of glucans from different races of *Phytophthora infestans* on defense reactions in potato tuber. European Journal of Plant Pathology **104:** 777-783.

Andrivon,D. 1995. Biology, ecology, and epidemiology of the potato late blight pathogen *Phytophthora infestans* in soil. Phytopathology **85:** 1053-1056.

Arias,J.A., Dixon,R.A., and Lamb,C.J. 1993. Dissection of the functional architecture of a plant defense gene promoter using a homologous *in vitro* transcription initiation system. Plant Cell **5:** 485-496.

Armstrong,M.R., Whisson,S.C., Pritchard,L., Bos,J.I.B., Venter,E., Avrova,A.O., Rehmany,A.P., Brooks,K., Cherevach,I., Hamlin,N., White,B., Fraser,A., Lord,A., Quail,M.A., Churcher,C., Hall,N., Berriman,M., Huang,S., Kamoun,S., Beynon,J.L., and Birch,P.J. 2005. An ancestral oomycete locus contains late blight avirulence gene *Avr3a*, encoding a protein that is recognized in host cytoplasm. Proceedings of the National Academy of Sciences of the United States of America. **102:** 7766-7771.

Avrova,A.O., Stewart,H.E., De Jong,W.D., Heilbronn,J., Lyon,G.D., and Birch,P.R. 1999. A cysteine protease gene is expressed early in resistant potato interactions

with *Phytophthora infestans*. Molecular Plant-Microbe Interactions **12:** 1114-1119.

Avrova,A.O., Taleb,N., Rokka,V.M., Heilbronn,J., Campbell,E., Hein,I., Gilroy,E.M., Cardle,L., Bradshaw,J.E., Stewart,H.E., Fakim,Y.J., Loake,G., and Birch,P.R. 2004a. Potato oxysterol binding protein and cathepsin B are rapidly up-regulated in independent defence pathway that distinguish R gene-mediated and field resistance to *Phytophthora infestans*. Molecular Plant Pathology **5:** 45-56.

Avrova,A.O., Venter,E., Birch,P.R., and Whisson,S.C. 2003. Profiling and quantifying differential gene transcription in *Phytophthora infestans* prior to and during the early stages of potato infection. Fungal Genetics and Biology **40:** 4-14.

Baker,B., Zambryski,P., Staskawicz,B., and Dineshkumar,S.P. 1997. Signaling in plant microbe interactions. Science **276:** 726-733.

Ballvora,A., Ercolano,M.R., Weiss,J., Meksem,K., Bormann,C.A., Oberhagemann,P., Salamini,F., and Gebhardt,C. 2002. The R1 gene for potato resistance to late blight (*Phytophthora infestans*) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. Plant Journal **30:** 361-371.

Beerhues,L. and Kombrink,E. 1994. Primary structure and expression of mRNAs encoding basic chitinase and 1,3-beta-glucanase in potato. Plant Molecular Biology **24 :** 353-367.

Beligni,M.V., Laxalt,A.M., and Lamattina,L. 1999. Temporal and spatial patterns of GAPDHC mRNA accumulation during an incompatible potato-*Phytophthora infestans* interaction, compared with a compatible interaction. Physiologica Plantarum **2:** 280-287.

Belkhadir,Y., Subramaniam,R., and Dangl,J.L. 2004. Plant disease resistance protein signaling: NBS-LRR proteins and their parters. Current Opinion in Plant Biology **7:** 391-399.

Bertini,L., Leonardi,L., Caporale,C., Tucci,M., Cascone,N., Deradino,I.D., Buonocore,V., and Caruso,C. 2003. Pathogen-responsive wheat PR4 genes are induced by activators of systemic acquired resistance and wounding. Plant Science **164:** 1067-1078.

Beyer,K., Jimenez,S.J., Randall,T.A., Lam,S., Binder,A., Boller,T., and Collinge,M.A. 2002. Characterization of *Phytophthora infestans* genes regulated during the interaction with potato. Molecular Plant Pathology **3:** 473-485.

Birch,P.R.J., Avrova,A.O., Armstrong,M., Venter,E., Taleb,N., Gilroy,E.M., and Whisson,S.C. 2003. The potato - *Phytophthora infestans* interaction transcriptome. Canadian Journal of Plant Pathology **25:** 226-231.

Birch,P.R.J., Avrova,A.O., Duncan,J.M., Lyon,G.D., and Toth,R.L. 1999. Isolation of potato genes that are induced during an early stage of the hypersensitive response to *Phytophthora infestans*. Molecular Plant-Microbe Interactions **12:** 356-361.

Birch,P.R.J. and Whisson,S.C. 2001. *Phytophthora infestans* enters the genomics era. Molecular Plant Pathology **2:** 257-263.

Bolwell,G.P. 1992. A role for phosphorylation in the down-regulation of phenylalanine ammonia lyase in suspension-cultured cells of French bean. Phytochemistry **31:** 4081-4086.

Bormann,C.A., Rickert,A.M., Castillo-Ruiz,R.A., Strahwald,J., Buhr,K., and Gebhardt,C. 2004. Tagging quantitative trait loci for maturity-correlated late blight resistance in tetraploid potato with PCR-based candidate gene markers. Molecular Plant-Microbe Interactions **17:** 1126-1138.

Bos,J.I.B., Armstrong,M., Whisson,S.C., Torto,T.A., Ochwo,M., and Birch,P.R.J. 2003. Intraspecific comparative genomics to identify avirulence genes from *Phytophthora* . New Phytologist **159:** 63-72.

Bostock,R.M., Kuc,J.A., and Laine,R.A. 1981. Eicosapentenoic and arachidonic acids from *Phytophthora infestans* elicits fungitoxic sesquiterpenes in the potato. Science **212:** 67-69.

Bostock,R.M., Schaeffer,D.A., and Hammer-schmidt,R. 1986. Comparsion of elicitor activities of arachidonic acid, fatty acids, and glucans from *Phytophthora infestans*. Physiological and Molecular Plant Pathology **29:** 339-360.

Bouarab,K., Melton,R., Peart,J., Baulcombe,D., and Osbourn,A. 2002. A saponin-detoxifying enzyme mediates suppression of plant defences. Nature **22:** 889-892.

Boyd,L.A., Smith,P.H., Green,R.M., and Brown,J.K.M. 1994. The relationship between the expression of defense-related genes and mildew development in barley. Molecular Plant-Microbe Interactions **7:** 401-410.

Brederode,F.T., Linthorst,H.J.M., and Bol,J.F. 1991. Diffferential induction of aquired resistance and PR gene expression in tobacco by virus infection, ethephon treatment, UV light and wounding. Plant Molecular Biology **17:** 1117-1125.

Brisson,L.F., Tenhaken,R., and Lamb,C. 1994. Function of oxidative cross-talking of cell wall structural proteins in plant disease resistance. Plant Cell **6:** 1703-1712.

Brouwer,D.J., Jones,E.S., and Clair,D.A. 2004. QTL analysis of quantitative resistance to *Phytophthora infestans* (late blight) in tomato and comparions with potato. Genome **47:** 475-492.

Brurberg,M.B., Hannukkala,A., and Hermansen,A. 1999. Genetic variability of *Phytophthora infestans* in Norway and Finland as revealed by mating type and fingerprint probe RG57. Mycological Research **103:** 1609-1615.

Buchter,R., Stromberg,A., Schmelzer,E., and Kombrink,E. 1997. Primary structure and expression of acidic (classII) chitinase in potato. Plant Molecular Biology **35:** 749-761.

Bulone,V., Chanzy,H., Gay,L., Girard,V., and Fevre,M. 1992. Characterization of chitin and chitin synthase from the cellulosic cell wall fungus *Saprolegnia monoica*. Experimental Mycology **16:** 8-21.

Castoria,R., Fanelli,C., Fabbri,A.A., and Passi,S. 1992. Metabolism of arachidonic acid involved in its eliciting activity in potato tuber. Physiological and Molecular Plant Pathology **41:** 127-137.

Chadha,K.C. and Brwon,S.A. 1974. Biosynthesis of phenolic acid in tomato plants infected with *Agrobacterium tumefaciens*. Canadian Journal of Botany **52:** 2041-2046.

Chandok,M.R., Ytterberg,A.J., van Wijk,K.J., and Klessig,D.F. 2003. The pathogen-inducible nitric oxide synthase (iNOS) is a variant of the P protein of the glycine decarboxylase complex. Cell **113:** 469-482.

Choi,D., Bostock,R.M., Avdiushko,S., and Hildebrand,D.F. 1994. Lipid-derived signals that discriminate wound- and pathogen-responsive isoprenoid pathways in plants: methyl jasmonate and the fungal elicitor arachidonic acid induce different 3-hydroxy-3-methylglutaryl-coenzyme A reductase genes and antimicrobial isoprenoids in *Solanum tuberosum L.* Proceedings of the National Academy of Sciences of the United States of America. **91:** 2329-2333.

Choi,D., Ward,B.L., and Bostock,R.M. 1992. Differential induction and suppression of potato 3-hydroxy-3-methylglutaryl coenzyme A reductase genes in response to *Phytophthora infestans* and to its elicitor arachidonic acid. Plant Cell **4:** 1333-1344.

Chycoski,C.I. and Punja,Z.K. 1996. Characteristics of populations of *Phytophthora infestans* from potato in Bristish Columbia and other regions of Canada during 1993 to 1995. Plant Disease **80:** 579-589.

Coffey,M. and Wilson,U. 1983. Histology and cytology of infection and disease caused by *Phytophthora* . In: *Phytophthora*: Its Biology, Taxonomy, Ecology and Pathology (eds D. C. Erwin, S. Bartnicki-Garcia and P. Tsao), pp 289-310. American Phytopathological Society. St Paul, Minnesota, USA.

Cohen,A., Gisi,U., and Mosinger,E. 1991a. Systemic resistance of potato plants againt *Phytophtora ifnestans* induced by unsaturated fatty acids. Physiological and Molecular Plant Pathology **38:** 255-264.

Cohen,Y., Gisi,U., and Mosinger,E. 1991b. Systemic acquired resistance of potato plants against *Phytophthora infestans* induced by unsaturated fatty acids. Physiological and Molecular Plant Pathology **38:** 255-264.

Cohen,Y., Gisi,U., and Niderman,T. 1993. Local and systemic protection against *Phytophthora infestans* induced in potato and tomato plants by jasmonic acid and jasmonic methyl ester. Phytopathology **83:** 1054-1062.

Collinge,M. and Boller,T. 2001. Differential induction of two potato genes, *Stprx2* and *stNAC*, in response to infection by *Phytophthora infestans* and to wounding. Plant Molecular Biology **46:** 521-529.

Collins,A., Milbourne,D., Ramsay,L., Meyer,R., Chatot-Balandras,C., Oberhagemann,P., Jong,A.J., Gebhardt,C., Bonnel,E., and Waugh,R. 1999. QTL for field resistance to late blight in potato are strongly correlated with maturity and vigour. Molecular Breeding **5:** 387-398.

Colon,L.T., Eijlander,R., Budding,D.J., Ijzendoorn,M.T., Pieters,M.M.J., and Hoogendoorn,J. 1993. Resistance to potato late blight (*Phytophthora infestans* (Mont.) de Bary) in *Solanum nigrum*, *S. villosum* and their sexual hybrids with *S. tuberosum* and *S. demissum*. Euphytica **66:** 64.

Colon,L.T., Turkensteen,L.J., Prummel,W., Budding,D.J., and Hoogendoorn,J. 1995. Durable resistance to late blight (*Phytophthora infestans*) in old potato cultivars. European Journal of Plant Pathology **101:** 387-397.

Cooke,D.E.L. and Lees,A.K. 2004. Markers, old and new, for examining *Phytophthora infestans* diversity. Plant Pathology **53:** 692-704.

Cooke,D.E.L., Young,V., Birch,P.R.J., Toth,R., Gourlay,F., Day,J.P., Carnegie,S.F., and Duncan,J.M. 2003. Phenotypic and genotypic diversity of *Phytophthora infestans* populations in Scotland (1995-97). Plant Pathology (Oxford) **52:** 181-192.

Coquoz,J.L., Buchala,A.J., and Metraux,J.P. 1998. The biosynthesis of salicylic acid in potato plants. Plant Physiology **117:** 1095-1101.

Coquoz,J.L., Buchala,A.J., Meuwly,P.H., and Metrux,J.P. 1995. Arachidonic acid induces local but not systemci synthesis of salicylic acid and confers systemic resistance in potato plants to *Phytophthora infestans* and *Alternaria solani*. Phytopathology **85:** 1219-1224.

Correll,D.S. 1962. *Tuberarium* of the Genus *Solanum*. In The Potato and its Wild Relatives. Texas Research Foundation. Renner, Texas. 606 pp.

Costet,L., Fritig,B., and Kauffmann,S. 2002. Scopoletin expression in elicitor-treated and tobacco mosaic virus infected tobacco plants. Plant Physiology **115:** 228-235.

Creelman,R.A. and Mullet,J.E. 1997. Biosynthesis and action of jasmonates in plants. Annual Review of Plant Physiology and Plant Molecular Biology **48:** 355-381.

Currier,W.W. 1981. Molecular controls in the resistance of potato to late blight. Trends in Biochemistry Science **6:** 191-194.

Cutt,J.R., Harpster,M.H., Dixon,D.C., Carr,J.P., Dunsmuir,P., and Klessig,D.F. 1989. Disease response to tobacco mosaic virus in transgenic tobacco plants that constitutively express the pathogenesis-related protein PR-1b gene. Virology **173:** 89-97.

Cuypers,B. and Hahlbrock,K. 1988. Immunohistological studies of compatible and incompatible interactions of potato leaves with *Phytophthora infestans* and of the non-host response to *Phytophthora megasperma*. Canadian Journal of Botany **66:** 700-705.

Daayf,F. and Platt,H.W. 1999. Assessment of mating types and resistance to metalaxyl of Canadian populations of *Phytophthora infestans* in 1997. American Journal of Potato Research **76:** 287-295.

Daayf,F. and Platt,H.W. 2000. Changes in metalaxyl resistance among glucose phosphate isomerase genotypes of *Phytophthora infestans* in Canada during 1997 and 1998. American Journal of Potato Research **77:** 311-318.

Daayf,F. and Platt,H.W. 2001a. Changes in metalaxyl resistance among glucose phosphate isomerase genotypes of *Phytophthora infestans* in Canada during 1997 and 1998. American Potato Journal **77:** 311-318.

Daayf,F. and Platt,H.W. 2001b. Cross-Canada potato late blight survey in 1999. Canadian Plant Disease Survey **81:** 141-143.

Daayf,F. and Platt,H.W. 2003. Differential pathogenicity on potato and tomato of *Phytophthora infestans* US-8 and US-11 strains isolated from potato and tomato. Canadian Journal of Plant Pathology **25:** 150-154.

Daayf,F., Platt,H.W., Mahuku,G., and Peters,R.D. 2001. Relationships between pathotypes and RAPDs, Gpi-allozyme patterns, mating types, and resistance to metalaxyl of *Phytophthora infestans* in Canada in 1997. American Journal of Potato Research **78:** 129-139.

Daayf,F., Platt,H.W., and Peters,R.D. 2000. Changes in mating types, resistance to metalaxyl, and Gpi-allozyme genotypes of *Phytophthora infestans* in Canadian provinces from 1996 to 1998. Canadian Journal of Plant Pathology **22:** 110-116.

Dangl,J.L., Dietrich,B., and Richberg,M.H. 1996. Death dont have no mercy: cell death programs in plant-microbe interactions. Plant Cell **8:** 1793-1807.

Dangl,J.L. and Jones,J.D.G. 2001. Plant pathogens and integrated defence reponses to infection. Nature **441:** 826-833.

Day,J.P., Wattier,R.A.M., Shaw,D.S., and Shattock,R.C. 2004. Phenotypic and genotypic diversity in *Phytophthora infestans* on potato in Great Britain, 1995-1998. Plant Pathology (Oxford) **53:** 303-315.

Delledonne,M., Xia,Y., Dixon,R.A., and Lamb,C. 1998. Nitric oxide functions as a signal in plant disease resistance. Nature **394:** 585-588.

Dempsey,D.A., shah,J., and Klessig,D.F. 2001. Salicylic acid and disease resistance in plants. Critical Reviews in Plant Science **18:** 547-575.

Despres,C., Subramaniam,R., Matton,D.P., and Brisson,N. 1995b. The activation of the potato PR-10a gene requires the phosphorylation of the nuclear Factor PBF-1. Plant Cell **7:** 589-598.

Dixon,R.A., Achnine,L., Kota,P., Liu,C.J., Reddy,M.S., and Wang,L.J. 2003. The phenylpropanoid pathway and plant defence - a genomics perspective. Molecular Plant Pathology **3:** 371-390.

Dodds,K.S. 1962. Classification of cultivated potatoes. In. The Potato and its Wild Relatives: Section *Tuberarium* of the Genus *Solanum*. Correll, D. S. Texas Research Foundation. Renner, Texas. 606 pp. 517-539.

Doke,N., Garas,N.A., and Kuc,J. 1979a. Effect on host hypersensitivity of supprssors released during the germination of *Phytophthora infestans* cystospores. Phytopathology **70:** 35-39.

Doke,N., Garas,N.A., and Kuc,J. 1979b. Partial characterization and aspects of the mode of action of a hypersensitivity-inhibiting factor (HIF) isolated from *Phytopthroa infestans.* Physiological Plant Pathology **15:** 127-140.

Doke,N. and Miura,Y. 1995. In vitro activation of NADPH-dependent O^{2-} generating system in a plasma membrane-rich fraction of potato tuber tissues by treatment with an elicitor from *Phytophthora infestans* or with digitonin . Physiological and Molecular Plant Pathology **46:** 17-28.

Doke,N., Ramirez,A.V., and Tomiyama,k. 1987. Systemic induction of resistance in potato plants against *Phytophthora infestans* by local treatment with hyphal wall components of fungus. Journal of Phytopathology **119:** 232-239.

Dubery,I.A., Teodorczuk,L.G., and Louw,A.E. 2000. Early responses in methyl jasmonate-preconditioned cells toward pathogen-derived elicitors. Molecular Cell Biology Research Communications **3:** 105-110.

Duncan,J.M. 1999. *Phytophthora infestans* - an abiding threat to our crops. Micrbiology Today **26:** 114-116.

Durner,J., Wendehenne,D., and Klessig,D.F. 1998. Defense gene induction in tobacco by nitric oxide, cyclic GMP and cyclic ADP-ribose. Proceedings of the National Academy of Sciences of the United States of America. **95:** 10328-10333.

Ebel,J. and Cosio,E.G. 1994. Elicitors of plant defense responses. International Review of Cytology. **148:** 1-36.

EL-Kharbotly,A., Leonards-Schippers,C., Huigen,D.J., Jacobsen,E., Pereira,A., Stiekema,W.J., Salamini,F., and Gebhardt,C. 1994. Segregation analysis and RFLP mapping of the R1 and R3 alleles conferring race-specific resistance to *Phytophthora infestans* in progeny of haploid potato parents. Molecular and General Genetics **242:** 749-754.

EL-Kharbotly,A., Palomino-Sanchez,C., Salamini,F., Jacobsen,E., and Gebhardt,C. 1996. R6 and R7 alleles of potato conferring race-specific resistance to *Phytophthora infestans* (Mont.) de Bary identified genetic loci clustering with the R3 locus on chromosome XI. Theoretical and Applied Genetics **92:** 880-884.

Ellis,B.E. and Amrhein,N. 1971. The 'NIH-shifting" during armatic *ortho*-hydroxylation in higher plants. Phytochemistry **10:** 3069-3072.

Ellis,J., Dodds,P., and Pryor,T. 2000. Function and evolution of plant disease resistance genes. Current Opinion in Plant Biology **3:** 278-284.

Erwin,D.C., Bartinicki-Garcia,S., and Tsao,P.H. 1983. *Phytophthora* : its biology, taxonomy, ecology, and pathology. *In* Erwin,D.C., Bartinicki-Garcia,S., and Tsao,P.H., eds. St. Paul, Minn. : American Phytopathological Society.

Erwin,D.C. and Ribeiro,O.K. 1996. *Phytophthora* diseases worldwide. APS press, St. Paul, Minn.

Ferris,V. 1955. Histological study of pathogen-suscept relations between *Phytophthora infestans* and derivatives of *Solanum demissum*. Phytopathology **45:** 546-552.

Feys,B.J. and Parker,J.E. 2000. Interplay of signaling pathways in plant disease resistance. Trends in Genetics **16:** 449-455.

Flier,W.G., Grunwald,N.J., Kroon,L.P.N.M., Sturbaum,A.K., van den Bosch,T.B.M., Garay-Serrano,E., Lozoya-Saldana,H., Fry,W.E., and Turkensteen,L.J. 2003. The population structure of *Phytophthora infestans* from the Toluca Valley of central Mexico suggests genetic differentiation between populations from cultivated potato and wild *Solanum* spp. Phytopathology **93:** 382-390.

Forbes,G.A., Goodwin,S.B., Drenth,A., Oyarzun,P., Ordonez,M.E., and Fay,W.E. 1998. A global marker database for *Phytophthora infestans*. Plant Disease **82:** 811-818.

Friends,J. 1991. The biochemistry and cell biology of interaction. In: Ingram,D. S. William,P. H. (eds.), Advances in Plant Pathology. Phytophthora infestans, the cause of late blight of potato, vol. 7, Academic Press 85-125.

Fritig,B., heitz,T., and Legrand,M. 1998. Antimicrobial proteins in induced plant defense. Current Opinion in Immunology **10:** 16-22.

Fry,W.E. and Goodwin,S.B. 1997a. Re-emergence of potato and tomato late blight in the United States. Plant Disease **81:** 1349-1357.

Fry,W.E., Goodwin,S.B., Dyer,A.T., Matuszak,J.M., Drenth,A., Tooley,P.W., Sujkowski,L.S., Koh,Y.J., Cohen,B.A., and Spielman,L.J. 1993. Historical and recent migrations of *Phytophthora infestans*: chronology, pathways, and implications. Plant Disease **77:** 653-661.

Fung,K.L., Zhao,K.J., He,Z.M., and Chye,M.L. 2002. Tobacco-expressed *Brassica juncea* chitinase BjCHL1 shows antifungal activity *in vitro*. Plant Molecular Biology **50:** 283-294.

Gabriel,D.W. 1999. Why do pathogens carry avirulence genes? Physiological and Molecular Plant Pathology **55:** 205-214.

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

Galis,I., Smith,J.L., and Jameson,P.E. 2004. Salicylic acid-, but not cytokinin-induced, resistance to WCIMV is associated with increased expression of SA-dependent resistance genes in *Phaseolus vulgaris*. Journal of Plant Physiology **161:** 459-466.

Garas,N.A., Doke,N., and Kuc,J. 1979. Suppression of the hypersensitive reaction in potato tubers by mycelial components from *Phytophthora infestans*. Physiological Plant Pathology **15:** 117-126.

Gaudet,D.A., Laroche,A., Frick,M., Davoren,J., Puchalski,B., and Ergon,A. 2000. Expression of plant defence-related (PR-protein) transcripts during hardening and dehardening of winter wheat. Physiological and Molecular Plant Pathology **57:** 15-24.

Gavino,P.D. and Fry,B.A. 2002. Diveristy in and evidence for selection on the mitochondrial geome of *Phytothora infestans*. Mycologia **94:** 781-793.

Gebhardt,C. and Valkonen,J.P. 2001. Organization of genes controlling disease resistance in the potato genome. Annual Review of Phytopathology **39:** 79-102.

Gobel,C., Feussner,I., Hamberg,M., and Rosahl,S. 2002. Oxylipin profiling in pathogen-infected potato leaves. Biochimica et Biophysica Acta **1584:** 55-64.

Goldstein,J.L. and Brwon,M.S. 1990. Regulation of the mevalonate pathway. Nature **343:** 425-430.

Goodwin,D.C. and Lee,S.B. 1993. Microwave miniprep of total genomic DNA from fungi, plants, protists and animals for PCR. Biotechniques **15:** 438, 441-2, 444.

Goodwin,S.B. 1997. The population genetics of *Phytophthora*. Phytopathology **87:** 462-473.

Goodwin,S.B. and Fry,W.E. 1994. Genetic analyses of interspecific hybrids between *Phytophthora infestans* and *Phytophthora mirabilis*. Experimental Mycology **18:** 20-32.

Goodwin,S.B., Schneider,R.E., and Fry,W.E. 1995. Use of cellulose-acetate electrophoresis for rapid identification of allozyme genotypes of *Phytophthora infestans*. Plant Disease **79:** 1181-1185.

Goodwin,S.B., Smart,C.D., Sandrock,R.W., Deahl,K.L., Punja,Z.K., and Fry,W.E. 1998. Genetic change within populations of *Phytophthora infestans* in the United States and Canada during 1994 and 1996: role of migration and recombination. Phytopathology **88:** 939-949.

Gornhardt,B., Rouhara,I., and Schmelzer,E. 2000. Cyst germination proteins of the potato pathogen *Phytophthora infestans* share homology with human mucins. Molecular Plant-Microbe Interactions **13:** 32-42.

Graham,M.Y., Weidner,J., Wheeler,K., Pelow,M.J., and Graham,T.L. 2003. Induced expression of pathogenesis-related protein genes in soybean by wounding and the *Phytophthora sojae* cell wall glucan elicitor. Physiological and Molecular Plant Pathology **63:** 141-149.

Griffith,G.W. and Shaw,D.S. 1998. Polymorphisms in *Phytophthora infestans*: four mitochondrial haplotypes are detected after PCR amplification of DNA from

pure cultures or from host lesions. Applied and Envionmental Microbiology **64:** 4007-4014.

Grube,R.C., Radwanski,E.R., and Jahn,M. 2000. Comparative genetics of disease resistance within the *solanaceae*. Genetics **155:** 873-887.

Gus-Mayer,S., Naton,B., Hahlbrock,K., and Schmelzer,E. 1998. Local mechanical stimulation induces components of the pathogen defense response in parsley. Proceedings of the National Academy of Sciences of the United States of America. **95:** 8398-8403.

Hahlbrock,K., Scheel,D., Logemann,E., Nurnberger,T., Parniske,M., Reinold,S., Sack,W.R., and Schmelzer,E. 1995. Oligopeptide elicitor-mediated defense gene activation in cultured parsley cells. Proceedings of the National Academy of Sciences of the United States of America. **92:** 4150-4157.

Hammerschmid,R. 1999. Phytoalexins: What have we learned after 60 years. Annual Review of Phytopathology **37:** 285-306.

Hammerschmidt,R. and Kuc,J. 1980. Lignification as a mechanism for induced systemic acquired resistance in cucumber. Physiological Plant Pathology **20:** 61-71.

Hammond-Kosack,K.E. and Jones,J.D.G. 1997. Plant disease resistance genes. Annual Review of Plant Physiology and Plant molecular Biology **48:** 575-607.

Hanson,K.R. and Havir,E.A. 1977. An introduction to the enzymology of phenylpropanoid biosynthesis. Recent Advances in Phytochemistry **12:** 91-137.

Harris,J.E. and Dennis,C. 1977. The effect of post infectional potato tuber metabolites and surfactants on zoospores of oomycetes. Physiological Plant Pathology **11:** 163-170.

Heath,M.C. 2000. Hypersensitive response-related death. Plant Molecular Biology **44:** 321-334.

Henderson,S.J. and Fiend,J. 1979. Increase in phenylalanine ammonia lyase and lignin-like compounds as race-specific resistance responses of potato tubers to *Phytophthora infestans*. Phytopathologische Zeitschrift **94:** 323-334.

Hermansen,A., Hannukkala,A., Naerstad,R.H., and Brurberg,M.B. 2000. Variation in populations of *Phytophthora infestans i*n Finland and Norway: mating type, metalaxyl resistance and virulence phenotype. Plant Pathology **49:** 11-12.

Hoegen,E., Stromberg,A., Pihlgren,U., and Kombrink,E. 2002. Primary structure and tissue specific expression of the pathogenesis-related protein PR-1b in potato. Molecular Plant Pathology **3:** 329-345.

Hooker,W.J. 1986. Compendium of Potato Diseases. American Phytopathological Society Press. St. Paul, Minnesota.

Hoshino,T., Chida,M., Yamaura,T., Yoshizawa,Y., and Mizutani,J. 1994. Phytoalexin induction in green pepper cell cultures treated with arachidonic acid. Phytochemistry **36:** 1417-1419.

Howe,P.A., Paiva,N.L., Sewalt,V.J.H., Elkind,N.L., Bate,Y., Lamb,C.J., and Dixon,R.A. 1996. Overexpression of L-pheylalanine ammonia lyase in transgenic tobaccoplants reveals control points for flux into phenylpanoid biosynthesis. Plant Physiology **112:** 1617-1624.

Huang,S., Edwin,A.G., Vossen,V.D., Kuang,H., Vleeshouwers,V.G.A.A., Zhang,N.W., Borm,T.J.A., Herman,J., and Richard,G.F.V. 2005. Comparative genomics enabled the isolation of the R3a late blight resistance gene in potato. Plant Journal **42:** 251-261.

Huang,S., Vleeshouwers,V.G., Werij,J.S., Hutten,R.C.B., Visser,R.G.F., and Jacobsen,E.J. 2004. The R3 resistance to *Phytophthora infestans* in potato is confered by two closely linked R genes with distinct specifities. Molecular Plant-Microbe Interactions **17:** 428-435.

Huitema,E., Vivianne,G.A.A., Vleeshouwers,V.G., Francis,D.V., and Kamoun,S. 2003. Active defence responses associated with non-host resistance of *Arabidopsis thaliana* to the oomycete pathogen *Phytophthora infestans*. Molecular Plant Pathology **4:** 487-500.

Huitema,E., Vleeshouwers,V.G.A.A., Cakir,C., Kamoun,S., and Govers,F. 2005. Differences in intensity and specificity of hypersensitive response induction in *Nicotiana* spp. by INF1, INF2A, and INF2B of *Phytophthora infestans*. Molecular Plant-Microbe Interactions **18:** 183-193.

Hutcheson,S.W. 1998. Current concepts of active defense in plants. Annual Review of Phytopathology **36:** 90.

Ichimura,K., Mizoguchi,T., Yoshida,R., Yuasa,T., and Shinozaki,K. 2000. Various abiotic stresses vapidly activate *Arabiodopsis* MPA kinase ATMPK4 and ATMPK6. Plant Journal **24:** 655-665.

Inglis,D.A., Powelson,M.L., and Dorrance,A.E. 1999. Effect of registered potato seed piece fungicides on tuber-borne *Phytophthora infestans*. Plant Disease **83:** 229-234.

Ishiguro,S., Kawai-Oda,A., Ueda,K., Nishida,I., and Okada,K. 2001. The DEFECTIVE IN ANTHER DEHISCENCE1 gene encodes a novel phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis,

which synchronizes pollen maturation, another dehiscence, and flower opening in *Arabidopsis*. Plant Cell 2191-2209.

Ishizka,N., Tomiyama,k., Katsui,N., Murai,A., and Masamune,T. 1969. Biogical activities of rishitin an antifungal compound isolated from diseased potato tubers and its derivatives *Phytophthora infestans*. Plant and Cell Physiology **10:** 183-192.

Istvan,E.S., Palnitkar,M., Buchanan,K., and Deisenhofer,J. 2000. Crystal structure of the catalytic portion of human HMG-CoA reductase: insights into regulation of activity and catalysis. The EMBO Journal **19:** 819-830.

Ito,I., Kato,N., and Uritani,I. 1984. Biochemistry of two new sesquiterpenoid phytoalexins from sweet potato roots *(Ipomoea batatas)*. Agricultural and Biological Chemistry **48:** 159-164.

James,R.V. and Fry,W.E. 1983. Potential for *Phytophthora infestans* populations to adapt to potto cultivars with rate-reducing resistance. Phytopathology **73:** 984-988.

Ji,C. and Kuc,J. 1996. Antifungal activity of cucumber beta-1,3-glucanase and chitinase. Physiological and Molecular Plant Pathology **49:** 257-265.

Joos,H.J. and Hahlbrock,K. 1992. Phenylalanine ammonia-lyase in potato (*Solanum tuberosum L.*). Genomic complexity, structural comparison of two selected genes and modes of expression. European Journal of Biochemistry **204:** 621-629.

Judelson,H.S. and Randall,T.A. 1998. Families of repeated DNA in the oomycete *Phytophthora infestans* and their distribution within the genus. Genome **41:** 605-615.

Judelson,H.S. and Roberts,S. 2002. Novel protein kinase induced during sporangial cleavage in the oomycete *Phytophthora infestans*. Eukaryotic Cell **1:** 687-695.

Kamoun,S. 2001. Nonhost resistance to *Phytophthora*: novel prospects for a classical problem. Current Opinion in Plant Biology **4:** 295-300.

Kamoun,S. 2003. Molecular genetics of pathogenic oomycetes. Eukaryotic Cell **2:** 191-199.

Kamoun,S., Huitema,E., and Vleeshouwers,V.G.A.A. 1999. Resistance to oomycetes: a general role for the hypersensitive response. Trends in Plant Science **4:** 196-200.

Kamoun,S., Klucher,K.M., Coffey,M.D., and Tyler,B.M. 1993. A gene encoding a host-specific elicitor protein of *Phytophthora parasitica*. Molecular Plant-Microbe Interactions **6:** 573-581.

Kamoun,S., Lindqvist,H., and Govers,F. 1997a. A novel class of elicitin-like genes from *Phytophthora infestans*. Molecular Plant-Microbe Interactions **10:** 1028-1030.

Kamoun,S., van West,P., de Jong,A.J., de Groot,K.E., Vleeshouwers,V.G., and Govers,F. 1997b. A gene encoding a protein elicitor of *Phytophthora infestans* is down-regulated during infection of potato. Molecular Plant-Microbe Interactions **10:** 13-20.

Kamoun,S., van West,P., Vleeshouwers,V.G., de Groot,K.E., and Govers,F. 1998. Resistance of *Nicotiana benthamiana* to *Phytophthora infestans* is mediated by the recognition of the elicitor protein INF1. Plant Cell **10:** 1413-1426.

Kang,Z. and Buchenauer,H. 2002. Immunocytochemical localization of beta-1,3-glucanase and chitinase in *Fusarium culmorum*-infected wheat spikes. Physiological and Molecular Plant Pathology **60:** 153.

Katagiri,F. 2004. A global view of defense gene expression regulation - a highly interconnected signaling network. Current Opinion in Plant Biology **7:** 506-511.

Kato,M., Mizubuti,E.S., Goodwin,S.B., and Fry,W.E. 1997. Sensitivity to protectant fungicides and pathogenic fitness of clonal lineages of *Phytophthora infestans in* the United States. Phytopathology **87:** 973-978.

Kato,M., Mizubuti,E.S., Muraya,K., Malik,K., Shiraishi,T., Ichinose,Y., and Yamada,N. 1995. Characterization of nuclear factors for elicitor-mediated activation of the promoter of the pea phenylalanine ammonia lyase gene 1. Plant Physiology **108:** 129-139.

Kawchuk,L.M., Hachey,J., Lynch,D.R., Kulcsar,F., van Rooigen,G., Waterer,D.R., Roberson,A., Kokko,E., Byers,R., Koward,R.J., and Fischer,R. 2001. Tomato Ve disease resistance genes encode cell surface-like receptors. Proceedings of the National Academy of Sciences of the United States of America. **98:** 6511-6515.

Kervinen,T., Peltonen,S., Teeri,T.H., and Karjalainen,R. 1998. Differential expression of phenylalanine ammonia-lyase genes in barley induced by fungal infection or elicitors. New Phytologist **139:** 293-300.

Kim,B.R., Nam,H.Y., Kim,S.U., Pai,T., and Chang,Y.J. 2004. Reverse transcription quantitative PCR of three genes with high homology encoding

3-hydroxy-methylglutaryl-CoA reductase in rice. Biotechnology Letters **26:** 985-988.

Kim,K.Y., Kwon,H.K., Kwon,S.Y., Lee,H.S., Hur,Y., Bang,J.W., Choi,K.S., and Kwak,S.S. 2000. Differential expression of four sweet potato peroxidase genes in response to abscisic acid and ethephon. Phytochemistry **54 :** 19-22.

Kim,Y.J. and Hwang,B.K. 2000. Pepper gene encoding a basic pathogenesis-related 1 protein is pathogen and ethylene inducible. Physiologica Plantarum **108:** 51-60.

Kleinig,H. 1989. The role of plastids in isoprenoid biosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology **40:** 39-59.

Klessig,D.F. and Malamy,J. 1994. The salicylic acid signal in plants. Plant Molecular Biology **26:** 1439-1458.

Klooster,J.W., Berg-Velthuis,G.G., West,P.v., and Govers,F. 2000. *tef1*, a *Phytophthora infestans* gene encoding translation elongation factor 1alpha. Gene **249:** 145-151.

Knapova,G. and Gisi,U. 2002a. Phenotypic and genotypic structure of *Phytophthora infestans* populations on potato and tomato in France and Switzerland. Plant Pathology **51:** 641-653.

Knight,V.I., Wang,H., Lincoln,J.E., Lulai,E.C., Gilchrist,D.G., and Bostock,R.M. 2001. Hydroperoxides of fatty acid induce programmed cell death in tomato protoplasts. Physiological and Molecular Plant Pathology **59:** 277-286.

Kolomiets,M.V., Chen,H., Gladon,R.J., Braun,E.J., and Hannapel,D.J. 2000. A leaf lipoxygenase of potato induced specifically by pathogen infection. Plant Physiology **124:** 1121-1130.

Kombrink,E. and Somssich,I.E. 1995. Defense responses of plants to pathogens. Advances in Botanical Research **21:** 1-34.

Kondo,K., Uritani,I., and Oba,K. 2003. Induction mechanism of 3-hydroxy-3-methylglutaryl CoA reductase in potato tuber and sweet potato root tissues. Bioscience, Biotechnology, and Biochemistry **8:** 17-24.

Kramer,R., Freytag,S., and Schmelzer,E. 1997. *In vitro* formation of infection structures of *Phytophthora infestans* is associated with synthesis of stage specific peptides. European Journal of Plant Pathology **103:** 43-53.

Kreft,S., Ravnikar,M., Mesko,P., Pungercar,J., Umek,A., Regar,I., and Strukelj,B. 1997. Jasmonic acid inducible aspartic proteinase inhibitors from potato. Phytochemistry (Oxford) **44:** 1001-1006.

Kruger,W.M., Szabo,L.J., and Zeyen,R.J. 2003. Transcription of the defense response genes chitinase IIb, PAL and peroxidase is induced by the barley powdery mildew fungus and is only indirectly modulated by R genes. Physiological and Molecular Plant Pathology **63:** 167-178.

Kuc,J. and Rush,J.S. 1985. Phytoalexins. Archives of Biochemistry and Biophysics **236:** 455-472.

Kumar,D. and Klessig,D.F. 2003. High-affinity salicylic acid-binding protein 2 is required for plant innate immunity and has salicylic acid-stimulated lipase activity. Proceedings of the National Academy of Sciences of the United States of America. **100:** 16101-16106.

kunkel,B.N. and Brooks,D.M. 2002. Cross talk between signaling pathways in pathogen defesne. Current Opinion in Plant Biology **5:** 325-331.

Lamari,L. 2002. Assess: Image analysis software for plant disease quantification. St Paul:MN, USA.

Lamb,C. 1994. Plant disease resistance genes in signal perception and transduction. Cell **76:** 419-422.

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

Lambert,D.H. and Currier,A.I. 1997. Difference in tuber root rot development for North American clones of *Phytophthora infestans*. American Potato Journal **74:** 39-43.

Latijnhouwers,M., Munnik,T., and Govers,F. 2002. Phospholipase D in *Phytophthora infestans* and its role in zoospore encystment. Molecular Plant-Microbe Interactions **15:** 939-946.

Lauge,R. and De Wit,P.J. 1998. Fungal avirulence genes: structure and possible functions. Fungal Genetics and Biology **24:** 285-297.

Lauge,R., Dmitriev,A.P., Joosten,M.A.H.J., and De Wit,P.J.G.M. 1998. Additional resistance gene(s) against *Cladosporium fulvum* present on the Cf-9 introgression segment are associated with strong PR protein accumulation. Molecular and Plant Microbe Interactions **11:** 301-308.

Laxalt,A.M., Cassia,R.O., Sanllorenti,P.M., Madrid,E.A., Andreu,A.B., Daleo,G.R., Conde,R.D., and Lamattina,L. 1996. Accumulation of cytosolic glyceraldehyde-3-phosphate dehydrogenase RNA under biological stress conditions and elicitor treatments in potato. Plant Molecular Biology **30:** 961-972.

Laxalt,A.M., Latjnhouwers,M., van Hulten,M., and Govers,F. 2002. Differential expression of G protein α and β subunits genes during development of *Phytophthora infestans*. Fungal Genetics and Biology **36:** 137-146.

Laxalt,A.M., Madrid,E.A., Beligni,M.V., and Lamattina,L. 1998. Anti-idiotypic antibodies can mimic the ability of *Phytophthora infestans* to induce the accumulation of transcripts associated with defence responses in potato. Physiological and Molecular Plant Pathology **53:** 135-148.

Lazarovits,G. and Higgins,V.J. 1976. Ultrastructure of susceptible, resistant, and immune reactions of tomato to races of *Cladosporum fulvum*. Candian Journal of Botany **54:** 235-249.

Lebreton,L., Lucas,J.M., and Andrivon,D. 1999. Aggressiveness and competitive fitness of *Phytophthora infestans* isolates collected from potato and tomato in France. Phytopathology **89:** 679-686.

Lee,S.W., Nazar,R.N., Powell,D.A., and Robb,J. 1992. Reduced PAL gene expression in *Verticillium*-infected resistant tomatoes. Plant Molecular Biology **18:** 352.

Leister,D., Ballvora,A., Salamini,F., and Gebhardt,C. 1996. A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. Nature Genetics **14:** 421-429.

Leonards-Schippers,C., Gieffers,W., Salamini,F., and Gebhardt,C. 1992. The R1 gene conferring race-specific resistance to *Phytophthora infestans* in potato is located on potato chromosome V. Molecular and General Genetics **233:** 278-283.

Leonards-Schippers,C., Gieffers,W., Schafer-Pregl,R., Ritter,E., Knapp,S.J., Salamini,F., and Gebhardt,C. 1994. Quantitative resistance to *Phytophthora infestans* in potato: a case study for QTL mapping in an allogamous plant species. Genetics **137:** 67-77.

Li,X., Eck,H.J., Rouppe,J.N.A.M., Rouppe van der Voort,J.N.A.M., Huigen,D.J., Stam,P., and Jacobsen,E. 1998. Autotetraploids and genetic mapping using common AFLP markers: the R2 allele conferring resistance to *Phytophthora infestans* mapped on potato chromosome 4. Theoretical and Applied Genetics **96:** 1121-1128.

Ligterink,W., Kroj,T., zur,N.U., Hirt,H., and Scheel,D. 1997. Receptor-mediated activation of a MAP kinase in pathogen defense of plants. Science **276:** 2054-2057.

Linke,C., Conrath,U., Jeblick,W., Betsche,T., Mahn,A., During,K., and Neuhaus,H.E. 2002. Inhibition of the plastidic ATP/ADP transporter protein primes potato

tubers for augmented elicitation of defense responses and enhances their resistance against *Erwinia carotovora*. Plant Physiology **129:** 1607-1615.

Linthorst,H.J.M. 1991. Pathogenesis-related proteins of plants. Critical Reviews in Plant Science **10:** 123-150.

Linthorst,H.J.M., Meuwissen,R.L., Kauffmann,S., and Bol,J.F. 1989. Constitutive expression of pathogenesis-related protein PR-1, GRP and PR-5 in tobacco has no effect on virus infection. Plant Cell **1:** 285-291.

Liu,D., Raghothama,K.G., Hasegawa,P.M., and Bressan,R.A. 1994. Osmotin overexpression in potato delays development of disease symptoms. Proceedings of the National Academy of Sciences of the United States of America. **91:** 1888-1892.

Liu,Z., Bos,J.I., Armstrong,M., Whisson,S.C., da Cunha,l., Torto-Alalibo,T., Win,J., Avrova,A.O., Wright,F., Birch,P.R., and Kamoun,S. 2005. Patterns of diversifying selection in the phytotoxin-like scr74 gene family of *Phytophthora infestans*. Molecular Biology and Evolution **0:** (in press).

Lulai,E.C. 2000. The effect of wound severity, type of tissue, and use of the tuber disk model system on arachidonic acid-induced rishitin accumulation in potato tube. American Potato Journal **77:** 303-310.

Lyon,G.D. 1972. Occurrence of rishitin and phytuberin in potato tubers inoculated with *Erwinia carotovora var. atroseptica*. Physiological Plant Pathology **2:** 411-416.

Lyon,G.D. 1980. Evidence that the toxic effect of rishitin may be due to membrane damage. Journal of Experimental Botany **31:** 957-966.

Madi,L. and Katan,J. 1998. *Penicillium janczewskii* and its metabolites, applied to leaves, elicit systemic acquired resistance to stem rot caused by *Rhizoctonia solani*. Physiological and Molecular Plant Pathology **53:** 163-175.

Mahuku,G., Peters,R.D., Platt,H.W., and Daayf,F. 2000. Random amplified polymorphic DNA (RAPD) analysis of *Phytophthora infestans* isolates collected in Canada during 1994 to 1996. Plant Pathology **49:** 252-260.

Malcolmson,J.F. and Black,W. 1966. New R genes in *Solanum demissum* L. and their complementary racs of *Phytophthora infestans* (Mont.) de Bary. Euphytica **15:** 199-203.

Maldonado,A.M., Doerner,P., Dixon,R.A., Lamb,C.J., and Cameron,R.K. 2002. A putative lipid transfer protein involved in systemic resistance signaling in *Arabidopsis*. Nature **419:** 399-403.

Marshall,J.S., Ashton,A.R., Govers,F., and Hardham,A.R. 2001. Isolation and characterization of four genes encoding pyruvate, phosphate dikinase in the oomycete plant pathogen *Phytophthora cinnamomi*. Current Genetics **40:** 73-81.

Martini,N., Egen,M., Runtz,I., and Strittmatter,G. 1993. Promoter sequences of a potato pathogenesis-related gene mediate transcriptional activation selectively upon fungal infection. Molecular & General Genetics : MGG **236 :** 179-186.

Matton,D.P. and Brisson,N. 1989. Cloning, expression, and sequence conservation of pathogenesis-related gene transcripts of potato. Molecular Plant-Microbe Interactions **2:** 325-331.

Mauch,F., Mauch-Mani,B., and Boller,T. 1988. Antifungal hydrolases in pea tissues. II. Inhibition of fungal growth by combinations of chitnase and β-1,3-glucanase. Plant Physiology **88:** 936-942.

May,K.J. and Ristaino,J.B. 2004. Identity of the mtDNA haplotype(s) of *Phytophthora infestans* in historical specimens from the Irish potato famine. Mycological research. **3:** 471-479.

Mayer,A.M. 1987. Polyphenol oxidases in plants. Phytochemistry **26:** 11-20.

Mazeyrat,F., Mouzeyar,S., Courbou,I., Badaoui,S., Roeckel-Drevet,P., Tourvieille-de-Labrouhe,D., and Ledoigt,G. 1999. Accumulation of defense related transcripts in sunflower hypocotyls (*Helianthus annuus L.*) infected with *Plasmopara halstedii*. European Journal of Plant Pathology **105:** 333-340.

McGee,J.D., Hamer,J.E., and Hodges,T.K. 2001. Characterization of PR-10 pathogenesis related protein family induced in rice during infection with *Magnaporthe grisea*. Molecular Plant-Microbe Interactions **14:** 877-886.

Meyer,R.C., Milbourne,D., Hackett,C.A., Bradshaw,J.E., McNichol,J.W., and Waugh,R. 1998. Linkage analysis in tetraploid potato and association of markers with quantitative resistance to late blight (*Phytophthora infestans*). Molecular and General Genetics **259:** 150-160.

Michelmore,R.W. and Meyers,B.C. 1998. Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Research **8:** 1113-1130.

Miller,J.S. and Johnson,D.A. 2000. Competitive fitness of *Phytophthora infestans* isolates under semiarid filed conditions. Phytopathology **90:** 220-227.

Mizubuti,E.S.G. and Fry,W.E. 1998. Temperature effects on developmental stages of isolates from three clonal lineages of *Phytophthora infestans*. Phytopathology **88:** 837-843.

Moffett,P., Farnham,G., Peart,J.R., and Baucombe,D.C. 2002. Interaction between domains of a plant NBS-LRR protein in disease resistance-related cell death. The EMBO Journal **21:** 4511-4519.

Morris,S.W., Vernooij,B., Titatarn,S., Starrett,M., Thomas,S., Wiltse,C.C., Frederiksen,R.A., Bhandhufalck,A., Hulbert,S., and Uknes,S. 1998. Induced resistance responses in maize. Molecular Plant-Microbe Interactions **11:** 643-658.

Mort-Bontemps,M., Gay,L., and Fevre,M. 1997. CHS2, a chitin synthase gene from the oomycete *Saprolegnia monoica*. Microbiology **143:** 2009-2020.

Mouton-Perronnet,F., Bruneteau,M., Denoroy,L., Bouliteau,P., Ricci,P., Bonnet,P., and Michel,G. 1995. Elicitin produced by an isolate of *Phytophthora parasitica* pathogenic to tobacco. Phytochemistry **38:** 41-44.

Mussig,C., Biesgen,C., Lisso,J., Uwer,U., Weiler,E.W., and Altmann,T. 2000. A novel stess-inducible 12-oxyphytodienoate reductase from *Arabidopsis thaliana* provides a potential link between Brassinosteroid-action and Jasmonic-acid synthesis. The Journal of Physiology **157:** 143-152.

Naderi,M. and Berger,P.H. 1997. Pathogenesis-related protein 1a is induced in potato virus Y-infected plants as well as by coat protein targeted to chloroplasts. Physiological and Molecular Plant Pathology **51:** 41-44.

Nandi,A., Welti,R., and shah,J. 2004. The arabidopsis thaliana dihydroxyacetone phosphate reductase gene SUPPRESSOR OF FATTY ACID DESATURASE DEFICIENCY1 is required for glycerolipid metabolism and for the activation of systemic acquired resistance. Plant Cell 465-477.

Nawrath,C. and Metraux,J.P. 1999. Salicylic acid induction deficient mutants of *Arabidopsis* express PR-2 and PR-5 and accumulate high levels of camalexin after pathogen inoculation. Plant Cell **11:** 1393-1404.

Nespoulous,C., Gaudemer,O., Huet,J.C., and Pernollet,J.C. 1999. Characterization of elicitin-like phospholipases isolated from *Phytophthora capsici* culture filtrate. FEBS Letters **452:** 400-406.

Nicholson,R.L. and Hammerschmid,R. 1992. Phenolic compounds and their role in disease resistance. Annual Review of Phytopathology **30:** 369-389.

Niderman,T., Genetet,I., Bruyere,T., Gees,R., Stintzi,A., Legrand,M., Fritig,B., and Mosinger,E. 1995. Pathogenesis-related PR-1 proteins are antifungal: isolation and characterization of three 14 KDa proteins of tomato and of a basic PR-1 of tobacco with inhibitory activity against *Phytophthora infestans*. Plant Physiology **108:** 17-27.

Nimchuk,Z., Eulgem,T., Holt,B.F., III, and Dangl,J.L. 2003. Recognition and response in the plant immune system. Annual Review of Genetics **37:** 579-609.

Noritake,T., Kawakita,K., and Doke,N. 1996. Nitric oxide induces phytoalexin accumulation in potato tuber tissues. Plant and Cell Physiology **37:** 113-116.

O'Brien,E. and McCully,M.E. 1981. The study of plant structure: Principles and selected methods. Melbourne: Termarcarphi Pty Ltd.

Oba,K., Kondo,K., Doke,N., and Uritani,I. 1985. Induction of 3-hydroxy-3-methylglutaryl CoA reductase in potato tubers after slicing, fungal infection or chemical treatment, and some properties of the enzyme. Plant Cell Physiology **26:** 873-880.

Oberhagemann,P., Chatot-Balandras,C., Schafer-Pregl,R., Wegener,D., Palomino,C., Salamini,F., Bonnel,E., and Gebhardt,C. 1999. A genetic analysis of quantitative resistance to late blight in potato: Towards marker-assisted selection. Molecular Breeding **5:** 399-415.

Oostendorp,M., kunz,W., Dietrich,B., and Staub,T. 2001. Induced disease resistance in plants by chemcials. European Journal of Plant Pathology **107:** 18-28.

Ordonez,M.E., Forbes,G.A., and Trognitz,B.R. 1997. Resistance to late blight in potato, a putative gene that suppress R genes and is elicited by specific isolates. Euphytica **95:** 167-172.

Ozeretskovskaya,O.L., Vasyukova,N.I., Perekhod,E.A., Chalenko,G.I., and Cerasimova,N.G. 2001. Plant resistance suppressors in the pathosystem formed by potato and the causual agent of late blight. Applied Biochemistry and Microbiology **37:** 506-511.

Paiva,N.L. 2000. An introduction to the bionsynthesis of chemicals used in plant-microbe communication. Journal of Plant Growth Regulation **19:** 131-143.

Park,D.S., Landini,S., and Graham,T.L. 2005. Induced distal defense potentiation against *Phytophthora sojae* in soybean. Physiological and Molecular Plant Pathology **60:** 293-310.

Parker,J.E., Aarts,N., Austin,M.A., Feys,B.J., Moisan,L.J., Muskett,P., and Rusterucci,C. 2001. Genetic analysis of plant disease resistance pathways. Novartis Foundation Symposium **236:** 153-161.

Peng,M. and Kuc,J. 1992. Peroxidase generated hydrogen peroxide as a source of antifungal activity. Phytopathology **82:** 696-699.

Peters,R.D., Forster,H., Platt,H.W., Hall,R., and Coffey,M.D. 2001. Novel genotypes of *Phytophthora infestans* in Canada during 1994 and 1995. American Journal of Potato Research **78:** 39-45.

Peters,R.D., Platt,H.W., and Hall,R. 1998. Characterization of changes in populations of *Phytophthora infestans* in Canada using mating type and metalaxyl sensitivity markers. Canadian Journal of Plant Pathology **20:** 259-273.

Peters,R.D., Platt,H.W., and Hall,R. 1999a. Hypotheses for the inter-regional movement of new genotypes of *Phytophthora infestans* in Canada. Canadian Journal of Plant Pathology **21:** 132-136.

Peters,R.D., Platt,H.W., and Hall,R. 1999b. Use of allozyme markers to determine genotypes of *Phytophthora infestans* in Canada. Canadian Journal of Plant Pathology **21:** 144-153.

Pieterse,C.M., Derksen,A.M., Folders,J., and Govers,F. 1994a. Expression of the *Phytophthora infestans* ipiB and ipiO genes *in planta* and *in vitro*. Molecular and General Genetics **244:** 269-277.

Pieterse,C.M., van West,P., Verbakel,H.M., Brasse,P.W., van den Berg-Velthuis GC, and Govers,F. 1994b. Structure and genomic organization of the ipiB and ipiO gene clusters of *Phytophthora infestans*. Gene **138:** 67-77.

Pieterse,C.M., van't Klooster,J., van den Berg-Velthuis GC, and Govers,F. 1995. NiaA, the structural nitrate reductase gene of *Phytophthora infestans*: isolation, characterization and expression analysis in *Aspergillus nidulans*. Current Genetics **27:** 359-366.

Platt,H.W. and Tai,G. 1998. Relationship between resistance to late blight in potato foliage and tubers of cultivars and breeding selections with different resistance levels. American Potato Journal **75:** 173-178.

Ponstein,A.S., Bres-Vloemans,S.A., Sela-Buurlage,M.B., van den Elzen,P.J.M., Melchers,L.S., and Cornelissen,B.J. 1994. A novel pathogne- and wound-inducible tobacco (Nicotiana tabacum) with antifungal activity. Plant Physiology **104:** 109-118.

Pozo,M.J., Cordier,C., Dumas-Gaudot,E., Gianinazzi,S., Barea,J.M., and Azcon-Aguilar,C. 2002. Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to *Phytophthora infection* in tomato plants. Journal of Experimental Botany **53 :** 525-534.

Preisig,C.L. and Kuc,J.A. 1985. Arachidonic acid-related elicitors of the hypersensitive response in potato and enhancement of their activities by glucans from *Phytophthora infestans* (Mont.) deBary. Archives of Biochemistry and Biophysics **236:** 379-389.

Przymusinski,R., Rucinska,R., and Gwozdz,E.A. 2004. Increased accumulation of pathogenesis-related proteins in lupine root in response to various abiotic stresses. Environmental and Experimental Botany **104:** 109-118.

Rahimi,S., Perry,R.N., and Wright,D.J. 1996. Identification of pathogenesis-related proteins induced in leaves of potato plants infected with potato cyst nematodes, *Globodera* species. Physiological and Molecular Plant Pathology **49:** 49-59.

Rance,I., Fournier,J., and Esquerre-Tugaye,M.T. 1998. The incompatible interaction between *Phytophthora parasitica var. nicotianae* race 0 and tobacco is suppressed in transgenic plants expressing antisense lipoxygenase sequences. Proceedings of the National Academy of Sciences of the United States of America. **95:** 6554-6559.

Rathjen,J.P. and Moffett,P. 2003. Early signal transduction events in specific plant disese resistance. Current Opinion in Plant Biology **6:** 300-306.

Ray,H. and Hammerschmid,R. 1998. Responses of potato tuber to infection by *Fusarium sambucinum*. Physiological and Molecular Plant Pathology **53:** 81-92.

Romeis,T., Piedras,P., Zhang,S., Klessig,D.F., Hirt,H., and Jones,J.D.G. 1999. Rapid, *Avr9* and *cf-9* dependent activation of MAP kinases in tobacco cell cultures and leaves: convergence of resistance gene, elicitor, wound and salicylate response. Plant Cell **11:** 273-287.

Ros,B., Thummler,F., and Wenzel,G. 2004. Analysis of differentially expressed genes in a susceptible and moderately resistant potato cultivar upon *Phytophthora infestans* infection. Molecular Plant Pathology **5:** 191-201.

Rose,J.K.C., Kam,K.S., Darvill,A.G., and Albersheim,P. 2002. Molecular cloning and characterization of glucanase inhibitor proteins: coevolution of a counter defence mechanism by plant pathogens. Plant Cell **14:** 1329-1345.

Ross,A.F. 1961. Localized acquired resistance to plant virus infection in hypersensitive host. Virology **14:** 329-339.

Ryals,J., Neuenschwander,U.H., Willites,M.G., Molina,A., Steiner,H.Y., and Hunt,M.D. 1996. Systemic aquired resistance. Plant Cell **8:** 1809-1819.

Ryan,C.A. and Pearce,G. 1998. Systemin: A polypeptide signal for plant defensive genes. Annual Review of Cell and Developmental Biology **14:** 1-17.

Sandbrink,J.M., Colon,L.T., Wolters,P.J.C.C., and Stiekema,W.J. 2000. Two related genotypes of *Solanum microdontum* carry different segregating alleles for field resistance to *Phytophthora infestans*. Molecular Breeding **6:** 215-225.

Sansome,E., Brasier,C.M., and Griffin,M.J. 1975. Chromosome size differences in *Phytophthora palmivora*, a pathogen of cocoa. Nature **255:** 704-705.

Schmelzer,E., Nation,B., Freytag,S., Rouhara,Y., Kuster,B., and Hahlbrock,K. 1995. Infection-induced rapid cell death in plants: a means of efficient pathogen defense. Candian Journal of Botany **73:** S426-S434.

Schroder,M., Hahlbrock,K., and Kombrink,E. 1992. Temporal and spatial patterns of 1,3-β–glucanase and chitinase induction in potato leaves infected by *Phytophthora infestans*. Plant Journal **2:** 161-172.

Seo,S., Sano,H., and Ohashi,Y. 1999. Jasmonate-based wound signal signal transduction requires activation of WIPK, a tobacco mitogen-activated protein kinase. Plant Cell **11:** 289-298.

shah,J. 2004. The salicylic acid loop in plant defense. Current Opinion in Plant Biology **6:** 365-371.

Shattock,R.C. and Shaw,D.S. 1975. Mutants of *Phytophthora infestans* resistant to, and dependent upon, antibiotics. Transactions of the British Mycological Society **64:** 29-41.

Shiraishi,T., Araki,M., Yoshioka,H., Kobayashi,I., Yamada,T., Ichino,Y., Kunoh,H., and Oku,H. 1991. In situ inhibition of ATPase activity in pea plasma membrane by a suppresor from a pea pathogen, *Mycosphaerella pinodes*. Plant Cell and Physiology **32:** 1067-1075.

Shirasu,K., Dixon,R.A., and Lamb,C. 1996. Signal transduction in plant immunity. Current Opinion in Immunology **8:** 3-7.

Shulaev,V., Leon,J., and Raskin,I. 1995. Is salicylic acid a translocated signal of systemic acquired resistance in tobacco? Plant Cell **7:** 1691-1701.

Si-Ammour,A., Mauch-Mani,B., and Mauch,F. 2003. Quantification of induced resistance agaisnt *Phytophthora* species expressing GPF as a vital marker: β-aminobutyric acid but not BTH protects potato and *Arabidopsis* from infection. Molecular Plant Pathology **4:** 237-248.

Simko,I. 2002. Comparative analysis of quantitative trait loci for foliage resistance *Phytophthora infestans* in tuber bearing species. American Potato Journal **79:** 125-132.

Smith,C.J. 1996. Accumulation of phytoalexin: defesne mechanism and stimulus response system. The New Phytologist **132:** 1-45.

Somssich,I.E., Bollmann,J., Hahlbrock,K., Kombrink,E., and Schulz,W. 1989. Differential early activation of defense-related genes in elicitor-treated parsley cells. Plant Molecular Biology **12:** 227-234.

Staskawicz,B., Ausubel,F.M., Baker,B.J., and Ellis,J.G. 1995. Molecular genetics of plant disease resistance. Science **268:** 661-667.

Sticher,L., Mauch-Mani,B., and Metraux,J.P. 1997. Systemic acquired resistance. Annual Review of Phytopathology **35:** 235-270.

Swiderski,M.R. and Innes,R.W. 2001. The *Arabidopsis* PBS1 resistance gene encodes a member of a novel protein kinase subfamily. Plant Journal **26:** 101-112.

Takakura,Y., Ishida,Y., Inoue,Y., Tsutsumi,F., and Kuwata,S. 2004. Induction of a hypersensitive response-like reaction by powdery mildew in transgenic tobacco expressing harpin(pss). Physiological and Molecular Plant Pathology **64:** 83-89.

Takemoto,D., Furuse,K., Doke,N., and Kawakita,K. 1997. Identification of chitinase and osmotin-like protein as actin binding proteins in suspension-cultured potato cells. Plant and Cell Physiology **38:** 441-448.

Tameling,W.I.L., Elzinga,S.D.J., Darmin,P.S., Vossen,J.H., Takken,F.L.W., Harling,M.A., and Cornelissen,B. 2002. The tomato R gene products I-2 and Mi-1 are functional ATP binding proteins with ATPase activity. Plant Cell **14:** 2929-2939.

Tanabe,T., Chamaillard,M., Ogura,Y., Zhu,L., Qiu,S., Masumoto,J., Ghosh,P., Moran,A., and Tromp,G. 2002. Regulatory regions and critical residues of NOD2 involved in muramyl dipeptide recognition. The EMBO Journal **7:** 1587-1597.

Tanaka,Y. and Uritani,I. 1976. Immunochemical studies on flucturation of phenylalanine ammonia lyase activity in sweet potato in response to cut injury. Journal of Biochemistry **9:** 130-135.

Tang,X., Frederick,R.D., Zhou,J., Halterman,D.A., Jia,Y., and Martin,G.B. 1996. Physical interaction of *avrPto* and the *Pto* kinase defines a recognition event involved in plant disease resistance. Science **274:** 2060-2063.

Tani,S., Kim,K.S., and Judelson,H.S. 2005. A cluster of NIF transcriptional regulators with divergent patterns of spore-specific expression in *Phytophthora infestans*. Fungal Genetics and Biology **42:** 42-50.

Tani,S., Yatzkan,E., and Judelson,H.S. 2004. Multiple pathways regulate the induction of genes during zoosporogenesis in *Phytophthora infestans*. Molecular Plant-Microbe Interactions **17:** 330-337.

Taylor,B. and Powell,A. 1982. Isolation of plant DNA and RNA. Focus **4:** 4-5.

Therrien,C.D. 1989. Nuclear DNA content, allozyme phenotypes and metalaxyl sensitivity of *Phytophthora infestans* from Japan. Mycological Research **97:** 945-950.

Thurston,H.D. 1971. Relationship of general resistance: late blight of potato. Phytopathology **61:** 620-626.

Tian,M.Y., Huitema,E., Cunha,L.D., Torto-Alalibo,T., and Kamoun,S. 2004. A kazal-like extracellular serine protease inhibitor from *Phytophthora infestans* targets the tomato pathogenesis-related protease P69B. Journal of Biological Chemistry **279:** 26370-26377.

Tonon,C., Andreu,A., Aued,M.E., Van Damme,M., Huarte,M., and Daleo,G.R. 1998. Defence reactions in two potato cultivars following infection with two races of *Phytophthora infestans*. Potato Research **41:** 319-325.

Tonon,C., Guevara,G., Oliva,C., and Daleo,G. 2002. Isolation of a potato acidic 39 KDa beta-1,3-glucanase with antifungal activity against *Phytophthora infestans* and analysis of its expression in potato cultivars differing in their degrees of field resistance. Journal of Phytopathology **150:** 189-195.

Tooley,P.W. and Therrien,C.D. 1987. Cytophotometrical determinationof the nuclear DNA content of 23 Mexican and 18 non-mexican isolates of *Phytophthora infestans*. Experimental Mycology **11:** 19-26.

Torto,T.A., Li,S., Styer,A., Huitema,E., Testa,A., Gow,N.A., van West,P., and Kamoun,S. 2003. EST mining and functional expression assays identify extracellular effector proteins from the plant pathogen *Phytophthora*. Genome Research **13:** 1675-1685.

Toyada,K., Shiraishi,T., Yoshioka,H., Yamada,T., Ichionse,Y., and Oku,H. 1992. Regulation of polyphosphoinositide metabolism in pea plasma membranes by elicitor and suppressor from a pea pathoen, *Mycosphaerella pinodes*. Plant Cell and Physiology **33:** 445-452.

Trognitz,F., Manosalva,P., Gysin,R., Ninio-Liu,D., Simon,R., del Herrera,M.R., Trognitz,B., Ghislain,M., and Nelson,R. 2002. Plant defense genes associated with quantitative resistance to potato late blight in *Solanum phureja* x dihaploid *S. tuberosum* hybrids. Molecular Plant-Microbe Interactions **15:** 587-597.

Turkensteen,L.J. 1993. Durable resistance of potatoes against *Phytophthroa infestans*. In Durability of disease resistance. Edited by T. Jacobs and J. E. parlevliet. Kluwer Academic Publisher. Dordrecht, the Netherlands. 115-124.

Turner,J.G., Ellis,J., and Devoto,A. 2002. The jasmonate signal pathway. Plant Cell **S:** 153-164.

Tyler,B.M. 2002. Molecular basis of recognition between *Phytophthora pathogens* and their hosts. Annual Review of Phytopathology **40:** 137-167.

Van Bel,A.J.E. and Gaupels,F. 2004. Pathogen-induced resistance and alarm signals in the phloem. Molecular Plant Pathology **5:** 495-504.

van der,L.T., Robold,A., Testa,A., 't Klooster,J.W., and Govers,F. 2001a. Mapping of avirulence genes in *Phytophthora infestans* with amplified fragment length polymorphism markers selected by bulked segregant analysis. Genetics **157:** 949-956.

van der,L.T., Testa,A., van 't,K.J., Berg-Velthuis,G., and Govers,F. 2001b. Chromosomal deletion in isolates of *Phytophthora infestans* correlates with virulence on R3, R10, and R11 potato lines. Molecular Plant-Microbe Interactions **14:** 1444-1452.

van Kan,J.A.L., Joosten,M.H.A.J., Wagemakers,C.A.M., van den Berg-Velthuis,G.C.M., and De Wit,P.J.G.M. 2005. Differential accumulation of mRNAs encoding extracellular and intracellular PR-proteins in tomato by virulent and avirulent races of *Cladosporium fulvum*. Plant Molecular Biology **20:** 513-527.

van Loon,L.C. 1999. Occurrence and properties of plant pathogenesis-related proteins. In: Pathogenesis-Related Proteins in Plants (Datta,S. K. and Muthukrishnan,S. , eds). Boca Raton:CRC Press, pp. 1-19.

van Loon,L.C., Pierpoint,W.S., Boller,T., and Conejero,V. 1994. Recommendations for naming plant pathogenesis-related proteins. Plant Molecular Biology Reporter **12:** 245-264.

van Loon,L.C. and van Kammen,A. 1970. Polyacrylamide disc electrophoresis of the soluble leaf proteins from *Nicotiana tobacum* var. "Samsun' and "Samsun NN" II. Changes in protein constitution after infection with tobacco mosaic virus. Virology **40:** 199-211.

van Loon,L.C. and van Strien,E.A. 1999. The families of pathogenesis-related proteins, their activies, and comparative analysis of PR-1 type proteins. Physiological and Molecular Plant Pathology **55:** 85-97.

Van Loon,L.C. and Van Strien,E.A. 1999. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 proteins. Physiological and Molecular Plant Pathology **55:** 85-97.

Van Pelt-Heerschap,H. and Smit-Bakker,O. 1999. Analysis of defense related proteins in stem tissue of carnation inoculated with a virulent and avirulent race of *Fusarium oxysporum f.sp. dianthi*. European Journal of Plant Pathology **105:** 681-691.

van West,P., Appiah,A.A., and Gow,N.A.R. 2003. Advances in research on root pathogenic oomycetes. Physiological and Molecular Plant Pathology **62:** 99-113.

van West,P., de Jong,A.J., Judelson,H.S., Emons,A.M., and Govers,F. 1998. The ipiO gene of *Phytophthora infestans* is highly expressed in invading hyphae during infection. Fungal Genetics and Biology **23:** 126-138.

van West,P. and Vleeshouwers,V.G.A.A. 2004. The *Phytophthora infestans*-host interaction. In: Plant Pathogen Interactions. Ed. : N. J. Talbot. Blackwell Scientific Publishers.

Vance,C.P. and Sherwood,R.T. 1980. Lignification as a mechanism of disease resistance. Annual Review of Phytopathology **18:** 259-288.

Velazhahan,R. and Muthukrishnan,S. 2003. Transgenic tobacco plant constitutively overexpressing a rice thaumatin-like protein (PR-5) show enchanced resistance to *Alternaria alternata*. Biologia Plantarum **47:** 347-354.

Veronesi,C., Rickauer,M., Fournier,J., Pouenat,M.L., and Esquerre-Tugaye,M.T. 1996. Lipoxygenase gene expression in the tobacco-*Phytophthora parasitica* nicotianae interaction. Plant Physiology **112:** 997-1004.

Verwoerd,T.C., Dekker,B.M., and Hoekema,A. 1989. A small scale procedure for the rapid isolation of plant RNAs. Nucleic Acids Research **25:** 2362.

Visker,M.H.P.W., Keizer,L.C.P., van Eck,H.J., Jacobsen,E., Colon,L.T., and Struik,P.C. 2003. Can the QTL for late blight resitance on potato chromosome 5 be attributed to foliage maturity type? Theoretical and Applied Genetics **106:** 317-325.

Vleeshouwers,V.G.A.A., Dooijeweert,W.v., Govers,F., and Kamoun,S. 2000a. The hypersensitive response is associated with host and non host resistance to *Phytophthora infestans*. Planta **210:** 853-864.

Vleeshouwers,V.G.A.A., Dooijeweert,W.v., Govers,F., Kamoun,S., and Colon,L.T. 2000b. Does basal PR gene expression in *Solanum* species contribute to non-specific resistance to *Phytophthora infestans*. Physiological and Molecular Plant Pathology **57:** 35-42.

Wada,M., Kato,H., Malik,K., Sriprasertsak,S., Ichinose,Y., Shiraishi,T., and Yamada,T. 1995. A suppressin from a Phytopathogenic fungus deactives

transcription of a plant defesne gene encoding phenylalanine ammonia lyase. Journal of Molecular Biology **249:** 513-519.

Walters,D., Cowley,T., and Mitchell,A. 2002. Methyl jasmonate alters polyamine metabolism and induces systemic protection against powdery mildew infection in barley seedlings. Journal of Experimental Botany **53:** 747-756.

Wang,X., El Hadrami,A., Adam,L., and Daayf,F. 2005a. Local and distal gene expression of *pr-1* and *pr-5* in potato leaves inoculated with isolates from the old (US-1) and the new (US-8) genotypes of *Phytophthora infestans* (Mont.) de Bary. Environmental and Experimental Botany ***In press***.

Wang,X., El Hadrami,A., Adam,L., and Daayf,F. 2005b. US-1 and US-8 genotypes of *Phytophthora infestans* differentially affect local, proximal and distal gene expression of phenylalnine ammonia lyase and 3-hydroxy,-3-methylglutaryl CoA reductase in potato leaves. Physiological and Molecular Plant Pathology **65:** 157-167.

Ward,E., Uknes,S., Williams,S., Dincher,S., Wiederhold,D., Alexander,D., Ahl-Goy,P., and Ryals,J. 1991. Coordinate gene activity in response to agents that induce systemic acquired resistance. Plant Cell **3:** 619-628.

Wastie,R.L. 1991. Breeding For resistance. In advances in plant pathology. Edited by D. S. Ingram and P. H. Williams. Academic Press. London. U. K. 193-224.

Way,H.M., Kazan,K., Goulter,K.C., Birch,R.G., and Manners,J.M. 2000. Expression of the *Shpx2* peroxdiase gene of *Stylosanthes humilis* in transgenic tobacco leads to enhanced resistance to *Phytophthora parasitica* pv. *nicotinanae* and *Cerocospora nicotianae*. Molecular Plant Pathology **1:** 223-232.

Wegener,C.B. 2002. Induction of defense responses against *Erwinia* soft rot by an endogenous pectate lyase in potatoes. Physiological and Molecular Plant Pathology **60:** 91-100.

Weisshaar,B. and Jenkins,G.I. 1998. Phenylpropanoid biosynthesis and its regulation. Current Opinion in Plant Biology **1:** 251-257.

Welling,L.L. 2001. Induced resistance: from the basic to the applied. Trends in Plant Science **6:** 445-447.

Werner,S., Steiner,R., Becher,A., Kortekamp,E., Zyprian,E., and Deising,H.B. 2002. Chitin synthesis during in planta growth and asexul propagation of the cellulosic oomycete and obligate biotrophic grapevine pathogen *Plasmopara viticola*. FEMS Microbiology Letters **208:** 169-173.

Whisson,S.C., Drenth,A., Maclean,D.J., and Irwin,J.A. 1995. Phytophthora sojae avirulence genes, RAPD, and RFLP markers used to construct a detailed genetic linkage map. Mol. Plant Microbe Interact. **8:** 988-995.

Whisson,S.C., van der,L.T., Bryan,G.J., Waugh,R., Govers,F., and Birch,P.R. 2001. Physical mapping across an avirulence locus of *Phytophthora infestans* using a highly representative, large-insert bacterial artificial chromosome library. Molecular Genetics and Genomics **266:** 289-295.

White,F.F., Yang,B., and Johnson,L.B. 2000. Prospects for understanding avirulence gene function. Current Opinion in Plant Biology **3:** 291-298.

Wilson,U.E. and Coffey,M.D. 1980. Cytological evaluation of general resistance to *Phytophthora infestans* in potato foliage. Annal of Botany **45:** 81-90.

Woloshuk,C.P., Meulenhoff,J.S., Sela-Buurlage,M., van dan Elzen,P.J.M., and Cornelissen,B.J.C. 1991. Pathogen-induced proteins with inhbitory activity toward *Phytophthora infestans*. Plant Cell **3:** 619-628.

Yamamoto,A., Katou,S., Yoshioka,H., Doke,N., and Kawakita,K. 2003. Nitrate reductase, a nitric oxide-producing enzyme: induction by pathgen signals. Journal of General Plant Pathology **69:** 218-229.

Yang,Y., shah,J., and Klessig,D.F. 1997. Signal perception and transduction in defense responses. Genes & Development **11:** 1621-1639.

Yao,K., De,L., V, and Brisson,N. 1995. Creation of a Metabolic Sink for Tryptophan Alters the Phenylpropanoid Pathway and the Susceptibility of Potato to *Phytophthora infestans*. Plant Cell **7:** 1787-1799.

Yao,M.K., Desilets,H., Charles,M.T., Boulanger,R., and Tweddell,R.J. 2003. Effect of mycorrhization on the accumulation of rishitin and solavetivone in potato plantlets challenged with Rhizoctonia solani. Mycorrhiza 333-336.

Yoshida,K., Kaothien,P., Matsui,T., Kawaoka,A., and Shinmyo,A. 2003. Molecular biology and application of plant peroxidase genes. Applied Microbiology and Biotechnology. **60:** 665-670.

Yoshioka,H., Miyabe,M., Hayakawa,Y., and Doke,N. 1996. Expression of genes for phenylalanine ammonia lyase and 3-hydroxy-3-methylglutaryl CoA reductase in aged potato tubers infected with *Phytophthora infestans*. Plant Cell Physiology **37:** 81-90.

Yoshioka,H., Shiraishi,T., Yamada,T., Ichionse,Y., and Oku,H. 1990. Suppression of pisatin production and ATPase activity in pea plasma membranes by orthovanadate, verapamil and suppressor from *Mycosphaerella pinodes*. Plant Cell and Physiology **31:** 1139-1146.

Yoshioka,H., Sugie,K., Park,H.J., Maeda,H.M., Tsuda,N., Kawakita,K., and Doke,N. 2001. Induction of plant gp91 *phox* homolog by fungal cell wall, arachidonic acid and salicylic acid in potato. Molecular Plant-Microbe Interactions **6:** 725-736.

Yoshioka,H., Yamada,N., and Doke,N. 1999. cDNA cloning of sesquiterpene cyclase and squalene synthase, and expression of the genes in potato tuber infected with *Phytophthora infestans*. Plant & Cell Physiology **40:** 993-998.

Young,S.A., Guo,A.G., Guikema,J.A., White,F.F., and Leach,J.E. 1995. Rice cationic peroxidase accumulates in xylem vessels during incompatible interactions with *Xanthomonas oryzae* pv *oryzae*. Plant Physiology **107:** 1333-1341.

Yu,D., Liu,C.H., Klessig,D.F., Fan,B.F., and Chen,Z. 1997. Is high basal level of salicylic acid important for disease resistance in potato? Plant Physiology **115:** 343-349.

Zareie,R., Melanson,D.L., and Murphy,P.J. 2002. Isolation of fungal cell wall degrading proteins from barley (*Hordeum vulgare L.*) leaves infected with *Rhynchosporium secalis*. Molecular and Plant Microbe Interactions **15:** 1031-1039.

Zhu,B., Chen,T.H., and Li,P.H. 1995. Activation of two osmotin-like protein genes by abiotic stimuli and fungal pathogen in transgenic potato plants. Plant Physiology **108:** 929-937.