Boosting Potato Defence Against Late Blight

A Study from Field to Molecule

Therése Bengtsson
Faculty of Landscape Planning, Horticulture and Agricultural Sciences
Department of Plant Protection Biology
Alnarp

Doctoral Thesis
Swedish University of Agricultural Sciences
Alnarp 2013

Acta Universitatis agriculturae Sueciae
2013:58

Cover: A hypersensitive response (HR)-like lesion in a BABA-treated leaflet of the potato cultivar Ovatio. The HR-like lesion is seen as a cluster of dead mesophyll cells, which are stained dark blue by trypan blue.
(photo: T. Bengtsson)

ISSN 1652-6880
ISBN (print version) 978-91-576-7854-6
ISBN (electronic version) 978-91-576-7855-3

Print: SLU Service/Repro, Alnarp 2013

Boosting Potato Defence Against Late Blight. A Study from Field to Molecule.

Abstract

For more than one century efforts has been made to obtain potato (*Solanum tuberosum*) cultivars resistant to late blight. However, introduced resistance has repeatedly been overcome by *Phytophthora infestans* (Mont) de Bary. Today late blight control is dependent on the frequent use of fungicides, but development of fungicide resistance and increasing fungicide restrictions by EU are of major concern. Methods with less fungicide requirement is therefore of crucial importance for a more environmentally sound and sustainable late blight control in the future.

In this study the potential of integrating BABA-induced resistance in existing late blight management with fungicides was investigated in field. The fungicide dose could be lowered with up to 25% when combined with BABA, without any decrease in late blight control or metabolic cost in terms of tuber yield. BABA was shown to directly activate basal defence responses and hormone signaling in potato. The BABA-induced hypersensitive-like lesions and major changes in the amino acid balance indicate that BABA induces resistance by stress imprinting.

Furthermore the potential of using a biosurfactant, produced by *Psuedomonas koreensis* strain 2.74, to control late blight in greenhouse was demonstrated. The biosurfactant was shown to have a direct effect on zoospores and also to induce PR-1 accumulation in the apoplast of potato leaves. Future experiments will reveal if the biosurfactant induces other defence mechanisms in potato.

This study demonstrated how integration of different control methods could lead to unchanged or even improved late blight control despite the decrease in fungicide dose.

Keywords: BABA, biosurfactant, disease control, field trials, induced resistance, integration, late blight, *Phytophthora infestans, Pseudomonas koreensis, Solanum tuberosum*

Author's address: Therése Bengtsson, SLU, Department of Plant Protection Biology, P.O. BOX 102, 230 53 Alnarp, Sweden
E-mail: therese.bengtsson@slu.se

Dedication

Till Dan-Ola och Viggo

It is good to have an end to journey toward; but it is the journey that matters in the end.

Ursula K. LeGuin

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Liljeroth, E., Bengtsson, T., Wiik, L. & Andreasson, E. (2010). Induced resistance in potato against *Phytophthora infestans* – effects of BABA in greenhouse and field tests with different potato varieties. *European Journal of Plant Pathology* 127(2), 171-183.

II Hultberg, M., Bengtsson, T. & Liljeroth, E. (2010). Late blight on potato is suppressed by the biosurfactant-producing strain *Pseudomonas koreensis* 2.74 and its biosurfactant. *Biocontrol* 55(4), 543-550.

III Bengtsson, T., Holefors, A., Witzell, J., Andreasson, E. & Liljeroth, E. (2013). Activation of defense responses to *Phytophthora infestans* in potato by BABA. *Plant Pathology*, DOI: 10.1111/ppa.12069.

IV Bengtsson, T., Liljeroth, E., Andreasson, E., Holefors, A., Caspersen, S., Alsanius, B. & Hultberg, M. Biosurfactant have the potential to induce defence against *Phytophthora infestans* in potato. (Manuscript).

V Bengtsson, T., Proux-Wéra, E., Levander, F., Resjö, S., Moushib, L., Hedley, P., Liljeroth, E., Alexandersson, E. & Andreasson, E. Proteomics and transcriptomics of BABA-induced resistance response in potato. (Manuscript)

The contribution of Therése Bengtsson to the papers included in this thesis was as follows:

I Planned and performed the greenhouse and laboratory part of the experiments together with co-authors and participated in evaluation of the data and writing of the manuscript.

II Planned and performed the experimental work together with co-authors and participated in evaluation of the data and writing of the manuscript.

III Planned and performed most of the experimental work, evaluated the data and wrote the manuscript together with co-authors.

IV Planned and performed the experimental work together with co-authors and participated in the writing of the manuscript together with co-authors.

V Planned the experiments together with co-authors. Performed a large part of the experimental work and wrote the manuscript together with co-authors.

Abbreviations

AABA	(DL)-2-aminobutyric acid or α-aminobutyric acid
ABA	abscisic acid
Avr	avirulence
BABA	(DL)-3-aminobutyric acid or β-aminobutyric acid
BTH	benzothiadiazole S-methyl ester
CC	coiled-coil
CM	cisgenetic modification
CMC	critical micelle concentration
CRN	crinkling and necrosis
DAMP	damage-associated molecular patterns
DuRPh	durable resistance against *Phytophthora* in potato
Et	ethylene
ETI	effector-triggered immunity
EU	European union
GABA	(DL)-4-aminobutyric acid or γ-aminobutyric acid
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GM	genetically modified
HR	hypersensitive response
INA	2,6-dichloroisonicotinic acid
IR	induced resistance
ISR	induced systemic resistance
JA	jasmonic acid
LRR	leucine rich repeats
MAMP	microbe-associated molecular patterns
MAPK	mitogen activated protein kinase
MPK	mitogen-activated protein kinases
NB	nucleotide binding
NO	nitric oxide

PAMP	pathogen-associated molecular patterns
PGPR/PGPF	plant-growth-promoting rhizobacteria/fungi
Phi	phosphite
PR	pathogenesis related
PRR	pattern recognition receptor
PTI	PAMP-triggered immunity
QTL	quantitative trait loci
R-gene	resistance-gene
RLK	receptor-like kinase
ROS	reactive oxygen species
SA	salicylic acid
SAR	systemic aquired resistance
TCA	tricarboxylic acid
TIR	TOLL/interleukin-1 receptor
tRNA	transfer RNA

1 Introduction

Potato is the third largest food crop in the world. In Sweden the area for potato cultivation constitute around 1% of the total agricultural area (SCB, 2012). However, among all cultivated agricultural crops in Sweden, potato has the highest applied amount of fungicide per hectar (SCB, 2012). This is mainly due to late blight caused by *Phytophthora infestans*, an oomycete that infects members of the *Solanaceae* family. Since the first late blight epidemic arise in the mid 19th century with dramatic consequences for the population of Ireland, intense efforts has been made to develop late blight-resistant potato cultivars. However, breeding for late blight resistance is time-consuming and the ability for *P. infestans* to rapidly overcome introduced resistance genes (R-genes) (Fry, 2008) has forced us to find new approaches.

In this thesis, the potential of integrating induced resistance in current management with fungicides, thereby reducing the amount of fungicides needed, is demonstrated in field and greenhouse. The field study revealed that when combining the inducing agent BABA with a fungicide, the fungicide dose could be lowered with 25% without losing effect in late blight control. BABA is a well-known inducer of plant defence, but the mechanism behind remains delusive. The current transcriptomic and proteomic study of BABA-IR in potato has provided us with tools to better understand how BABA treatment affects the potato defence on a molecular level. Studies within this thesis have also shown the potential of using biosurfactants in controlling late blight and that they induce defence responses in potato.

Together these findings will help us to understand how IR can be used in practice and hopefully contribute to new approaches for combined control methods against the late blight disease.

2 Background

2.1 Potato (*Solanum tuberosum* L.)

2.1.1 History and origin

Potato (*Solanum tuberosum* L.) belongs to the family of *Solanaceae*. Vegetables as tomato, pepper and eggplant, and ornamental plants as *Petunia* and *Nicotiana* are also members of the *Solanaceae* family.

Potatoes were domesticated already 8000 years ago, in the Andes of southern Peru around Lake Titicaca and this place is also accepted as the origin of potato. Today, there still exist a wide variety of wild relatives of the species with a great diversity in this region (Spooner *et al.*, 2005).

The Europeans first discovered potato in 1532, when Francisco Pizarro and his conquistadors conquered what now is Peru (Hawkes & Francisco-Ortega, 1993). Columbus did not explore these areas and the potato was therefore introduced later to Europe than many other crops from the New World (Hawkes & Francisco-Ortega, 1993). The earliest record of cultivated potato in Europe was in the Canary Islands in 1567 (Rios *et al.*, 2007; Hawkes & Francisco-Ortega, 1993). From here it spread throughout Europe and rest of the world, but it took more than a century before it was accepted as a major food crop. In the beginning it was mostly used as an ornamental plant in botanical gardens.

Olof Rudbeck who planted potato in Uppsala Botanical garden around 1655 was most probably the first one that brought it to Sweden. However not until about 70 years later, Jonas Alströmer began to cultivate potato on his farm in Alingsås. Alströmer also tried to convince the farmers about the potential of the tuber without greater success. It was not until the Swedish soldiers returned back home from the Pomerian war (1757-1762), where they had come to appreciate potato, as the production increased. Another factor behind the increasing production was the discovery of the possibility to produce alcohol

and flour from potato (Osvald, 1965). This discovery by Eva Ekeblad made her, as the first woman ever, elected to the Royal Swedish Academy of Sciences in 1748.

Recent DNA studies of historical herbarium specimens and landraces from India and the Canary Islands has revealed that the Andean potato predominated in Europe in the 1700s, but Chilean potato introduced to Europe in the beginning of 1800s soon became predominant (Ames & Spooner, 2008; Rios *et al.*, 2007). The chloroplast DNA data from the study of Ríos et al. (2007) further revealed that 99% of existing potatoes today have Chilean germplasm.

The original name of potato comes from the Quechua-Inca word ¨papa¨. However, this word was never adopted in Europe. In Italy it become known as tartouffli (truffle) and in France pomme de terre. Both 'patata' (Spain) and 'potato' (England) has derived from a combination of batata, which is the name of sweet potato (*Ipomoea batatas*), and the word papa.

2.1.2 Production and nutritional value

Potato is grown in more than 100 countries and is the third most important food crop in the world after wheat and rice. It is very adaptable and is cultivated both in temperate, subtropical and tropical conditions. Between 1991-2007, the potato production levels in the developed nations of Europe, North America and the former Soviet Union have declined from 183,13 to 159,89 million tonnes (FAO, 2008). In contrast, the production increased from 84,86 to 165,41 million tonnes in countries belonging to Asia, Africa and Latin America during the same period (FAO, 2008). The top three world leaders in potato production 2011 included China (88.4 million tonnes), India (42.3 million tonnes) and the Russian federation (32.7 million tonnes) (FAOSTAT, 2011).

Potatoes are rich in minerals like potassium, phosphorus and magnesium as well as vitamins like B1, B3, B6 and vitamin C (Camire *et al.*, 2009). One single potato (150 g) can meet half of the daily adult requirement of vitamin C. In addition to this, potatoes also contain dietary antioxidants, fibers, high quality proteins and carbohydrates. The best preparation method to preserve the vitamin C level is to boil the potato with the skin, however losses of other vitamins and minerals are less during baking (Prokop & Albert, 2008). However, potatoes also contain toxic glucoalkaloids like solanine and chaconine that often occurs just beneath the skin. To keep the levels of glucoalkaloids low, potatoes should be stored in a dark and cool place (Prokop & Albert, 2008).

2.2 *Phytophthora infestans* and the late blight disease

> "I shall never forget the change in one week in August. On the first occasion, on an official visit of inspection, I had passed over thirty-two miles thickly studded with potato fields in full bloom. The next time the face of the whole country had changed; the stalk remained bright green, but the leaves were all scorched black. It was the work of a single night. Distress and fear were pictured on every countenance and there was a great rush to dig and sell, or consume the crop by feeding pigs and cattle, fearing in a short time they would prove unfit for any use". (Captain Robert Mann, Coastguard officer in County Clare, 1846)

Figure 1. Late blight infection in a potato field. Photo: E. Liljeroth.

2.2.1 History and origin

The origin of *Phytophthora infestans*, the pathogen causing late blight in potato, is still a matter of controversy. Mexico has been suggested as the centre of origin of *P. infestans*, due to the occurrence of both mating types and high genetic and phenotypic variation (Andrivon, 1996). The other more recent upcoming theory, based on studies of mitochondrial and nuclear loci in *P. infestans* and its close relative *P. andina*, points to the Andes as the centre of origin (Gomez-Alpizar *et al.*, 2007).

The first late blight epidemic arose in the American east coast in 1843 and from there it spread all over Europe with a remarkably speed. In Ireland, potato constituted the main source of food and "potato murrain", as late blight then was called, got devastating consequences. The "Irish Potato Famine" led to starvation and death of more than 1 million people in Ireland and emmigration of 1.2 million Irish citizens. Even today the population of Ireland is not as big as before the famine.

Figure 2. Great famine memorial in Dublin. Photo: M. Gotte.

At first there was a controversy of whether a fungus of the *Botrytis* family was the cause of the late blight disease or rather a consequence of the disease. It was not until Anton de Bary in the beginning of 1860s described the life cycle of the pathogen, that it was classified as a fungus and later he also named it *Phytophthora infestans* the Greek words for 'plant destroyer' (Large, 1946).

A second migration of late blight to Europe occurred in the late 1970s, via a large import from Mexico of potatoes for fresh consumption (Niederhauser, 1991). The migration brought a second mating type designated A2 together with new genotypes with rare alleles (Fry, 2008). Prior to the second migration, the populations of *P. infestans* outside of Mexico were dominated by a single

clonal lineage, US-1 and consisted of only the A1 mating type (Goodwin *et al.*, 1994). The occurrence of both mating types, which makes sexual reproduction possible, was thought to lead to a more diverse genotypic variation, as seen in Mexico (Grünwald & Flier, 2005). However, the existing populations of *P. infestans* in Europe are still dominated by clonal lineages and a limited number of genotypes (Gisi *et al.*, 2011), except for the Nordic countries where sexual reproduction and genotypic variation occur (Brurberg *et al.*, 2011).

2.2.2 Taxonomy and biology

Phytophtora infestans (Mont.) De Bary is belonging to the class of oomycetes (water molds) of the kingdom Stramenopila and is taxonomically closely related to golden-brown algae and diatoms and is not a fungus as it first was classified as (Judelson & Blanco, 2005; Erwin & Ribeiro, 1996). The cell wall of true fungi consists of chitin, whereas the cell wall of oomycetes mainly contains cellulose and other glucans. *P. infestans* is a hemibiotrophic pathogen, which means that it, during infection, first has an initial biotrophic phase where it forms specialized feeding structures, like haustoria. This is followed by a nectrophic phase, where secondary hyphae are killing the tissue for nutrient acquisition (Perfect & Green, 2001). The pathogen mostly infects the potato foliage, but can also attack the stem and cause brown rot in the tubers (Fig. 3).

The project to sequence the genome of *P. infestans* was completed in 2009 and revealed that the genome, with a size of 240 megabases (Mb), consisted of an extremely high repeat content (74%) and highly mobile transposable elements (Haas *et al.*, 2009). Many of the genes within the dynamic repeat-rich regions belonged to fast-evolving pathogenicity effectors such as the RXLR and Crinkler families, which could explain its rapid evolutionary changes and effector gene expansions (Haas *et al.*, 2009).

Figure 3. Late blight symptoms on a leaf, tuber and stem. Photo: (from left): T. Bengtsson, F. Reslow and L. Wiik.

2.2.3 The infection stages and life cycle

The life cycle of *P. infestans* can be divided into an asexual and sexual part (Fig. 4). The asexual reproduction allows rapid dispersal and generation to take place and can be repeated many times during the season (Fry, 2008), whereas the sexual phase result in increased genetic variation and helps the pathogen to survive between seasons.

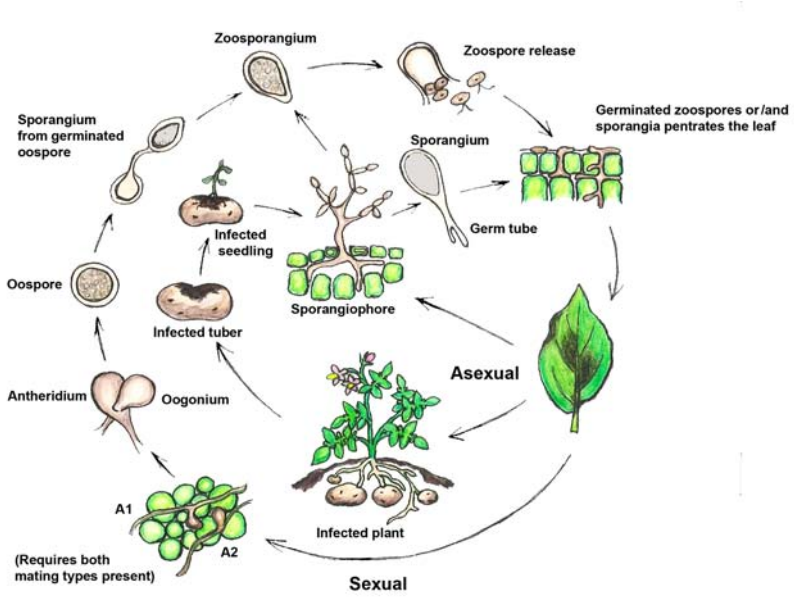

Figure 4. The life cycle of *Phytophthora infestans*. Illustration: H. P. Hovmalm.

In the absence of a compatible mating type, *P. infestans* reproduce asexually by the formation of specialised hyphae called sporangiophores. The branched sporangiophore emerges through the stomata of stem and leaf and produces sporangia (or zoosporangia) (Fig. 4). Sporangia are often released in the morning when it is becoming warmer and there is a drop in humidity. They can then be spread by wind to a nearby plant, where they at the right condition (20-25°C, nutrients available) germinate directly and cause infection or in case of lower temperatures (10-15°C, absence of nutrients) release 3-8 biflagellate motile zoospores (zoosporogenesis) (Fig. 5) (Fry, 2008; Grenville-Briggs *et al.*, 2005). The zoospores are wall-less and motile for a short time before they encyst, germinate and penetrate the plant. Infection by encysted zoospores is referred to as indirect germination (Grenville-Briggs *et al.*, 2005). Both sporangia and zoospores form germ tubes and appressoria prior to penetration (Tucker & Talbot, 2001). The mycelium grows intra- and intercellularly and

occasionally haustoria formation occurs inside the cells (Grenville-Briggs *et al.*, 2005). The whole leaf foliage of a field can be totally wilted within a week after infection. In addition to leaf damage, the sporangia and zoospores can also infect tubers, leading to a reduced harvest. Subsequently, the tubers will be a source of inoculum for the coming season.

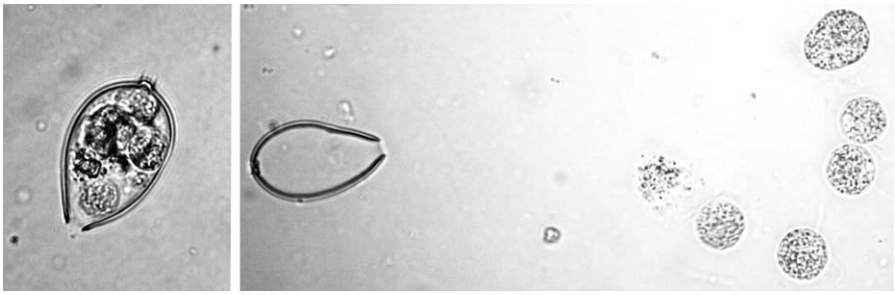

Figure 5. A sporangium before and after the release of zoospores. Photo: E. Liljeroth.

P. infestans is heterothallic and have two mating types, A1 and A2, for sexual reproduction. The sexual spore, called oospore, is formed after fertilization between the oogonium (female organ) and the antheridium (male organ). The oospore formation occurs more frequently in stems than in leaves. A possible explanation for this could be that the stems are able to resist a blight attack for a longer period (Andrivon, 1995). The oospores are thick-walled and very robust and can survive for several years in soil (Mayton *et al.*, 2000). They are able to survive very low temperatures but are more sensitive against higher temperatures (12 h at 40°C) (Fay & Fry, 1997). During the germination of an oospore, a germ tube is formed, by which a sporangium is produced. Just like in the asexual cycle, infection can occur both directly by the sporangium itself or indirect via the release of zoospores.

2.3 Management of the late blight disease

It is now more than 150 years since the first late blight epidemic occurred in Europe, but still the disease has the farmers and breeders in its grip. Today a combination of methods, like crop rotation, resistance breeding, chemical treatments, top-killing of the foliage prior to harvest and the use of high quality seed potato, are needed to keep late blight under control. There are also forecast systems, which aims at predicting when the weather is suitable for the pathogen and thus recommend when to spray the fields with fungicides. The control measures and yield losses due to late blight has been estimated to an annual cost of M € 4800 globally (Haverkort *et al.*, 2008).

2.3.1 Resistance breeding

Breeders have during more than a century tried to develop cultivars with race-specific (a.k.a. qualitative, vertical) resistance against *P. infestans*. Resistance genes (R-genes) from a wild Solanum species, *Solanum demissum* Lindl., were introduced in *Solanum tuberosum* by classical breeding (Umaerus & Umaerus, 1994). *P. infestans*, with its ability to rapidly evolve new virulent races in response to selection pressure, has today overcome all of the 11 R-genes introduced from *S. demissum* (Fry, 2008). Recently, breeders have tried to obtain more durable resistance by pyramiding (combining serveral R-genes) from wild Solanum relatives (Tan *et al.*, 2010). For the pathogen to overcome this, mutations at several avirulence (Avr) loci would be required.

Figure 6. Besksöta (*Solanum dulcamara*), a wild relative to *Solanum tuberosum*, found flowering (purple flowers) next to the sea in Lomma. Photo: Å. Lankinen.

Race-non-specific resistance (a.k.a. quantitative, horizontal, field or partial) is believed to be more durable due to its polygenic nature. A plant with quantitative resistance will not be totally immune but confer equal protection against all races and result in a lower selection pressure on the pathogen (Van der Plank, 1963). The breeding for quantitative resistance is challenging since the mechanisms behind the resistance are unknown and several genes or quantitative trait loci (QTL) are involved. In addition, the QTLs behind the

resistance have also been associated with undesirable traits, like lateness in maturity (Visker *et al.*, 2005). It is important to remember that disease resistance is only one among several other traits like, yield, storability, shape, taste, texture, color, resistance to browning, starch content, glycoalkaloid content etc. to consider in potato breeding. Besides all of these traits that need to be considered, traditional potato breeding is time consuming and accompanied with additional problems such as linkage drag, differences in ploidy levels and Endosperm Balance Number (Johnston *et al.*, 1980). When stacking of several R-genes is needed the time-span is prolonged and the breeding complicated, hence the chance to win the arms race against *P. infestans* becomes diminished. Stacking could be facilitated with genetically engineering by introduction of transgenes, e.g. genes from non-crossable species, or cisgenes, e.g. genes from the species itself or from a crossable species (Jacobsen & Schouten, 2009). In Europe the opposition against the genetic modification (GM)-approach still is strong and the way from development to release of a GM-crop is complicated, slow and expensive. In March 2010, after a 12-year approval process, the EU commission approved commercial use of the GM starch potato, named Amflora. BASF has also with the use of transgenic modification developed a variety, named Fortuna, which harbor two resistance genes (van der Vossen *et al.*, 2005; Van Der Vossen *et al.*, 2003) introduced from the wild relative *Solanum bulbocastanum* into the potato variety Fontane. However, already two years after the EU approval of Amflora, BASF stopped marketing GM varieties in Europe due to the lack of public acceptance and political resistance. Breeders are now hoping that the use of cisgenetic modifications (CM), without the use of selection markers such as antibiotic resistance genes, will prove acceptance by the public and facilitate the legalisation of CM varieties (Holme *et al.*, 2013; Haverkort *et al.*, 2009). In 2006, a research program called Durable Resistance against *Phytophthora* in potato (DuRPh), continuing over 10 years, was started at Wageningen University and Research Centre (Haverkort *et al.*, 2009; Haverkort *et al.*, 2008). The DuRPh program is relying on the CM approach without the use of selection markers.

2.3.2 Use of fungicides

The potato production is highly dependent on the use of fungicides. At the same time, the consumer's demand for organic and locally produced potato is increasing. The usual spraying frequency against late blight in Sweden is once per week starting from when the plant reaches a height of 20 cm or even earlier when there is risk for early infections. As a consequence, potato fields in Sweden are in average exposed to seven chemical treatments during a season

and during a rainy summer even more (SCB, 2011). The recommendations from the Swedish Board of Agriculture are to use contact fungicides that protect locally and not can be taken up by the plant, such as Shirlan and Ranman. In case of infected soil or seed potato, the recommendations are to use systemic or translaminar fungicides such as Acrobat, Epok, Ridomil Gold, Tattoo and Revus. Translaminar fungicides are taken up by the sprayed upper side of the leaf and are then transported to the lower unsprayed side of the leaf, in contrast to systemic fungicides that are absorbed by the leaf and transported through the xylem vessels, either short or long distances, within the plant. In Sweden, 2011, the area used for potato cultivation reached just above 1 % of the total cultivated agricultural area (SCB, 2012). Nonetheless, potato has the highest applied amount of fungicide per hectar among all cultivated agricultural crops in Sweden mainly due to late blight (SCB, 2012).

2.4 Plant innate immunity

Plants constantly have to face threats from pathogens like fungi, virus, bacteria, nematodes, insects etc., yet disease is an unusual outcome in nature. Most pathogens have a restricted host range but occasionally, under the right conditions, the pathogen succeeds to infect/infest a host plant. To defend themselves, plants have evolved an arsenal of constitutive and inducible defence mechanisms. Constitutive defences include pre-formed barriers such as cell walls, wax layers, thorns and secondary metabolites. The inducible defence is activated when the plant senses an intruder and includes the production of toxins, defence proteins and the hypersensitive response (HR). The plants inducible defence is part of the innate immune system, which is divided into two types based on the recognition of evolutionary conserved molecules from the pathogen (see Fig. 7).

2.4.1 PAMP-triggered immunity (PTI)

One part of the two-way innate immune system in plants is PAMP-triggered immunity (PTI), which is based on the recognition of pathogen-associated molecular patterns (PAMPs), or as more recently called microbe-associated molecular patterns (MAMPs), by the plant pattern recognition receptors (PRRs) located in the plasma membrane of the host (Chisholm *et al.*, 2006; Jones & Dangl, 2006) (Fig. 7). PAMPs/MAMPs are recognized as non-self molecular signatures, often evoultionary conserved in a certain class of pathogen, such as chitin for fungi, glucan for oomycetes and flagellin for bacteria (Boller & Felix, 2009). PTI can also indirectly be activated as a result

from the damage caused by microbes, so called damage-associated molecular patterns (DAMPs) (Boller & Felix, 2009).

PRRs often contain an extracellular domain of LRR (leucine rich repeats) that can sense PAMPs/MAMPs or DAMPs, a transmembrane domain and an intracellular serine/threonine kinase domain that transmits the signal (Zipfel, 2008). Belonging to this category are the receptor-like kinases (RLKs) (Shiu & Bleecker, 2003). The signal results in the activation of a wide set of downstream defence responses such as cell wall reinforcement, mitogen activated protein kinase (MAPK) cascades, production of reactive oxygen species (ROS) and induction of defence gene expression (Ingle *et al.*, 2006).

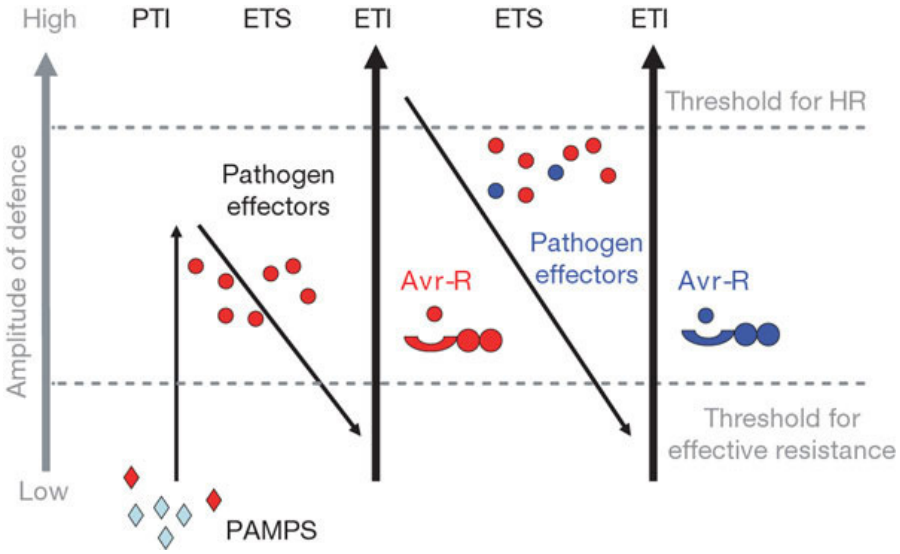

Figure 7. Schematic representation of the co-evolutionary zig-zag model of plant-pathogen defence strategies. In phase 1, plant pattern recognition receptors (PRRs) perceive highly conserved non-self molecules (MAMPs/PAMPs) or damage-associated molecular patterns (DAMPs) and activate PAMP-triggered immunity (PTI). In phase 2, the pathogen interferes with PTI through the release of effectors, which if successful results in effector-triggered susceptibility (ETS). In phase 3, a race-specific effector (red) is recognized by the plant resistance protein (R1) and activates effector-triggered immunity (ETI) often associated with the hypersensitive response (HR). Phase 2 and 3 are then repeated over and over in an ongoing arms race between the plant and the pathogen, with selection of new resistance proteins and effector molecules. Reproduced with permission from Macmillan Publishers Ltd: Nature. Jones & Dangl, copyright 2006.

One example of a *P. infestans* PAMP are Pep-13, a motif from a transglutaminase (GP42), that induces the oxidative burst and lead to salicylic acid (SA) and jasmonic acid (JA) accumulation, defence gene expression and HR in potato (Halim *et al.*, 2004). Other known *P. infestans* PAMPs are INF1,

an elicitin with sterol carrier activity (Kamoun *et al.*, 1998; Mikes *et al.*, 1998), and scr74, a phytotoxin-like protein that triggers HR within the host (Liu *et al.*, 2005).

2.4.2 Effector-triggered immunity (ETI)

PTI often stops the infection at an early stage before the pathogen gains a hold in the plant. However, in some cases the pathogen is able to suppress the PTI by release of effectors, leading to effector-triggered susceptibility (ETS) (Chisholm *et al.*, 2006; Jones & Dangl, 2006) (Fig. 7). Subsequently, in order for the plants to survive, they evolved a more specialized recognition system with resistance (R) genes, encoding R proteins that either direct or indirect can recognize a specific effector. This leads to an incompatible reaction called effector-triggered immunity (ETI) that often is associated with HR (Chisholm *et al.*, 2006; Jones & Dangl, 2006) (see Fig. 7). Typical R proteins are NB-LRRs named after their nucleotide binding (NB) and leucine rich repeat (LRR) domains, but other domains such as coiled-coil (CC) and TOLL/interleukin-1 receptor (TIR) can also be present (McHale *et al.*, 2006). Recently, 435 NB-LRRs (putative R-genes) was identified in the *Solanum tubersoum* group phureja DM1-3516 R44 genotype (Lozano *et al.*, 2012).

2.4.3 *P. infestans* effectors

Effectors released by the pathogen are classified as extracellular (apoplastic) or intracellular (cytoplasmic) effectors based on the place for expression within the host (Kamoun, 2006) (Fig. 8). The recently published genome of *P. infestans* revealed, highly mobile transposable elements and large families of putative effectors (Haas *et al.*, 2009), most of them found in untranslated repeat rich regions. This could enable changes and expansions of the effector repertoire. However, the underlying function of most of them still remains unknown.

Apoplastic effectors

Apoplastic effectors have N-terminal signal peptides for secretion and C-terminal effector module(s) (Damasceno *et al.*, 2008; Tian *et al.*, 2007; Tian *et al.*, 2005; Tian *et al.*, 2004). Most of them target proteases and glucanases, thus plant defence related proteins. The *P. infestans* effectors EPI1 and EPI10 inhibit subtilisin-like protease P69B (Tian *et al.*, 2005; Tian *et al.*, 2004), while EPIC1 and EPIC2B inhibit different cysteine proteases like C14, PIP1 and Rcr3, in tomato (Kaschani *et al.*, 2010; Song *et al.*, 2009; Tian *et al.*, 2007). Glucanase inhibitor protein (GIP) is a *P. infestans* effector that inhibits Endo-beta-1, 3 glucanases, thus prevent the degradation of pathogen cell wall

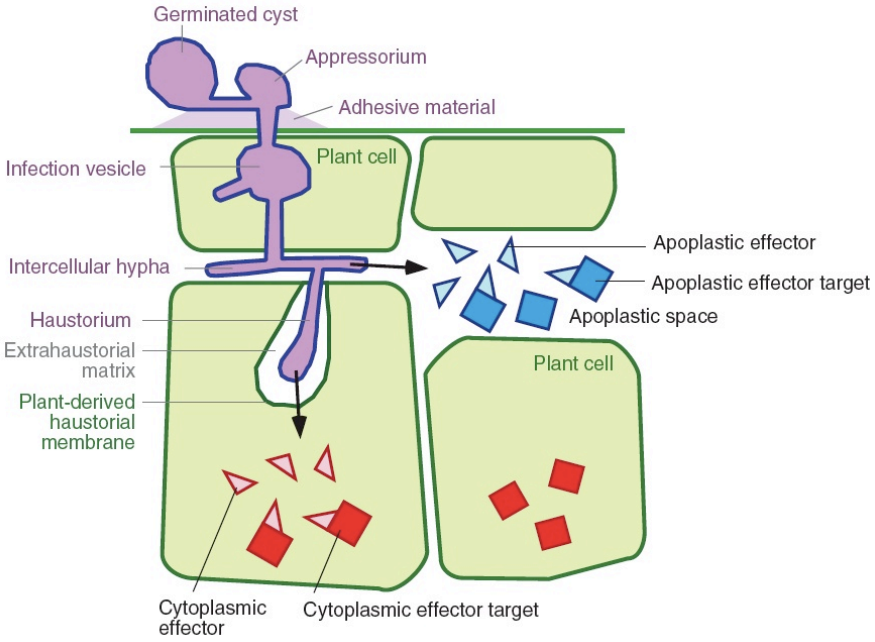

Figure 8. Schematic view of a host infected by *P. infestans* secreting both apoplastic and cytoplasmic effectors. Note that the scale is not reflecting the reality. Republished with permission of ANNUAL REVIEWS, INC from "A Catalogue of the Effector Secretome of Plant Pathogenic Oomycetes", Kamoun, Annual Review of Phytopathology 44(1), copyright 2006; permission conveyed through Copyright Clearance Center, Inc.

components that can act as elicitors of host defence mechanisms (Bishop et al., 2005).

Cytoplasmic effectors

The cytoplasmic effectors have just as the apoplastic ones, N-terminal signal peptides for secretion, but also for translocation inside the host cell. The C-terminal domain is involved in the biochemical effector activity (Schornack *et al.*, 2009). It is specific motifs in the N-terminal region following the signal peptides that defines where it should be translocated inside the cell. For instance RXLR effectors are characterized by a specific amino acid sequence (Arg-X-Leu-Arg, where X denotes any amino acid) (Dou *et al.*, 2008; Whisson *et al.*, 2007). RXLR is also one of the main cytoplasmic effectors and hundreds of them are present in the genome of *P. infestans* (Haas *et al.*, 2009). The RXLR family, IPIO, consists of several classes (I, II and III), where class I and II can be recognized by a R-gene (RB or Rpi-blb1) from *Solanum*

bulbocastanum, thus leading to HR and resistance against isolates of *P. infestans* (Halterman *et al.*, 2010; Champouret *et al.*, 2009; Song *et al.*, 2003). In contrast, *P. infestans* harbouring class III variants of IPIO (IPI-O4) has been shown to overcome RB resistance, by either avoiding recognition or interfering with the resulting HR (Champouret et al., 2009; Halterman et al., 2010).
Another *P. infestans* RXLR effector, is AVR3a that occurs in two forms: $AVR3A^{KI}$ and $AVR3A^{EM}$ (Bos *et al.*, 2010). AVR3a function as a virulence factor that targets and stabilizes the plant U-box E3 ligase CMPG1 resulting in HR inhibition (Bos *et al.*, 2010). $AVR3A^{KI}$ is recognized by the corresponding potato resistance protein R3a and strongly suppresses infestin 1 (INF1)-triggered cell death (ICD), whereas $AVR3A^{EM}$ avoids recognition thus only results in a weak HR suppression (Bos *et al.*, 2010).

The second predominant family of *P. infestans* cytoplasmic effectors is the Crinkler family (CRN for CRinkling and Necrosis). The first CRN protein was found in *P. infestans* and was named based on the leaf crinkling and cell death phenotype observed when expressed *in planta* (Torto *et al.*, 2003). Their biochemical activity is still largely unknown, however a recent study have shown that many of them are phosphorylated (van Damme *et al.*, 2012). This has yielded an interest in exploring how CRNs modulates post-translational processes of the host and thus interferes with the host defence signalling (Howden & Huitema, 2012).

2.5 Induced resistance (IR) in plants

Both abiotic and biotic stimuli can activate the inducible defence within the plant, leading to an increased resistance towards pathogens and herbivores both locally and systemically, a phenomenon termed induced resistance (IR).

The first reports about the IR phenomenon were published in the early 1900s. In 1952 Gilpatrick and his colleague, observed a reduction in virus symptoms on *Dianthus barbatus* L. plants (eng. Sweet-William plant, sv. borstnejlika), if the plants previously had been exposed for the same virus (Gilpatrick & Weintraub, 1952). A similar observation was made in tobacco (*Nicotiana tabacum*) 1961, where inoculation of the lower leaves with tobacco mosaic virus, resulted in induced resistance to a secondary infection within the upper leaves, a phenomenon termed systemically aquired resistance (SAR) (Ross, 1961). Another form of IR is induced systemic resistance (ISR), which is acquired when the plant rhizosphere are colonized by plant-growth-promoting rhizobacteria/fungi (PGPR/PGPF) (Shoresh *et al.*, 2010; van Loon, 2007). In addition, previous exposure to insects, avirulent nematode species

and endophytes has also been shown to induce plant resistance (Kang *et al.*, 2007; Bostock, 2005; Kosaka *et al.*, 2001).

IR does usually not result in fully resistant plants and the effect has shown to be dependent on various factors such as genotype, application method and surrounding environment (Walters *et al.*, 2011; Liljeroth *et al.*, 2010; Sharma *et al.*, 2010) (Fig. 9). Since resistance is quickly overcome by many pathogens and significant reduction of pesticides is a goal for the European Union (EU) (Hillocks, 2012), there is an urgent demand for alternative approaches. Future agricultural practices are headed towards the use of more sustainable and environmentally sound control systems that often requires integration of several approaches.

Despite many years of research within the area of IR, the use in practise is minimal. Farmers are used to high disease control, thus the possibility to use IR, which is associated with lower disease control, is less tempting. Even if pest/pathogen/abiotic stress control not can rely solely on IR, it still may have a great potential to be used in an integrated approach. For example, the abiotic agent β-aminobutyric acid (BABA) applied in combination with a fungicide, could lower the amount of fungicide needed for late blight control with up to 25% in a potato field (Liljeroth *et al.*, 2010). Recently, the use of potassium phosphite (Phi) another abiotic inducing agent, has shown great potential to reduce downey mildew (*Plasmopara viticola*) infection in grapewine fields (Pinto *et al.*, 2012). In fact it showed to be superior to fungicide treatments when applied alone, with obtained disease control around 40 % (Pinto et al., 2012). In addition, Pinto et al. (2012) also showed that Phi application was an economical viable option to fungicide use. Potato field trials conducted during 2011 and 2012, has confirmed the potential of using Phi (Liljeroth *et al.*, 2012). Results from the two years have shown that a synergistic effect can be reached when combining Phi with the fungicide Shirlan (Liljeroth *et al.*, 2012). In other words, the fungicide dose could be reduced up to 50%, when Phi was added to the treatment, without affecting the efficacy (Liljeroth *et al.*, 2012). Interestingly, for some varieties, Phi applied alone, resulted in a lower percent infection than obtained when solely Shirlan was applied (Liljeroth *et al.*, 2012). These results clearly demonstrate the potential of integrating IR in existing management strategies and the importance of using the best responding varieties.

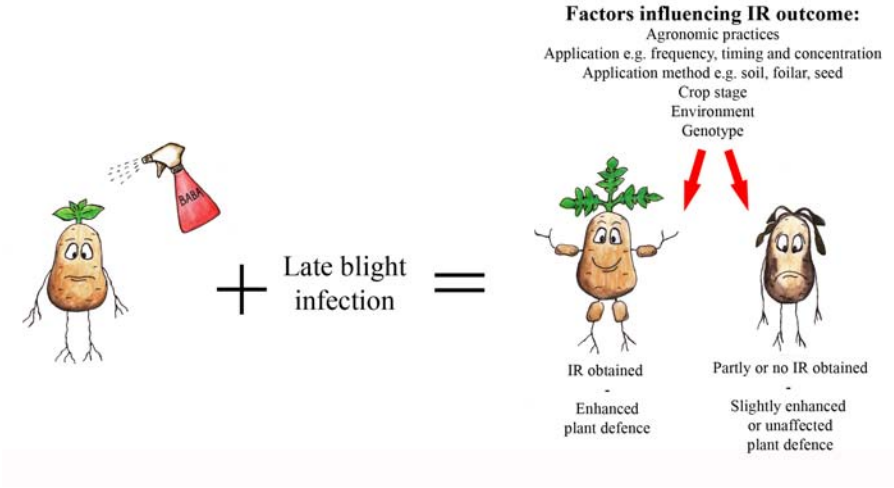

Figure 9. Illustration over factors influencing the outcome of induced resistance (IR) in plants, with BABA, late blight and potato as example. Note that the IR-response is partial and not so clear as illustrated in the figure. Illustration: H. P. Hovmalm.

The plant defence does not always become directly activated upon stimuli instead it can become activated first upon subsequent exposure to stress. This is referred to as priming and is often associated with a faster and stronger induction of the plant defence when exposed for subsequent abiotic and/or biotic stress (Conrath, 2011; Conrath *et al.*, 2006). Beckers et al. (2009) has shown that two inactive proteins of mitogen-activated protein kinases (MPKs), MPK3 and MPK6, have a role in priming in Arabidopsis. These signaling proteins were activated in primed Arabidopsis and associated with an enhanced defence gene expression upon infection (Beckers *et al.*, 2009). In a recent publication an extracellular subtilase, SBT3.3, that acts as a switch for a SA-dependent immune priming in Arabidopsis, has been identified (Ramírez *et al.*, 2013). Furthermore, SBT3.3 became upregulated and primed the defence when epigenetic control was constrained, thus suggesting a role for epigenetic control in the regulation of plant immunity. In general, IR by priming of the defence is considered to be the better strategy, due to the higher allocation cost associated with a constitutively activated defence (Walters & Heil, 2007; van Hulten *et al.*, 2006).

2.5.1 Systemic aquired resistance (SAR)

The term SAR refers to the systemically broad-spectrum defence induced in tissue distal from a local pathogen infection. However, SAR can also be activated by numerous of abiotic agents like the defence hormone salicylic acid

(SA) and its synthetic analogs 2, 6-dichloroisonicotinic acid (INA) and benzothiadiazole S-methyl ester (BTH) (Durrant & Dong, 2004; Ryals *et al.*, 1996). SAR is often associated with the priming, accumulation of pathogenesis-related (PR) proteins and defence against hemibiotrophic and biotrophic pathogens (Glazebrook, 2005; Durrant & Dong, 2004).

Since the phenomenon of SAR was described and phrased in the 1960s (Ross, 1961; Chester, 1933), intense research has been conducted to understand which components that are involved in SAR activation and signal transmission. Most of the questions still need to be answered, but recent progress has been made. Several review articles have been published to summarize the latest findings and address the fundamental questions regarding SAR (Fu & Dong, 2013; Pajerowska-Mukhtar *et al.*, 2013; Shah & Chaturvedi, 2013; Dempsey & Klessig, 2012; Spoel & Dong, 2012).

Pajerowska-Mukthar et al. (2012) suggested that changes in amino acid homeostasis induced by ETI could play a role in initiating SAR signalling. In their study they found that TL1-binding factor 1 (TBF1), which contains two up-stream open reading frames enriched for phenylalanine, was translated upon ETI induction by *P. syringae* pv. *maculicola* (*Psm*) ES4326/AvrRpt2. A significant increase of the TBF1 transcript was observed within 30 minutes after *Psm* inoculation hence it might be one of the earliest triggering responses for SAR. Several candidates are suggested to have a role in the mobile signalling for SAR, such as methyl salicylic acid (MeSA), pipecolic acid (PiP), azelaic acid (AzA), glycerol-3-phosphate (G3P), abietane diterpenoid dehydroabietinal (DA) and a lipid transport protein named DIR1 (defective in induced resistance 1) (Shah & Chaturvedi, 2013). Among these AzA, G3P and DA all require DIR1 to induce SAR (Shah & Chaturvedi, 2013). Furthermore the transcription co-factor nonexpressor of PR genes 1 (NPR1), a master regulator of plant and required for activation of pathogenesis related (PR)-proteins, together with SA and the suggested SA receptors NPR3 and NPR4, all seems to be important players and parts of a complex network leading to SAR establishment (Fu & Dong, 2013, Pajerowska-Mukhtar et al., 2013). The effect of SAR can be long lasting and recent reports indicate that the memory of SAR even can be inherited to the next generation (Luna *et al.*, 2012; Slaughter *et al.*, 2012).

2.5.2 Induced systemic resistance (ISR)

Induced systemic resistance (ISR) is synonymous to SAR, but was termed ISR to facilitate the separation of pathogen- and PGPR/PGPF-induced resistance. Occurrence of non-pathogenic and plant growth-promoting bacteria and fungus in the rhizhosphere, can lead to an enhanced defence in above ground plant

parts called ISR (Pieterse *et al.*, 1998). Commonly studied PGPR/PGPF species mediating ISR are *Pseudomonas*, *Bacillus* and *Trichoderma* spp. (Walters *et al.*, 2013). ISR, like SAR, constitute defence against a broad spectrum of pathogens, but also towards insects and against abiotic stress (Yang *et al.*, 2009; Van Oosten *et al.*, 2008; van Loon *et al.*, 1998). Several "omics" studies have recently been conducted in order to obtain a deeper knowledge of the mechanisms behind ISR (van de Mortel *et al.*, 2012; Weston *et al.*, 2012; Walker *et al.*, 2011; Van der Ent *et al.*, 2009; Verhagen *et al.*, 2004). In contrast to SAR, establishment of ISR is most often dependent on components of the JA and/or ethylene (Et) signaling pathway (Pieterse *et al.*, 1998), pathways associated with defence against necrotrophic pathogens (Glazebrook, 2005). However, this is not always the case. In some pathosystems ISR can be dependent also on SA signaling or even require both pathways to function (van de Mortel *et al.*, 2012; Yoshioka *et al.*, 2012; Conn *et al.*, 2008; Tjamos *et al.*, 2005; Audenaert *et al.*, 2002). In addition to the hormone metabolism, components of the secondary-, carbohydrate- and amino acid-metabolism have shown to be involved in ISR (Weston *et al.*, 2012).

The mechanism involved in ISR-related priming seems also to be regulated by different pathways, depending on the pathosystem as well as the inducing agent. For instance, ISR-related priming in Arabidopsis by *P. fluorescens* WCS417r or BABA, has been shown to involve control by NPR1-dependent signalling pathways (Van der Ent *et al.*, 2009). However, the same study showed that the two inducers resulted in distinct sets of priming-responsive genes suitable as specific markers for priming. For example, a putative *cis*-element was strongly over-represented in the promoters of 21 NPR1-dependent, BABA-induced WRKY genes (Van der Ent *et al.*, 2009).

2.5.3 BABA-IR

Among abiotic IR-inducers, BABA is one of the most well known agents. Papavizas and Davey discovered it already in 1963, when they found that BABA could reduce root rot of peas caused by *Aphanomyces euteiches*. Since then numerous of studies have shown that BABA can induce resistance in many plant species, spanning over different families, against a broad range of pathogens but also against abiotic stress like drought and salt (Table 1).

The ability of BABA to induce resistance in plants directly or indirectly is usually not associated with a direct antifungal or antibacterial activity. However, reports of direct toxicity against fungal pathogens exists (Šašek *et al.*, 2012; Zhang *et al.*, 2011; Marcucci *et al.*, 2010; Fischer *et al.*, 2009; Tavallali *et al.*, 2008; Porat *et al.*, 2003), but has been suggested to be

dependent on the dose and presence of organic nitrogen in the culture medium (Šašek *et al.*, 2012; Fischer *et al.*, 2009). The concentrations required for antifungal activity has also been shown to be significantly higher than the optimal concentration for BABA-IR (Porat *et al.*, 2003).

Recently it has been demonstrated that BABA-primed defence, just as SAR, can become inherited by following plant generations (Slaughter *et al.*, 2012). Progeny of BABA-treated Arabidopsis showed a stronger expression of defence-related genes and enhanced disease resistance against the bacteria *Pseudomonas syringae* pv *tomato* and the obligate oomycete *Hyaloperonospora arabidopsidis* (Slaughter *et al.*, 2012). No BABA could be detected in the progeny at the time of challenge inoculation, thus a direct antimicrobial effect of BABA could be ruled out as the cause of the observed priming.

Table 1. *Selection of studies of BABA-induced resistance (BABA-IR) in various plant-abiotic/biotic interactions*

Family	Plant Species	Common name	Type of stress	Subsequent exposure for:	Reference
Asteraceae	*Cynara cardunculus (artichoke)*	Artichoke	Fungus	*Sclerotinia sclerotiorum*	Marcucci et al. 2010
	Helianthus annuus L.	Sunflower	Fungus	*Puccinia helianthi*	Amzalek and Cohen 2007[1]
			Oomycete	*Plasmopara helianthi*	Tosi et al. 2000
				Plasmopara halstedii	Nandeeshkumar et al. 2009[1]
	Lactuca sativa L.	Lettuce	Oomycete	*Bremia lactucae*	Cohen 2007[1]
					Cohen et al. 2010
					Cohen et al. 2011
					Pajot et al. 2001
Alliaceae	*Allium cepa* L.	Onion	Fungus	*Botrytis allii/Botrytis cinerea*	Polyakovskii et al. 2008
Brassicaceae	*Arabidopsis thaliana L.*	Thale cress	Abiotic stress	Acid rain	Liu et al. 2011
				NaCl	Ton et al. 2005
			Bacteria	*Pectobacterium carotovorum*	Po-Wen et al. 2013
				Pseudomonas fluorescence	Van der Ent et al. 2009
				Pseudomonas syringae	Flors et al. 2008
					Návarová et al. 2012
					Singh et al. 2012
					Ton et al. 2005
					Tsai et al. 2011
					Van Hulten et al. 2006
					Zimmerli et al. 2000
			Fungus	*Alternaria brassicicola*	Flors et al. 2008
					Ton and Mauch-Mani 2004
				Botrytis cinerea	Zimmerli et al. 2001
				Plectosphaerella cucumerina	Ton and Mauch-Mani 2004
			Insect	*Brevicoryne brassicae/Plutella xylostella*	Hodge et al. 2006
			MAMP	*Flg22*	Singh et al. 2012
			Oomycete	*Hyaloperonospora parasitica*	Ton et al. 2005
					Van Hulten et al. 2006

Family	Plant Species	Common name	Type of stress	Subsequent exposure for:	Reference
				Peronospora parasitica	Zimmerli et al. 2000
				Phytophthora brassicae	Si-Ammour et al. 2003
				Phytophthora infestans	Si-Ammour et al. 2003
	Brassica juncea L.	Brown mustard	Fungus	*Alternaria brassicae*	Kamble and Bhargava 2007[1]
	Brassica napus L.	Salad rape	Fungus	*Leptosphaeria maculans*	Šašek et al. 2012
				Verticillium longisporum	Kamble et al. 2013
			Insect	*Myzus persicae/Brevicoryne brassicae/Plutella xylostella*	Hodge et al. 2006
	Brassica nigra L.	Black mustard	Insect	*Trichoplusia ni*	Hodge et al. 2006
	Brassica oleracea (L.)	Broccoli	Bacteria	*Pseudomonas fluorescens*	Pajot and Silue 2005
		Calabrese	Insect	*Myzus persicae/Brevicoryne brassicae/Trichoplusia ni/Plutella xylostella*	Hodge et al. 2006
		Savoy cabbage		*Myzus persicae/Plutella xylostella*	Hodge et al. 2006
		Spring cabbage		*Brevicoryne brassicae/ Trichoplusia ni*	Hodge et al. 2006
		Cauliflower	Oomycete	*Peronospora parasitica*	Silué et al. 2002
	Sinapis alba L.	White mustard	Insect	*Myzus persicae/Brevicoryne brassicae/Trichoplusia ni/ Plutella xylostella*	Hodge et al. 2006
Bromeliaceae	*Ananas cosmosis* L.	Pineapple	Nematode	*Meloidogyne javanica/Rotylenchulus reniformis*	Chinnasri et al. 2006
Cucurbitaceae	*Cucumis sativus L.*	Cucumber	Fungus	*Colletotrichum lagenarium*	Walz and Simon 2009
				Colletotrichum orbiculare	Jeun et al. 2004
					Jeun et al. 2007
			Nematode	*Meloidogyne javanica*	Sahebani et al. 2010
			Oomycete	*Pseudoperonospora cubensis*	Walz and Simon 2009
					Baider and Cohen 2003
	Cucurbita pepo L.	Squash	Oomycete	*Phytophthora capsici*	Kone et al. 2009
Fabaceae	*Glycine max L.*	Soybean	Abiotic stress	Cadmium	Hossain et al. 2012
	Medicago sativa L.	Alfalfa	Insect	*Acyrthosiphon pisum*	Hodge et al. 2005
	Phaseolus coccineus L.	Runner Bean	Insect	*Acyrthosiphon pisum*	Hodge et al. 2005
	Pisum sativum L.	Pea	Fungus	*Uromyces pisi*	Barilli et al. 2010a
					Barilli et al. 2010b
					Barilli et al. 2012
			Insect	*Acyrthosiphon pisum*	Hodge et al. 2005

Family	Plant Species	Common name	Type of stress	Subsequent exposure for:	Reference
	Trifolium pratense L.	Red clover	Insect	*Acyrthosiphon pisum*	Hodge et al. 2005
	Vicia faba var. major L.	Broad Bean	Insect	*Acyrthosiphon pisum*	Hodge et al. 2005
	Vicia faba var. minor L.	Tic bean	Insect	*Acyrthosiphon pisum*	Hodge et al. 2005
					Hodge et al. 2011
Lamiaceae	*Ocimum basilicum* L.	Basil	Oomycete	*Peronospora belbahrii*	Mersha et al. 2013
Malvaceae	*Corchorus capsularis L.*	Jute	Fungus	*Macrophomina phaseolina*	Ray et al. 2011
Poaceae	*Pennisetum glaucum* L.	Pearl millet	Fungus	*Sclerospora graminicola*	Shailasree et al. 2001
					Shailasree et al. 2007
	Triticum aestivum L.	Spring wheat	Abiotic stress	Drought	Du et al . 2012
	Triticum aestivum L.	Wheat	Fungus	*Fusarium graminearum*	Zhang et al. 2007
Rosaceae	*Malus domestica Borkh.*	Apple	Bacteria	*Erwinia amylovora*	Hassan and Buchenauer 2007
			Fungus	*Alternaria alternata*	Reuveni et al. 2003 [1]
				Penicillium expansum	Zhang et al. 2011
	Malus pumila	Crabapple	Abiotic stress	Drought	Macarisin et al. 2009
Rutaceae	*Citrus aurantifolia L.*	Lime	Bacteria	*Xanthomonas citri*	Sharifi -sirchi et al. 2011
	Citrus paradisi L.	Grapefruit	Fungus	*Penicillium digitatum*	Porat et al. 2003
	Citrus sinensis L.	Orange	Fungus	*Penicillium italicum*	Tavallali et al. 2008
	Citrus paradisi x Poncirus trifoliata	Swingle citrumelo	Insect	*Diaphorina citri*	Tiwari et al. 2013
Solanaceae	*Capsicum annuum L.*	Pepper	Fungus	*Colletotrichum coccodes*	Hong et al. 1999
			Oomycete	*Phytophthora capsici*	Hwang et al. 1997
					Lee et al. 2000
					Sunwoo et al. 1996
	Lycopersicon esculentum Mill	Tomato	Bacteria	*Clavibacter michiganensis*	Baysal et al. 2005
					Hassan and Buchenauer 2008
				Pseudomonas syringae	*Pseudomonas syringae*
				Ralstonia solanacearum	Hassan and Abo-Elysour 2013
			Fungus	*Fusarium oxysporum*	Chamsai et al. 2004
				Oidium neolycopersici	Worrall et al. 2012
			Nematode	*Meloidogyne javanica*	Fatemy et al. 2012
					Oka et al. 1999
					Sahebani and Hadavi 2009

Family	Plant Species	Common name	Type of stress	Subsequent exposure for:	Reference
			Oomycete	*Phytophthora infestans*	Cohen 1994b
					Cohen et al. 1994
					Jeun et al. 2001
	Nicotiana tabacum L.	Tobacco	Oomycete	*Peronospora tabacina*	Cohen 1994a
			Virus	*Tobacco mosaic virus*	Lazzarato et al .2009
					Siegrist et al. 2000
	Solanum tuberosum L.	Potato	Fungus	*Fusarium solani*	Olivieri et al. 2009
			Oomycete	*Phytophthora brassicae*	Si-Ammour et al. 2003
				Phytophthora infestans	Altamiranda et al. 2008
					Andreu et al. 2006
					Baider and Cohen 2003
					Bengtsson et al. 2013
					Cohen 2002[1]
					Eschen-Lippold et al. 2010
					Floryszak-Wieczorek et al 2012
					Kim and Jeun 2007
					Liljeroth et al. 2010[1]
					Olivieri et al. 2009
					Si-Ammour et al. 2003
Vitaceae	*Vitis vinifera L.*	Grapewine	Abiotic stress	OG elicitor	Dubreuil -Maurizi et al. 2010
			Oomycete	*Plasmopara viticola*	Cohen et al. 1999
					Dubreuil-Maurizi et al. 2010
					Hamiduzzaman et al. 2005
					Reuveni et al. 2001[1]
					Slaughter et al. 2008
Zigiberaceae	*Zingiber officinale*	Ginger	Oomycete	*Pythium aphanidermatum*	Karmakar et al. 2003

1. Whole experiment or parts of it conducted in field.

The BABA molecule

BABA is a non-protein amino acid and a derivative of carboxylic acid. It has a carboxyl group at the first carbon atom and an amino group positioned at the third carbon atom, thus the name (DL)-3-aminobutyric acid (β-aminobutyric acid) (see Fig. 10). BABA is a racemate that can consist of both R- and S-enantiomers, where the IR-effect has been shown to depend mostly on the R-enantiomer (Cohen *et al.*, 2011; Chamsai, 2004; Silué *et al.*, 2002) (see Fig. 10). Further more, the 3-(β)-position of the amino group is crucial for BABA activity in lettuce, since the two isomers 2-aminobutyric acid (AABA) and 4-aminobutyric acid (GABA) were unable to induce resistance against *Bremia lactuacae* (Cohen *et al.*, 2011; Cohen *et al.*, 2010) (see Fig. 10). This has been confirmed by several other studies conducted in rape, sunflower, pepper, grapewine, cauliflower, tomato and tobacco where AABA and GABA were either less efficient or unable to induce resistance (Šašek *et al.*, 2012; Silué *et al.*, 2002; Siegrist *et al.*, 2000; Cohen *et al.*, 1999; Hong *et al.*, 1999; Tosi *et al.*, 1998). Thus, as suggested by Cohen et al. (2011), a specific stereoscopic arrangement of the amino group on carbon 3 might be crucial for the binding and activity of BABA. BABA has earlier been shown to be able to bind to protein(s) in the cell wall of tomato, tobacco, potato and grapwine (Cohen *et al.*, 1999; Cohen & Gisi, 1994). However, no BABA-specific receptor(s) has so far been discovered.

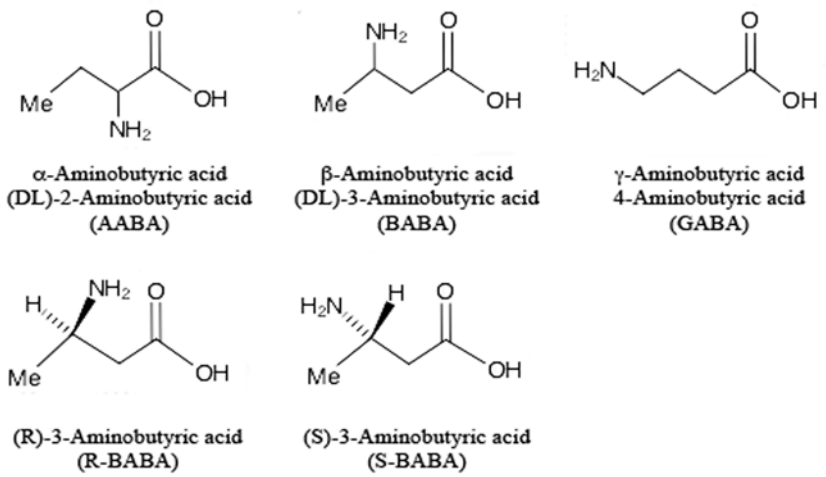

Figure 10. Chemical structures of: α-, β- and γ-aminobutyric acid and BABA enantiomers.

Natural occurence

BABA does normally not occur naturally in nature, but there are two reports of BABA found in plants. The first report came in 1992, when Gamliel and Katan found BABA in the root exudates from tomato. The second and latest report came in 2009, when Pfautsch et al. (2009) found BABA present in the phloem and wood of *Eucalyptus regnans* and two *Acacia* species. In addition Barrado et al. (2009) have found BABA present in Spanish wines, when analysing wines for the presence and quantity of eight amino acids. In contrast, the BABA isomer, GABA, is widely abundant in both plants and animals. GABA was found present in a plant for the first time more than 60 years ago, when it was identified in potato tubers (Steward *et al.*, 1949). In animals GABA, which is present in the brain, acts as an important neurotransmittor. It is synthesized in a short pathway called the GABA shunt (Bouché & Fromm, 2004, which in addition to animals can be found also in bacteria, fungi and plants. The pathway bypasses two steps of the tricarboxylic (TCA) cycle, hence the name GABA shunt. Several roles has been suggested for GABA and the GABA shunt in plants such as; contributing to the C:N balance, regulation of cytosolic pH, protection against oxidative stress and insects as well as a role in signaling and as an osmoregulator (Bouché & Fromm, 2004).

BABA transport

Early studies of BABA-IR found out that BABA can not only be taken up by the plant through the root system and the abaxial surface of a leaf, but can also be transported in both a basipetal and acropetal direction within the plant (Návarová *et al.*, 2012; Cohen *et al.*, 1999; Cohen & Gisi, 1994). Furthermore, studies conducted in tomato, tobacco and Arabidopsis, using ^{14}C-labeled BABA revealed that BABA not is metabolized within the plant (Jakab *et al.*, 2001; Cohen & Gisi, 1994). In a study by Cohen et al. (2010) the translocation of BABA correlated with the observed systemic resistance. BABA has recently also been found unmetabolized in a pea aphid that have been feeding on a bean treated with BABA and in the pea aphid parasitoid (Hodge *et al.*, 2011).

Mechanisms involved in BABA-IR

The mechanisms responsible for the obtained BABA-IR in plants remain unclear. Mechanisms such as reactive oxygen species, HR, callose deposition, lignin and PR-protein accumulation as well as biosynthesis of secondary metabolites, HR- and lignin-related enzymes and of enzymes related to plant secondary metabolism, have been reported to be involved in BABA-IR (Justyna & Ewa, 2013). Other parts of the plant defence with a documented role in BABA-IR are the hormone and amino acid signaling pathways (Justyna

& Ewa, 2013). However, BABA-induced defence mechanisms seem to a high degree be specific to the pathosystem. For instance the SA signaling pathway has been shown to be involved in BABA-IR against virus and biotrophic pathogens in plant species such as pepper, tomato and potato, all belonging to the solanaceae family (Eschen-Lippold *et al.*, 2010; Siegrist *et al.*, 2000; Hwang *et al.*, 1997). However, the JA signaling pathway, but not the SA and ABA (Abscicic acid), was active in BABA-IR against downey mildew in grapewine (Hamiduzzaman *et al.*, 2005). In Arabidopsis, ABA-dependent priming for callose has been demonstrated to be involved in BABA-IR against two necrotrophic pathogens, *Alternaria brassicicola* and *Plectosphaerella cucumerina,* but also SA-dependent signaling has been reported for BABA-IR in Arabidopsis, then against *P. syringae* DC3000 (Van der Ent et al., 2009; Ton & Mauch-Mani, 2004). Thus the impact of BABA on hormone signaling is complex and acts via interplay of several hormones.

Recently it has become clear that BABA also can cause major alterations in plant amino acid balance, induce stress-responsive energy sensor protein kinases, induce anthocyanin accumulation and reduce vegative growth in Arabidopsis (Wu *et al.*, 2010). Responses which all were restored or inhibited by L-Glutamine. These findings suggest that BABA prime plants by stress imprinting. The same group has also demonstrated that the BABA-responsive L-type lectin receptor kinase-VI.2 (LecRK-VI.2) is needed for full BABA-IR and priming of PTI in Arabidopsis (Singh *et al.*, 2012). Another suggested master regulator of BABA-induced priming in Arabidopsis is the putative aspartyl tRNA synthetase, IBI. This was suggested in a recent report by Luna and colleagues, whom also suggested that two separate pathways exist in Arabidopsis for control of BABA-IR by priming and BABA-induced stress (induced by high BABA concentrations) respectively (Luna *et al.*, 2013). Furthermore their preliminary results indicated that BABA-IR in tomato might be regulated in a similar manner.

Advances in proteomic techniques for quantitative protein identification have facilitated the search for proteins involved in BABA-IR. A proteomic study conducted in crabapple in 2009, compared changes induced by ABA and BABA treatment during drought stress (Macarisin *et al.*, 2009). Results revealed that BABA-IR against drought shared some patterns of protein expression with the ABA-treated sample, but some were unique to BABA. The BABA-primed drought tolerance in crabapple were also suggested to involve changes in cell wall enzymes and suppression of lignin pathway (Macarisin *et al.*, 2009). Jelonek and colleagues used a proteomic approach for identification of possible molecular markers for primed defence mediated by nitric oxide (NO) in potato (Arasimowicz-Jelonek *et al.*, 2012). This since an earlier study

by the same group showed that some inducers caused a rapid increase in NO synthesis in primed potato leaves (Floryszak-Wieczorek *et al.*, 2012). In the proteomic study, proteins induced in potato leaves after treatment with four different inducers, BABA, GABA, laminarin and 2, 6-dichloroizonicotinic acid (INA) and in potato leaves treated with the NO-donor, GSNO, were identified. Results from 2-DE analysis and mass spectrometry revealed accumulation of 25 proteins after treatment with the four inducers, 13 protein spots in common for all inducers and GSNO and five leaf proteins only induced by BABA and GSNO (Arasimowicz-Jelonek *et al.*, 2012). The last five were identified as glyceraldehyde 3-phosphate dehydrogenase (GAPDH), fructose-biphosphate aldolase, a chloroplastic oxygen-involving enhancer protein 2, a cytosolic nucleoside diphsophate kinase and a hypothetical protein.

Another comparative proteomics study between BABA and BTH, conducted in pea against *Uromyces pisi*, showed that BTH and BABA operated via different mechanisms (Barilli *et al.*, 2012). BABA activated proteins within the phenolic biosynthesis pathway, whereas BTH seemed to induce more defence- and stress-related proteins.

Costs and benefits related to plant growth and development

A recent publication reported a trade-off between yield-improved cultivars and the ability to mount induced resistance and further suggested that the different response between genotypes to inducers is due to domestication (Córdova-Campos *et al.*, 2012). In general direct activation of plant defence is associated with high allocation costs (Walters & Heil, 2007; van Hulten *et al.*, 2006). However, whether the induction will cost or not might depend on the concentration used. For instance, pearl millet and sunflower seeds treated with BABA, resulted in taller plants with higher fresh weight and larger leaf area as well as increased seed germination and seedling vigor, respectively (Nandeeshkumar *et al.*, 2009; Shailasree *et al.*, 2001). On the other hand, higher concentrations resulted in inhibited seed germination and inferior seedling condition. Studies conducted in potato, has shown to result in improved or unchanged tuber yield (Liljeroth *et al.*, 2010; Olivieri *et al.*, 2009). To confirm that BABA-IR not has a cost in growth and reproduction, experiments need to be conducted in a disease- and stress-free environment.

If the cost outweights the benefits of a constitutively activated defence may also depend on other factors than concentration. The magnitude of the disease pressure is an important factor, where the benefits from the obtained IR can be superior to the cost in growth and development in case of high disease pressure. In addition, it might be that a clonally propagated crop like potato suffers less from a potential cost than sexually propagated crops, since a cost

associated with seed production and germination not will be a problem. However, a cost in terms of tuber vitality can presently not been ruled out. The cost will also depend on the way and timing of application, where treatment of seed and younger plants more likely will suffer from a cost than older plants treated slightly before harvest.

2.6 Biosurfactants

Surfactants are amphiphilic molecules, which mean that they possess both hydrophilic and lipophilic properties. They adsorb preferentially at the interface between fluid phases (oil/water or air/water) and reduce surface (liquid-air) and interfacial (liquid-liquid) tension, thus allowing the two phases to mix and interact. This happens at surfactant concentrations above the critical micelle concentration (CMC), when micelles, bilayers and vesicles are formed (Pacwa-Plociniczak *et al.*, 2011) (Fig. 11). The CMC is affected by temperature, pH and ionic strength (Mulligan, 2005). Due to the surfactants foaming capacity and ability to reduce surface tension and facilitate solubility etc., synthetic surfactants are widely used in the industry as adhesives, emulsifiers, de-emulsifiers, penetrants and as flocculating, wetting and foaming agents (Mulligan & Gibbs, 2004).

Surfactants that are produced by microorganisms are named biosurfactants and are considered to have lower toxicity compared to many synthetic surfactants due to faster bio-degradability (Lin, 1996). Biosurfactants also have high specificity and can function under extreme conditions (Sachdev & Cameotra, 2013). For commercialization of biosurfactants there is a problem to obtain an economical large-scale production due to expensive substrates, low yields, unpure products and limited product concentrations (Makkar *et al.*, 2011). Wastes from the agricultural industry are considered to have great potential to be used as substrate to a relative low cost (Makkar *et al.*, 2011).

Biosurfactants have been shown to have many different roles in nature. For example they have a role in increasing surface area and bioavailability of hydrophobic water-insoluble substrates, binding of heavy metals, pathogenesis, antimicrobial activity, regulating the (de)-attachment of microorganisms to and from surfaces, emulsifier production, quorum sensing as well as a role as bioemulsifiers in biofilm (Ron & Rosenberg, 2001).

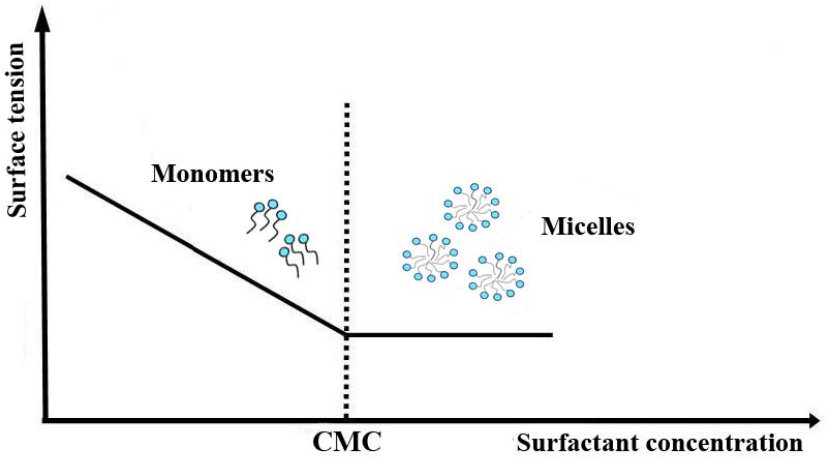

Figure 11. Graph over the relationship between surfactant concentration and surface tension. Surfactant monomers are gathered in micelles when the critical micelle concentration (CMC) is reached. The graph is adapted from Pacwa-Plociniczak et al., 2011 (http://creativecommons.org/licenses/by/3.0/).

2.6.1 Different biosurfactant classes and their role in disease control

Biosurfactants are divided in following groups: glycolipids, phospholipids, fatty acids, neutral lipids, lipopeptides, polymeric and particulate compounds (Mulligan, 2005) (Table 2). Some biosurfactants are low-molecular weight molecules that can lower surface and interfacial tension, whereas the high-molecular weight molecules can bind tightly to surfaces (Ron & Rosenberg, 2001).

One of the earliest reports of a role for biosurfactants in disease control came from Stanghellini and Miller (1997). In their study, rhamnolipids, a well-known group of biosurfactants, caused zoospore lysis by intercalation into the zoospore membrane. Rhamnolipids produced by *P. aeruginosa* have also been shown to induce resistance against *Botrytis cinerea* in grapevine and are characterized as MAMPs by Varnier et al. (2009). Other identified biosurfactants with ability to induce plant defence against pathogens are the lipopeptides, surfactin, fengycin and massetolide A (Jourdan *et al.*, 2009; Ongena *et al.*, 2007; Tran *et al.*, 2007). In the study by Tran et al. (2007), massetolide A purified from *P. fluorescens* SS101 and applied to the lower leaves of tomato, was able to induce systemic resistance against *P. infestans* by reducing the size of the lesions in the upper leaves.

The mechanisms behind biosurfactants elicitation of ISR in plants are still unknown, but it has been suggested that surfactin, from *Bacillus subtilis*, transiently disturb the plant plasma membrane rather than bind to a specific receptor, thereby triggering a defence response (Jourdan *et al.*, 2009).

Table 2. Biosurfactant classes[1]

Glycolipids	Fatty acids, phospholipids and neutral lipids	Lipopeptides	Polymeric compounds	Particulate compounds
Rhamnolipids	Corynomycolic acids	Surfactin	Emulsan	Vesicles
Trehalolipids	Spiculisporic acid	Lichenysin	Alasan	Whole microbial cells
Sophorolipids	Phosphatidylethanolamine	Massetolide A	Biodispersan	
Mannosylerythritol -lipids		Viscosin	Liposan	
		Serrawettin	Mannoprotein	
		Iturin		
		Fengycin		

1. Table adapted from Pacwa-Plociniczak et al., 2011 (http://creativecommons.org/licenses/by/3.0/).

3 Aim and objectives

The phenomenom of induced resistance (IR) is well known, but the mechanisms behind are still not fully understood. Most studies of IR have been conducted under controlled environments and it remains unclear whether the effect will be maintained in the field. One goal with this thesis was to investigate if integration of induced resistance to existing strategies could be a way to decrease the amount of fungicides needed for late blight control in the field. Another goal was to elucidate how the potato defence is affected by BABA on a molecular level. The more specific objectives were to:

- determine if BABA applied in combination with a fungicide could contribute to late blight control in field grown potato (Paper I).

- find out if application of a *Pseudomonas koreensis* strain or its biosurfactant could decrease late blight infection in detached potato leaves (Paper II).

- determine if BABA induced resistance in potato acts through priming or by direct activation of defence mechanisms (Paper III).

- find out if *P. koreensis* or its biosurfactant can prevent late blight infection of intact potato plants and if they can induce defence responses (Paper IV).

- obtain transcriptomic and proteomic data that could help to better understand the mechanisms behind BABA induced resistance in potato (Paper V).

4 BABA-IR to *P. infestans* in potato

4.1 The potential of combining BABA with fungicides to control late blight in potato (paper I)

Induced plant resistance against abiotic and biotic stress due to application of BABA is well studied under controlled environments in greenhouse, but only a few studies have been conducted in field (Table 2). More studies conducted in field are needed to find out if application of BABA or other inducing agents could be an alternative to fungicide application. Late blight is an extremely severe disease of potato and it is not likely that fungicides entirely could be exchanged with BABA for the purpose of late blight control, but by integrating BABA in existing management strategies, the total amount of fungicide could potentially be lowered.

The results from the field studies conducted in 2007 and 2008 revealed that weekly foliar application of BABA had a small effect on the late blight infection, with 1-3 days delay of the infection process. However, when BABA was combined with a 20-25% reduced dose of the fungicide Shirlan and applied weekly to the foliar in field, the effect was just as good as with full dose of Shirlan (Fig. 12).

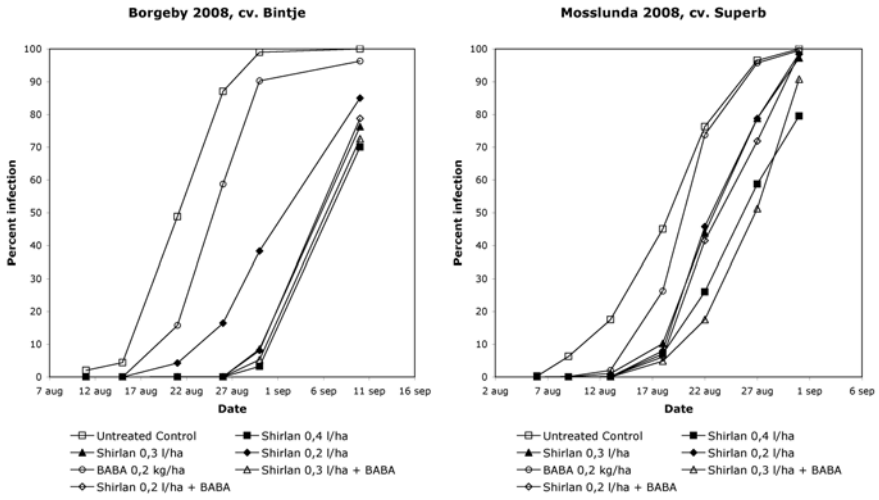

Figure 12. Development of *Phythophthora infestans* infections in field experiments conducted at two different locations with the potato cvs. Bintje and Superb. The plants were treated with different doses of Shirlan and BABA once per week. Reproduced from Liljeroth et al., 2010 with kind permission from Springer Science and Business media.

This effect, confirmed in greenhouse experiments, was shown to be additive rather than synergistic as reported of in other studies (Baider & Cohen, 2003). In the present greenhouse study foliar treatment was more efficient than soil application of BABA. Furthermore, results from both the field and greenhouse experiments, showed a clear dose-response effect and cultivar difference in response to BABA. The partially resistant cultivars responded better than the susceptible cultivar Bintje. However, since the susceptible cultivar Desiree also responded well to BABA as shown in paper V, the original level of resistance might not correlate with the level of IR obtained by BABA. Differences in inducibility between cultivars and independence of degree of partial resistance level has been reported for BABA in tomato (Sharma et al., 2010) and is something that must be considered when integrating IR in control strategies. Knowledge about varieties that respond differently to BABA, might reveal important mechanisms behind BABA inducibility.

The durability of the obtained disease control when applying IR is another important factor to consider. The greenhouse experiments showed that the observed diesease reduction, which where in the range of 40-50% by foliar treatment with, 10mM BABA, had a durability of 4-5 days before the effect declined. This has the consequence that BABA would need to be applied at least once per week, which is the normal praxis for fungicide application in

Sweden. Whether even more frequent applications of BABA to field grown potatoes would improve late blight control further, remains to be investigated.

One of the most important findings was that weekly application of BABA, at most 13 applications, did not cause any significant change in tuber yield. However, a metabolic cost caused by BABA cannot be ruled out. To do so, the experiment would need to be conducted in a totally disease free environment without outer stress exposure. However for potato, which is most often exposed for late blight, it is important that BABA treatment does not affect the yield in an environment with high disease pressure. Costs in terms of canopy growth or seed viability would be of less importance since potato is a clonally propagated crop.

A reduction of fungicide use by 20-25% may sound insignificant, but still means less fungicides spread in nature. One thing to keep in mind is that the field experiments are conducted under an extremely high infection pressure, due to rows with uncontrolled infector plants with the susceptible cultivar, Bintje, in the middle of the field (Fig. 13).

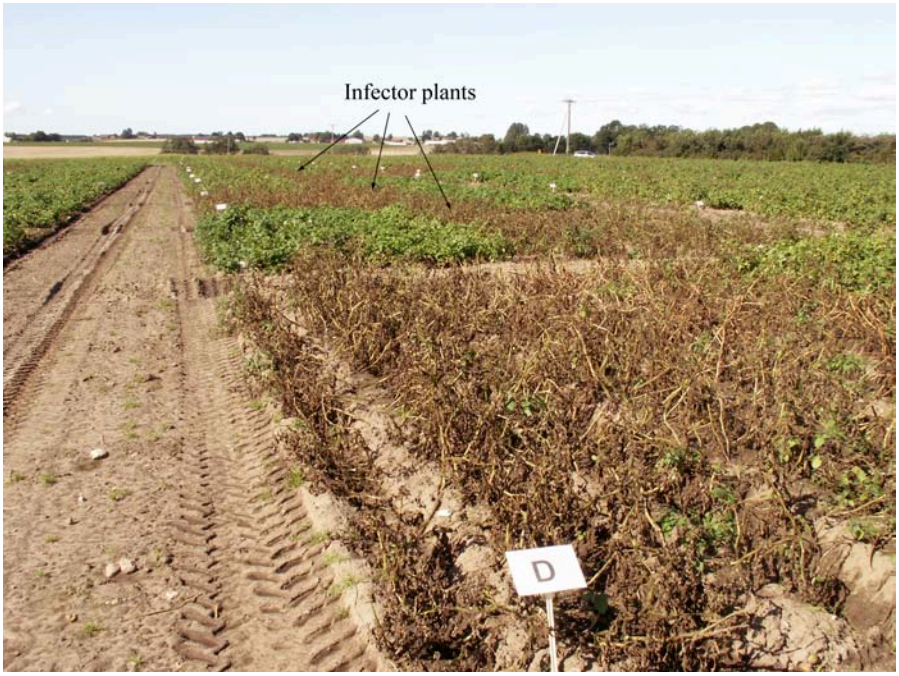

Figure 13. Photo taken at the field trial in Borgeby, August 2008. The arrows show the row with infector plants consisting of untreated plants of cv. Bintje. Photo: E. Liljeroth.

It is not unrealistic to speculate that the infection process could be delayed longer in a common agricultural field, where all plants are treated and only cultivars that respond well to BABA are used. Another observation was that BABA appeared to reduce sporulation of *P. infestans* in greenhouse trials. This effect was most probably diminished in the field trials due to high sporulation within the infector plants, but might have an impact on the epidemics in common agricultural fields.

Integration of IR, by e.g. BABA, in combination with the best responsive cultivars and with a lower fungicide concentration, may be a more durable protection strategy since that would take longer time for *P. infestans* to overcome.

4.2 Direct activation of basal defence mechanisms and HR-like lesions (paper III)

As demonstrated in the first study (paper I), BABA has the potential to induce resistance against *P. infestans* in potato both in greenhouse and in field, but the inducibility varies between cultivars. Even if BABA-IR is a well studied phenomenon (Table 1), the mechanisms involved still puzzles and has been shown to differ depending on the plant-pathosystems (Justyna & Ewa, 2013).

Microscopy, secretome and HPLC analysis of BABA-treated leaflets were performed in two potato cultivars (cv.) Bintje and Ovatio, during late blight infection. This in order to further understand how BABA affects basal defence responses such as HR, H_2O_2 production, PR-1 accumulation, callose deposition and phenol composition. As seen in the previous study (paper I) the well inducible cv. Ovatio developed small necrotic spots all over the leaves two days after foliar treatment with BABA, while no such lesions were observed on leaves of the less inducible cv. Bintje prior to infection (Fig. 14). The results from this study revealed that these necrotic spots resembles HR lesions with production of H_2O_2 within the epidermal cells, and consists of clusters of dead mesophyll cells surrounded by callose depositions. Interestingely, HR-like lesions were visible also in cv. Bintje 24 hours after detachment of the leaflets from the plant. In Bintje, H_2O_2 production and callose deposition within the HR-like lesions occured only when the leaflets subsequently were infected with *P. infestans,* in contrast to Ovatio where this response was induced irregardless of infection. Clusters with dead cells within the HR-like lesions were visible also in non-inoculated Bintje.

Figure 14. Potato leaves 48 h after treatment with 10 mM BABA, with HR-like lesions visible in cv. Ovatio. Left; cv. Bintje, right; cv. Ovatio. Photo: T. Bengtsson.

PR-1 occurred at a low basal level in both cultivars 48 h after treatment with water or BABA at concentrations below 10 mM. After treatment with the BABA concentration needed for effective late blight control in potato, 10 mM, PR-1 was further accumulated in the apoplast, to a higher degree in cv. Ovatio than in cv. Bintje. The response of phenolic compounds also varied between the two cultivars. In cv. Ovatio, the levels of arbutin and three chlorogenic acids significantly increased after BABA treatment, whereas in Bintje, BABA only caused a significant increase of arbutin. Subsequent late blight infection did not further affect the composition of phenolics.

As the results from this study implies, IR in potato by 10 mM BABA, seem to act through direct activation of the basal defence responses rather than through priming. This finding together with the development of HR-like lesions, leads to the question if the observed BABA-IR in potato simply is a result of BABA toxicity. It might be that it is a matter of dose-dependency. Higher concentration would most likely result in killing the plant, whereas a lower concentration will be just enough to trigger the plant to a defence "ready state"-mode.

In a recent performed experiment, HR-like lesions were observed macroscopically in 17 of 26 tested potato varieties two days after foliar treatment with 10 mM BABA (Table 3). The preliminary results suggest that the appearance of HR-like lesions do not correlate with the inherent level of partial resistance. Results from analysis by mass spectrometry of the secretome

from all of the mentioned 26 varieties will soon be available. Hopefully, these results can reveal protein changes in common for the varieties developing HR-like lesions. If the difference in inducibility between potato varieties depends on differences in sensitivity for stress, in basal defence mechanisms, in recognition of BABA or in morphology e.g. different leaf structure that may affect the uptake of BABA, remains to be investigated. The HR-like lesions are most likely not the main function, if they have any function at all in BABA-IR. However, they might correlate with a varieties's degree of inducibility. This is something that will be tested among the 26 varieties (Table 3). If so, it would facilitate the screening of varieties to be selected for IR-management in agriculture and/or future breeding of IR responsive cultivars.

4.3 BABA-induced changes of potato transcriptome and apoplast secretome (paper V)

This study of BABA-IR in potato indicates that BABA act through direct activation of defence responses in potato, and the effect depends on both variety and dose. The results also points to that BABA treatment could stress potato plants. The transcriptome and secretome of potato leaves of cv. Desiree treated with 1 or 10 mM BABA, was therefore analyzed in order to obtain insight in the molecular changes following treatment with BABA.

The 10 mM BABA treatment caused major changes in gene expression with 3272 up- and 2106 downregulated transcripts and also changes in the apoplastic protein abundance with 50 up and 41 downregulated. In contrast, only six transcripts and 24 proteins in total were affected by 1mM BABA and only one protein a mutT/nudix domain protein was upregulated by both concentrations. This low overlap between transcript regulation and apoplast protein abundance, confirms the value of using a combined approach in the search for molecular markers.

After 10 mM BABA a major accumulation of PR-proteins and changes within the amino acid and hormone metabolism were evident. A general amino acid stress induced by BABA has earlier been observed in Arabidopsis (Singh *et al.*, 2010) and present results suggest that this also occur in potato. Interestingly the sterol biosynthesis, part of the mevalonate pathway, were repressed whereas the sesquiterpene phytoalexin biosynthesis, another branch of the mevalonate pathway, was induced. A negative correlation between sterols and non-host resistance to *P. infestans* has recently been reported by Kopischke et al. (2012), thus this down-regulation might be a crucial step in BABA-IR against *P. infestans*. It might also play a role in BABA-IR against

Table 3. *Varieties treated with 10 mM BABA and screened for development of macroscopic HR-like lesions*

Resistance to late blight on foliage	Potato variety	Development of macroscopic HR-like lesions
High to very high[1,4]	Sarpo Mira	+
High to very high[1,4]	Toluca	-
High[3]	SW04-3262	+
High[3]	SW03-2402	-
High[3]	SW04-2662	-
Medium to high[2]	Ovatio	+
Medium to high[2]	Superb	+
Medium to high[3]	SW04-2081	-
Medium to high[4]	Tivoli	+
Medium[1]	Magnum Bonum	-
Medium[1]	Asterix	-
Medium[1,2,4]	Desiree	+
Medium[1,4]	Sava	+
Medium[3]	SW04-2669	+
Medium[4]	Jutlandia	-
Medium[4]	Hanna	+
Low to medium[1,4]	King Edward	+
Low to medium[3]	SW01-1224	+
Low to medium[3]	SW03-2385	-
Low to medium[4]	Fakse	+
Low[1,2]	Bintje	-
Information not found	Royal	+
Information not found	Senna	+
Information not found	Ballerina	+
Information not found	Maestro	+
Information not found	Vivi	+

1. Information retrieved from The European cultivated Potato Database.
2. Observations from our own performed detached leaf assays (paper I, V).
3. Results from field trials, personal communication Ulrika Carlson-Nilsson, SLU, Alnarp.
4. Information retrieved from www.Euroblight.net.

other oomycetes, since oomycetes are depending on their hosts for acquisition of sterol compounds essential for reproduction (Hendrix, 1970).

The major changes observed at the transcript level after treatment with 10 mM BABA might suggest yield penalty. However, no influence on potato yield, in terms of tuber yield, was observed in the previous study conducted in field (paper I). Thus, the results from this study might provide us with possible candidates or markers for improved resistance without major influence on potato yield.

5 The use of a biosurfactant for late blight control in potato (paper II, IV)

Treatment of potato leaves with *Pseudomonas koreensis* strain 2.74 or its biosurfactant 24 h prior to late blight inoculation resulted in statistically significant disease reduction. The biosurfactant was tested for the ability to inhibit mycelial growth of *P. infestans*. Results showed that only the highest concentration (1mg/ml) was toxic to *P. infestans* mycelia, and therefore other factors than mycelial inhibition most likely explain the significantly reduced infection obtained by the lower biosurfactant concentrations. No effect on sporangia production could be seen in pure culture after treatment with the biosurfactant.

Mixing the biosurfactant with the inoculum for 5 min before applying it to the detached potato leaves, resulted in a clear disease reduction. Thus the effect of the biosurfactant on zoospores was evident and the mechanism behind it might be destabilization of the zoospore membrane leading to lysis of the *P. infestans* zoospores similar as for *P. quercina* zoospores (unpublished results) (Figure 15).

In contrast to the study with detached leaflets, where both the bacteria strain and the biosurfactant had an effect on the late blight infection, only the biosurfactant was effective when using intact plants (paper IV). It could be that a higher bacterial concentration is needed when using intact plants. Analysis of the secretome in the apoplast of Ovatio showed that the biosurfactant also induced secretion of PR-1 and other unidentified proteins. Further identification and quantification of the secretome might reveal an answer to if the biosurfactant can induce other potato defence responses.

Taken together the results from experiments with *P. koreensis* and its biosurfactant again demonstrate the different responses among varieties and the importance to select the best suitable variety.

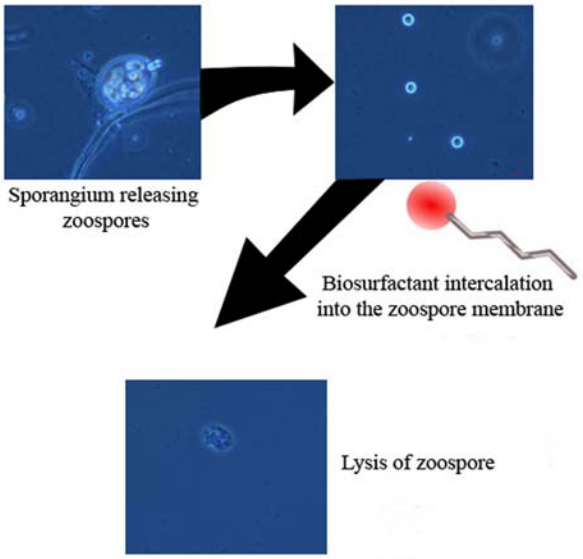

Figure 15. Illustration showing how the biosurfactant used in this study causes lysis of zoospores of *Phytophthora quercina, a pathogen to oak*. Illustration: M. Hultberg, Photos: K. Blümenstein.

6 Conclusions

Combining different control strategies in potato cultivation can result in lower fungicide usage without decreased efficacy in disease control.

- *BABA applied in combination with a fungicide can contribute to late blight control in field grown potato*
The late blight control in field was maintained with the 20-25% lower dose of fungicide, when it was combined with BABA. No cost penalty in terms of reduced tuber yields due to BABA application was observed. BABA applied to the potato foliage reduced late blight infection with up to 40% in detached leaf assays and were more efficient than soil application. The effect was both dose- and cultivar-dependent and lasted up to five days post treatment.

- *Treatment with 10 mM BABA activates defence responses in potato*
BABA treatment at a concentration of 10 mM, led to PR-1 accumulation within the apoplast and increased levels of phenolic substances. Treatment with 10 mM BABA also resulted in H_2O_2 formation and clusters of dead mesophyll cells within HR-like lesions, which were surrounded by callose. The impact of BABA on the potato defence responses differed between the two cultivars Ovatio and Binte, with more pronounced effects in cv. Ovatio.

- *BABA treatment causes a massive activation of the potato defence*
Treatment of the potato leaf canopy with 10 mM BABA resulted in more than 5000 differentially regulated transcripts and 90 proteins with a differentially changed abundance. In contrast only six transcripts and 24 proteins were differentially regulated by 1 mM BABA. Treatment with 10 mM BABA caused major changes in genes involved in hormone and amino acid metabolism and induction of several known PR-proteins. In addition several

transcripts within the sterol biosynthesis were downregulated. The only protein upregulated by both concentrations were a MutT/nudix domain protein.

- *The Pseudomonas koreensis strain 2.74 and its biosurfactant can decrease late blight infection in detached leaf assays*
Application of *P. koreensis* and its biosurfactant 24 h prior to infection caused significant reduction of late blight lesions on detached potato leaves. A clear disease reduction was also evident when mixing the inoculum with the biosurfactant during 5 min prior to infection, indicating a direct effect on the zoospores. No toxic effect of the biosurfactant was observed on *P. infestans* sporangia production in vitro and only the highest concentration (1mg/ml) significantly reduced mycelia growth rate. The obtained level of disease control varied between the cultivars.

- *The biosurfactant is able to decrease late blight infection and induce PR-1 accumulation within the leaf apoplast of intact potato plants in greenhouse*
Pretreatment of intact potato plants with the biosurfactant, but not with the bacteria strain, decreased late blight infection. In addition the biosurfactant showed an induced accumulation of PR-1 and other unidentified proteins in the apoplast of cv. Ovatio, indicating that the biosurfactant can activate basal defence responses in potato.

7 Future perspectives

Incorporation of IR to current disease management programmes by applying an inducing agent alone or in combination with a fungicide could be a more durable strategy with lower usage of fungicides. Late blight control today is dependent on frequent applications of fungicide. However, restrictions for fungicide use are becoming more constrained within EU, at the same time, as there is a prevailing problem with fungicide resistance. Thus alternative strategies are needed for late blight control in the future. IR could be an alternative, especially for IP-cultivation. BABA is presently an expensive chemical, but there are other abiotic inducing agents such as phosphite available on the market to a relatively low cost. Phosphite has been shown to have a good effect in field trials against *e.g.* potato late blight. Since direct activation of plant defence most often has a metabolic cost for the plant, it is important to find out if this could have a negative effect on the yield before integrating IR in a system. In the case of potato no yield penalty was seen in present field trials, maybe because the IR effect, *i.e.* decreased disease, overweighted the costs. However, possible yield penalties under disease free conditions need to be evaluated in the future.

Results from the transcriptome and secretome analysis provide a large resource to search for mechanisms responsible for the BABA-IR. For example, it would be interesting to further investigate the role of the MutT/nudix domain protein as well as of the sterol biosynthesis in BABA-IR against e.g. *P. infestans* in potato. In addition, the ongoing screening of the secretome of different cultivars treated with BABA will hopefully provide an important tool to find BABA-specific markers. It will be interesting to find out if the BABA-induced HR-like lesions can be used as a tool to find cultivars that respond well to BABA. This would be a cheap and easy way for cultivar selection. Future experiments to find out if breeding for yield-improved cultivars is reached at the expense of the potato plant's capacity to express IR as seen in

beans (Córdova-Campos *et al.*, 2012), would further help breeders with cultivar selection.

Evidence is pointing to an inheritance of BABA-IR to the progeny (Slaughter *et al.*, 2012). It would therefore be of high interest to investigate if IR applied in the production of seed tubers would have an effect on late blight infection in the subsequent crop.

The direct effect on zoospores, the possible capacity to mount defence responses to late blight in potato and the low toxicity of the biosurfactant makes it an interesting candidate for disease control. Future experiment conducted in field with applications of the biosurfactant alone or in combination with an inducing agent will determine if the biosurfactant has a future in agriculture.

Since IR is host-specific it is to a high degree influenced by the genotype and environment. Therefore, studies such as this are important to increase the knowledge of how IR can be used in practice and what factors should be taken into consideration. It is also important to prove to farmers that it is possible to reduce the amount of fungicide without affecting the efficacy of disease control or the yield. However, the economical gain from using IR is something that has to be proven before IR will become accepted as a regular crop protection strategy.

References

Altamiranda, E.A.G., Andreu, A.B., Daleo, G.R. & Olivieri, F.P. (2008). Effect of beta-aminobutyric acid (BABA) on protection against Phytophthora infestans throughout the potato crop cycle. *Australasian Plant Pathology* 37(4), 421-427.

Ames, M. & Spooner, D.M. (2008). DNA from herbarium specimens settles a controversy about origins of the European potato. *American Journal of Botany* 95(2), 252-257.

Andreu, A.B., Guevara, M.G., Wolski, E.A., Daleol, G.R. & Caldiz, D.O. (2006). Enhancement of natural disease resistance in potatoes by chemicals. *Pest Management Science* 62(2), 162-170.

Andrivon, D. (1995). Biology, ecology, epidemiology of the potato late blight pathogen Phytophthora infestans in soil. *Phytopathology* 85(10), 1053-1056.

Andrivon, D. (1996). The origin of Phytophthora infestans populations present in Europe in the 1840s: A critical review of historical and scientific evidence. *Plant Pathology (Oxford)* 45(6), 1027-1035.

Arasimowicz-Jelonek, M., Kosmala, A., Janus, Ł., Abramowski, D. & Floryszak-Wieczorek, J. (2012). The proteome response of potato leaves to priming agents and S-nitrosoglutathione. *Plant Science*.

Audenaert, K., Pattery, T., Cornelis, P. & Höfte, M. (2002). Induction of Systemic Resistance to Botrytis cinerea in Tomato by Pseudomonas aeruginosa 7NSK2: Role of Salicylic Acid, Pyochelin, and Pyocyanin. *Molecular Plant-Microbe Interactions* 15(11), 1147-1156.

Baider, A. & Cohen, Y. (2003). Synergistic interaction between BABA and mancozeb in controlling Phytophthora infestans in potato and tomato and Pseudoperonospora cubensis in cucumber. *Phytoparasitica* 31(4), 399-409.

Barilli, E., Prats, E. & Rubiales, D. (2010a). Benzothiadiazole and BABA improve resistance to Uromyces pisi (Pers.) Wint. in Pisum sativum L. with an enhancement of enzymatic activities and total phenolic content. *European Journal of Plant Pathology* 128(4), 483-493.

Barilli, E., Rubiales, D. & Castillejo, M.Á. (2012). Comparative proteomic analysis of BTH and BABA-induced resistance in pea (Pisum sativum) toward infection with pea rust (Uromyces pisi). *Journal of Proteomics*.

Barilli, E., Sillero, J.C. & Rubiales, D. (2010b). Induction of systemic acquired resistance in pea against rust (Uromyces pisi) by exogenous application of biotic and abiotic inducers. *Journal of Phytopathology* 158(1), 30-34.

Barrado, E., Rodriguez, J. & Castrillejo, Y. (2009). Determination of primary amino acids in wines by high performance liquid magneto-chromatography. *Talanta* 78(3), 672-675.

Baysal, Ö., Gürsoy, Y.Z., Örnek, H. & Duru, A. (2005). Induction of oxidants in tomato leaves treated with dl-β-amino butyric acid (BABA) and infected with Clavibacter michiganensis ssp. michiganensis. *European Journal of Plant Pathology* 112(4), 361-369.

Beckers, G.J.M., Jaskiewicz, M., Liu, Y., Underwood, W.R., He, S.Y., Zhang, S. & Conrath, U. (2009). Mitogen-Activated Protein Kinases 3 and 6 Are Required for Full Priming of Stress Responses in Arabidopsis thaliana. *The Plant Cell Online* 21(3), 944-953.

Bengtsson, T., Holefors, A., Witzell, J., Andreasson, E. & Liljeroth, E. (2013). Activation of defence responses to Phytophthora infestans in potato by BABA. *Plant Pathology*, DOI 10.1111/ppa.12069.

Bishop, J.G., Ripoll, D.R., Bashir, S., Damasceno, C.M.B., Seeds, J.D. & Rose, J.K.C. (2005). Selection on Glycine β -1,3-Endoglucanase Genes Differentially Inhibited by a Phytophthora Glucanase Inhibitor Protein. *Genetics* 169(2), 1009-1019.

Boller, T. & Felix, G. (2009). A Renaissance of Elicitors: Perception of Microbe-Associated Molecular Patterns and Danger Signals by Pattern-Recognition Receptors. *Annual Review of Plant Biology* 60(1), 379-406.

Bos, J.I.B., Armstrong, M.R., Gilroy, E.M., Boevink, P.C., Hein, I., Taylor, R.M., Zhendong, T., Engelhardt, S., Vetukuri, R.R., Harrower, B., Dixelius, C., Bryan, G., Sadanandom, A., Whisson, S.C., Kamoun, S. & Birch, P.R.J. (2010). Phytophthora infestans effector AVR3a is essential for virulence and manipulates plant immunity by stabilizing host E3 ligase CMPG1. *Proceedings of the National Academy of Sciences*.

Bostock, R.M. (2005). Signal Crosstalk and Induced Resistance: Straddling the Line Between Cost and Benefit. *Annual Review of Phytopathology* 43(1), 545-580.

Bouché, N. & Fromm, H. (2004). GABA in plants: just a metabolite? *Trends in plant science* 9(3), 110-115.

Brurberg, M.B., Elameen, A., Le, V.H., Nærstad, R., Hermansen, A., Lehtinen, A., Hannukkala, A., Nielsen, B., Hansen, J., Andersson, B. & Yuen, J. (2011). Genetic analysis of Phytophthora infestans populations in the Nordic European countries reveals high genetic variability. *Fungal Biology* 115(4–5), 335-342.

Camire, M.E., Kubow, S. & Donnelly, D.J. (2009). Potatoes and Human Health. *Critical Reviews in Food Science and Nutrition* 49(10), 823-840.

Champouret, N., Bouwmeester, K., Rietman, H., van der Lee, T., Maliepaard, C., Heupink, A., van de Vondervoort, P.J.I., Jacobsen, E., Visser, R.G.F., van der Vossen, E.A.G., Govers, F. & Vleeshouwers, V.G.A.A. (2009). Phytophthora infestans Isolates Lacking Class I ipiO Variants Are Virulent on Rpi-blb1 Potato. *Molecular Plant-Microbe Interactions* 22(12), 1535-1545.

Chamsai, J. (2004). Mode of action of the resistance-inducing 3-aminobutyric acid in tomato roots against Fusarium wilt. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 111(3), 273.

Chester, K.S. (1933). The Problem of Acquired Physiological Immunity in Plants. *The Quarterly Review of Biology* 8(3), 275-324.

Chinnasri, B., Sipes, B.S. & Schmitt, D.P. (2006). Effects of Inducers of Systemic Acquired Resistance on Reproduction of Meloidogyne javanica and Rotylenchulus reniformis in Pineapple. *J Nematol* 38(3), 319-25.

Chisholm, S.T., Coaker, G., Day, B. & Staskawicz, B.J. (2006). Host-Microbe Interactions: Shaping the Evolution of the Plant Immune Response. *Cell* 124(4), 803-814.

Cohen, Y. (1994a). 3-Aminobutyric acid induces systemic resistance against Peronospore tabacina. *Physiological and Molecular Plant Pathology* 44(4), 273-288.

Cohen, Y. (1994b). Local and systemic control of Phytophthora infestans in tomato plants by DL-3-amino-n-butanoic acids. *Phytopathology* 84(1), 55-59.

Cohen, Y., Baider, A., Gotlieb, D. & Rubin, E. (2007). Control of Bremia lactucae in field-grown lettuce by DL-3-amino-n-butanoic acid (BABA).

Cohen, Y. & Gisi, U. (1994). Systemic translocation of 14C-DL-3-aminobutyric acid in tomato plants in relation to induced resistance against Phytophthora infestans. *Physiological and Molecular Plant Pathology* 45(6), 441-456.

Cohen, Y., Niderman, T., Mosinger, E. & Fluhr, R. (1994). Beta-Aminobutyric acid induces the accumulation of pathogenesis-related proteins in tomato (Lycopersicon esculentum L.) plants and resistance to late blight infection caused by Phytophthora infestans. *Plant Physiology (Rockville)* 104(1), 59-66.

Cohen, Y., Reuveni, M. & Baider, A. (1999). Local and systemic activity of BABA (DL-3-aminobutyric acid) against Plasmopara viticola in grapevines. *European Journal of Plant Pathology* 105(4), 351-361.

Cohen, Y., Rubin, A. & Kilfin, G. (2010). Mechanisms of induced resistance in lettuce against Bremia lactucae by DL-β-amino-butyric acid (BABA). *European Journal of Plant Pathology* 126(4), 553-573.

Cohen, Y., Rubin, A.E. & Vaknin, M. (2011). Post infection application of DL-3-amino-butyric acid (BABA) induces multiple forms of resistance against Bremia lactucae in lettuce. *European Journal of Plant Pathology* 130(1), 13-27.

Cohen, Y.R. (2002). beta-aminobutyric acid-induced resistance against plant pathogens. *Plant Disease* 86(5), 448-457.

Conn, V.M., Walker, A.R. & Franco, C.M.M. (2008). Endophytic Actinobacteria Induce Defense Pathways in Arabidopsis thaliana. *Molecular Plant-Microbe Interactions* 21(2), 208-218.

Conrath, U. (2011). Molecular aspects of defence priming. *Trends in plant science* 16(10), 524-531.

Conrath, U., Beckers, G.J.M., Flors, V., García-Agustín, P., Jakab, G., Mauch, F., Newman, M.-A., Pieterse, C.M.J., Poinssot, B., Pozo, M.J., Pugin, A., Schaffrath, U., Ton, J., Wendehenne, D., Zimmerli, L. & Mauch-Mani, B. (2006). Priming: Getting Ready for Battle. *Molecular Plant-Microbe Interactions* 19(10), 1062-1071.

Córdova-Campos, O., Adame-Álvarez, R.M., Acosta-Gallegos, J.A. & Heil, M. (2012). Domestication affected the basal and induced disease resistance in common bean (Phaseolus vulgaris). *European Journal of Plant Pathology* 134(2), 367-379.

Damasceno, C.M.B., Bishop, J.G., Ripoll, D.R., Win, J., Kamoun, S. & Rose, J.K.C. (2008). Structure of the Glucanase Inhibitor Protein (GIP) Family from Phytophthora Species Suggests Coevolution with Plant Endo-β-1,3-Glucanases. *Molecular Plant-Microbe Interactions* 21(6), 820-830.

Dempsey, D.M.A. & Klessig, D.F. (2012). SOS – too many signals for systemic acquired resistance? *Trends in plant science* 17(9), 538-545.

Dou, D., Kale, S.D., Wang, X., Chen, Y., Wang, Q., Wang, X., Jiang, R.H.Y., Arredondo, F.D., Anderson, R.G., Thakur, P.B., McDowell, J.M., Wang, Y. & Tyler, B.M. (2008). Conserved C-Terminal Motifs Required for Avirulence and Suppression of Cell Death by Phytophthora sojae effector Avr1b. *The Plant Cell Online* 20(4), 1118-1133.

Du, Y.-L., Wang, Z.-Y., Fan, J.-W., Turner, N.C., Wang, T. & Li, F.-M. (2012). β-Aminobutyric acid increases abscisic acid accumulation and desiccation tolerance and decreases water use but fails to improve grain yield in two spring wheat cultivars under soil drying. *Journal of Experimental Botany* 63(13), 4849-4860.

Dubreuil-Maurizi, C., Trouvelot, S., Frettinger, P., Pugin, A., Wendehenne, D. & Poinssot, B. (2010). beta-Aminobutyric Acid Primes an NADPH Oxidase-Dependent Reactive Oxygen Species Production During Grapevine-Triggered Immunity. *Molecular Plant-Microbe Interactions* 23(8), 1012-1021.

Durrant, W.E. & Dong, X. (2004). SYSTEMIC ACQUIRED RESISTANCE. *Annual Review of Phytopathology* 42(1), 185-209.

Erwin, D.C. & Ribeiro, O.K. (1996). *Phytophthora diseases worldwide*. American Phytopathological Society, St. Paul, MN. ISBN 9780890542125.

Eschen-Lippold, L., Altmann, S. & Rosahl, S. (2010). dl-Œ≤-Aminobutyric Acid‚ÄìInduced Resistance of Potato Against Phytophthora infestans Requires Salicylic Acid but Not Oxylipins. *Molecular Plant-Microbe Interactions* 23(5), 585-592.

FAO *International Year of the Potato (IYP): Potato World*. [online] Available from: http://www.potato2008.org.

FAOSTAT [online] Available from: http://faostat3.fao.org.
Fatemy, S., Moslemi, F. & Bernard, F. (2012). Seed treatment and soil drench with dl-β-amino butyric acid for the suppression of Meloidogyne javanica on tomato. *Acta Physiologiae Plantarum* 34(6), 2311-2317.
Fay, J. & Fry, W. (1997). Effects of hot and cold temperatures on the survival of oospores produced by United States strains ofPhytophthora infestans. *American Potato Journal* 74(5), 315-323.
Fischer, M.J.C., Farine, S., Chong, J., Guerlain, P. & Bertsch, C. (2009). The direct toxicity of BABA against grapevine ecosystem organisms. *Crop Protection* 28(8), 710-712.
Flors, V., Ton, J., van Doorn, R., Jakab, G., Garcia-Agustin, P. & Mauch-Mani, B. (2008). Interplay between JA, SA and ABA signalling during basal and induced resistance against Pseudomonas syringae and Alternaria brassicicola. *Plant Journal* 54(1), 81-92.
Floryszak-Wieczorek, J., Arasimowicz-Jelonek, M., Milczarek, G., Janus, L., Pawlak-Sprada, S., Abramowski, D., Deckert, J. & Billert, H. (2012). Nitric Oxide-Mediated Stress Imprint in Potato as an Effect of Exposure to a Priming Agent. *Molecular Plant-Microbe Interactions* 25(11), 1469-1477.
Fry, W. (2008). Phytophthora infestans: the plant (and R gene) destroyer. *Molecular Plant Pathology* 9(3), 385-402.
Fu, Z.Q. & Dong, X. (2013). Systemic Acquired Resistance: Turning Local Infection into Global Defense. *Annual Review of Plant Biology* 64(1), 839-863.
Gamliel, A. & Katan, J. (1992). Influence of seed and root exudates on fluorescent pseudomonads and fungi in solarized soil. *Phytopathology* 82(3), 320-327.
Gilpatrick, J.D. & Weintraub, M. (1952). An Unusual Type of Protection with the Carnation Mosaic Virus. *Science* 115(3000), 701-702.
Gisi, U., Walder, F., Resheat-Eini, Z., Edel, D. & Sierotzki, H. (2011). Changes of Genotype, Sensitivity and Aggressiveness in Phytophthora infestans Isolates Collected in European Countries in 1997, 2006 and 2007. *Journal of Phytopathology* 159(4), 223-232.
Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. In: *Annual Review of Phytopathology*. pp. 205-227. Palo Alto: Annual Reviews. (Annual Review of Phytopathology; 43). ISBN 0066-4286978-0-8243-1343-2.
Gomez-Alpizar, L., Carbone, I. & Ristaino, J.B. (2007). An Andean origin of Phytophthora infestans inferred from mitochondrial and nuclear gene genealogies. *Proceedings of the National Academy of Sciences of the United States of America* 104(9), 3306-3311.
Goodwin, S.B., Cohen, B.A. & Fry, W.E. (1994). Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. *Proceedings of the National Academy of Sciences of the United States of America* 91(24), 11591-11595.

Grenville-Briggs, L.J., Avrova, A.O., Bruce, C.R., Williams, A., Whisson, S.C., Birch, P.R.J. & Van West, P. (2005). Elevated amino acid biosynthesis in Phytophthora infestans during appressorium formation and potato infection. *Fungal Genetics and Biology* 42(3), 244-256.

Grünwald, N.J. & Flier, W.G. (2005). The Biology of Phytophthora infestans at Its Center of Origin*. *Annual Review of Phytopathology* 43(1), 171-190.

Haas, B.J., Kamoun, S., Zody, M.C., Jiang, R.H.Y., Handsaker, R.E., Cano, L.M., Grabherr, M., Kodira, C.D., Raffaele, S., Torto-Alalibo, T., Bozkurt, T.O., Ah-Fong, A.M.V., Alvarado, L., Anderson, V.L., Armstrong, M.R., Avrova, A., Baxter, L., Beynon, J., Boevink, P.C., Bollmann, S.R., Bos, J.I.B., Bulone, V., Cai, G., Cakir, C., Carrington, J.C., Chawner, M., Conti, L., Costanzo, S., Ewan, R., Fahlgren, N., Fischbach, M.A., Fugelstad, J., Gilroy, E.M., Gnerre, S., Green, P.J., Grenville-Briggs, L.J., Griffith, J., Grunwald, N.J., Horn, K., Horner, N.R., Hu, C.-H., Huitema, E., Jeong, D.-H., Jones, A.M.E., Jones, J.D.G., Jones, R.W., Karlsson, E.K., Kunjeti, S.G., Lamour, K., Liu, Z., Ma, L., MacLean, D., Chibucos, M.C., McDonald, H., McWalters, J., Meijer, H.J.G., Morgan, W., Morris, P.F., Munro, C.A., O,ÄôNeill, K., Ospina-Giraldo, M., Pinzon, A., Pritchard, L., Ramsahoye, B., Ren, Q., Restrepo, S., Roy, S., Sadanandom, A., Savidor, A., Schornack, S., Schwartz, D.C., Schumann, U.D., Schwessinger, B., Seyer, L., Sharpe, T., Silvar, C., Song, J., Studholme, D.J., Sykes, S., Thines, M., van de Vondervoort, P.J.I., Phuntumart, V., Wawra, S., Weide, R., Win, J., Young, C., Zhou, S., Fry, W., Meyers, B.C., van West, P., Ristaino, J., Govers, F., Birch, P.R.J., Whisson, S.C., Judelson, H.S. & Nusbaum, C. (2009). Genome sequence and analysis of the Irish potato famine pathogen Phytophthora infestans. *Nature* 461(7262), 393-398.

Halim, V.A., Hunger, A., Macioszek, V., Landgraf, P., Nuernberger, T., Scheel, D. & Rosahl, S. (2004). The oligopeptide elicitor Pep-13 induces salicylic acid-dependent and -independent defense reactions in potato. *Physiological and Molecular Plant Pathology* 64(6), 311-318.

Halterman, D.A., Chen, Y., Sopee, J., Berduo-Sandoval, J. & Sánchez-Pérez, A. (2010). Competition between Phytophthora infestans Effectors Leads to Increased Aggressiveness on Plants Containing Broad-Spectrum Late Blight Resistance. *PLoS ONE* 5(5), e10536.

Hamiduzzaman, M.M., Jakab, G., Barnavon, L., Neuhaus, J.M. & Mauch-Mani, B. (2005). beta-Aminobutyric acid-induced resistance against downy mildew in grapevine acts through the potentiation of callose formation and jasmonic acid signaling. *Molecular Plant-Microbe Interactions* 18(8), 819-829.

Hassan, M. & Buchenauer, H. (2007). Induction of resistance to fire blight in apple by acibenzolar-S-methyl and DL-3-aminobutyric acid. *Journal of Plant Diseases and Protection* 114(4), 151-158.

Hassan, M. & Buchenauer, H. (2008). Enhanced control of bacterial wilt of tomato by DL-3-aminobutyric acid and the fluorescent Pseudomonas isolate CW2. *Journal of Plant Diseases and Protection (JPDP)* 5(115), 199-207.

Hassan, M.E. & Abo-Elyousr, K.M. (2013). Activation of tomato plant defence responses against bacterial wilt caused by Ralstonia solanacearum using DL-3-aminobutyric acid (BABA). *European Journal of Plant Pathology* 136(1), 145-157.

Haverkort, A.J., Boonekamp, P.M., Hutten, R., Jacobsen, E., Lotz, L.A.P., Kessel, G.J.T., Visser, R.G.F. & Vossen, E.A.G. (2008). Societal Costs of Late Blight in Potato and Prospects of Durable Resistance Through Cisgenic Modification. *Potato Research* 51(1), 47-57.

Haverkort, A.J., Struik, P.C., Visser, R.G.F. & Jacobsen, E. (2009). Applied Biotechnology to Combat Late Blight in Potato Caused by Phytophthora Infestans. *Potato Research* 52(3), 249-264.

Hawkes, J.G. & Francisco-Ortega, J. (1993). The early history of the potato in Europe. *Euphytica* 70(1-2), 1-7.

Hendrix, J.W. (1970). Sterols in Growth and Reproduction of Fungi. *Annual Review of Phytopathology* 8(1), 111-130.

Hillocks, R.J. (2012). Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. *Crop Protection* 31(1), 85-93.

Hodge, S., Pope, T.W., Holaschke, M. & Powell, G. (2006). The effect of β-aminobutyric acid on the growth of herbivorous insects feeding on Brassicaceae. *Annals of Applied Biology* 148(3), 223-229.

Hodge, S., Thompson, G.A. & Powell, G. (2005). Application of DL-β-aminobutyric acid (BABA) as a root drench to legumes inhibits the growth and reproduction of the pea aphid Acyrthosiphon pisum (Hemiptera: Aphididae). *Bulletin of Entomological Research* 95(05), 449-455.

Hodge, S., Ward, J., Galster, A., Beale, M. & Powell, G. (2011). The effects of a plant defence priming compound, β-aminobutyric acid, on multitrophic interactions with an insect herbivore and a hymenopterous parasitoid. *BioControl* 56(5), 699-711.

Holme, I.B., Wendt, T. & Holm, P.B. (2013). Intragenesis and cisgenesis as alternatives to transgenic crop development. *Plant Biotechnology Journal* 11(4), 395-407.

Hong, J.K., Hwang, B.K. & Kim, C.H. (1999). Induction of Local and Systemic Resistance to Colletotrichum coccodes in Pepper Plants by dl -β-Amino-n-Butyric Acid. *Journal of Phytopathology* 147(4), 193-198.

Hossain, Z., Makino, T. & Komatsu, S. (2012). Proteomic study of β-aminobutyric acid-mediated cadmium stress alleviation in soybean. *Journal of Proteomics* 75(13), 4151-4164.

Howden, A.J.M. & Huitema, E. (2012). Effector-triggered post-translational modifications and their role in suppression of plant immunity. *Frontiers in Plant Science* 3.

Hwang, B.K., Sunwoo, J.Y., Kim, Y.J. & Kim, B.S. (1997). Accumulation of Œ≤-1,3-glucanase and chitinase isoforms, and salicylic acid in the DL-Œ≤-amino-n-butyric acid-induced resistance response of pepper stems toPhytophthora capsici. *Physiological and Molecular Plant Pathology* 51(5), 305-322.

Ingle, R.A., Carstens, M. & Denby, K.J. (2006). PAMP recognition and the plant–pathogen arms race. *BioEssays* 28(9), 880-889.

Jacobsen, E. & Schouten, H. (2009). Cisgenesis: an important sub-invention for traditional plant breeding companies. *Euphytica* 170(1-2), 235-247.

Jakab, G., Cottier, V., Toquin, V., Rigoli, G., Zimmerli, L., Metraux, J.-P. & Mauch-Mani, B. (2001). beta-aminobutyric acid-induced resistance in plants. *European Journal of Plant Pathology* 107(1), 29-37.

Jeun, Y., Park, K., Kim, C., Fowler, W. & Kloepper, J. (2004). Cytological observations of cucumber plants during induced resistance elicited by rhizobacteria. *Biological Control* 29(1), 34-42.

Jeun, Y.C. & Buchenauer, H. (2001). Infection structures and localization of the pathogenesis-related protein AP24 in leaves of tomato plants exhibiting systemic acquired resistance against Phytophthora infestans after pre-treatment with 3-aminobutyric acid or tobacco necrosis virus. *Journal of Phytopathology (Berlin)* 149(3-4), 141-153.

Jeun, Y.C., Kim, K.W., Kim, K.D. & Hyun, J.W. (2007). Comparative Ultrastructure of Cucumbers Pretreated with Plant Growth-promoting Rhizobacteria, DL-3-aminobutyric Acid or Amino Salicylic Acid after Inoculation with Colletotrichum orbiculare. *Journal of Phytopathology* 155(7-8), 416-425.

Johnston, S.A., Nijs, T.P.M., Peloquin, S.J. & Hanneman, R.E., Jr. (1980). The significance of genic balance to endosperm development in interspecific crosses. *Theoretical and Applied Genetics* 57(1), 5-9.

Jones, J.D.G. & Dangl, J.L. (2006). The plant immune system. *Nature* 444(7117), 323-329.

Jourdan, E., Henry, G., Duby, F., Dommes, J., Barthélemy, J.-P., Thonart, P. & Ongena, M. (2009). Insights into the defense-related events occurring in plant cells following perception of surfactin-type lipopeptide from Bacillus subtilis. *Molecular Plant-Microbe Interactions* 22(4), 456-468.

Judelson, H.S. & Blanco, F.A. (2005). The spores of Phytophthora: weapons of the plant destroyer. *Nat Rev Micro* 3(1), 47-58.

Justyna, P.-G. & Ewa, K. (2013). Induction of resistance against pathogens by β-aminobutyric acid. *Acta Physiologiae Plantarum*, 1-14.

Kamble, A. & Bhargava, S. (2007). beta-aminobutyric acid-induced resistance in Brassica juncea against the necrotrophic pathogen Alternaria brassicae. *Journal of Phytopathology (Berlin)* 155(3), 152-158.

Kamble, A., Koopmann, B. & von Tiedemann, A. (2013). Induced resistance to Verticillium longisporum in Brassica napus by β-aminobutyric acid. *Plant Pathology* 62(3), 552-561.

Kamoun, S. (2006). A Catalogue of the Effector Secretome of Plant Pathogenic Oomycetes. *Annual Review of Phytopathology* 44(1), 41-60.

Kamoun, S., van West, P., Vleeshouwers, V.G.A.A., de Groot, K.E. & Govers, F. (1998). Resistance of Nicotiana benthamiana to Phytophthora infestans Is Mediated by the Recognition of the Elicitor Protein INF1. *The Plant Cell Online* 10(9), 1413-1425.

Kang, S.H., Cho, H., Cheong, H., Ryu, C., Kim, J.F. & Park, S. (2007). Two bacterial entophytes eliciting both plant growth promotion and plant defense on pepper (Capsicum annuum L.). *J Microbiol Biotechnol* 17(1), 96-103.

Karmakar, N., Ghosh, R. & Purkayastha, R. (2003). Plant defence activators inducing systemic resistance in Zingiber officinale Rosc. against Pythium aphanidermatum (Edson) Fitz. *Indian Journal of Biotechnology* 2(4), 591-595.

Kaschani, F., Shabab, M., Bozkurt, T., Shindo, T., Schornack, S., Gu, C., Ilyas, M., Win, J., Kamoun, S. & van der Hoorn, R.A.L. (2010). An Effector-Targeted Protease Contributes to Defense against Phytophthora infestans and Is under Diversifying Selection in Natural Hosts. *Plant Physiology* 154(4), 1794-1804.

Kim, H.-J. & Jeun, Y.-C. (2007). Infection structures on the infected leaves of potato pre-inoculated with bacterial strains and DL-3-amino butyric acid after challenge inoculation with Phytophthora infestans. *Plant Pathology Journal* 23(3), 203-209.

Kone, D., Csinos, A., Jackson, K. & Ji, P. (2009). Evaluation of systemic acquired resistance inducers for control of Phytophthora capsici on squash. *Crop Protection* 28(6), 533-538.

Kopischke, M., Westphal, L., Schneeberger, K., Clark, R., Ossowski, S., Wewer, V., Fuchs, R., Landtag, J., Hause, G., Dörmann, P., Lipka, V., Weigel, D., Schulze-Lefert, P., Scheel, D. & Rosahl, S. (2012). Impaired sterol ester synthesis alters the response of Arabidopsis thaliana to Phytophthora infestans. *The Plant Journal* 73(3), 456-468.

Kosaka, H., Aikawa, T., Ogura, N., Tabata, K. & Kiyohara, T. (2001). Pine Wilt Disease Caused by the Pine Wood Nematode: The Induced Resistance of Pine Trees by the Avirulent Isolates of Nematode. *European Journal of Plant Pathology* 107(7), 667-675.

Large, E.C. (1946). *The advance of the fungi*: Dover Publications.

Lazzarato, L., Trebbi, G., Pagnucco, C., Franchin, C., Torrigiani, P. & Betti, L. (2009). Exogenous spermidine, arsenic and beta-aminobutyric acid modulate tobacco resistance to tobacco mosaic virus, and affect local and systemic glucosylsalicylic acid levels and arginine decarboxylase gene expression in tobacco leaves. *Journal of Plant Physiology* 166(1), 90-100.

Lee, Y.K., Hong, J.K., Hippe-Sanwald, S. & Hwang, B.K. (2000). Histological and ultrastructural comparisons of compatible, incompatible and DL-β-amino-n-butyric acid-induced resistance responses of pepper stems to

Phytophthora capsici. *Physiological and Molecular Plant Pathology* 57(6), 269-280.

Liljeroth, E., Bengtsson, T., Wiik, L. & Andreasson, E. (2010). Induced resistance in potato to Phytphthora infestans-effects of BABA in greenhouse and field tests with different potato varieties. *European Journal of Plant Pathology* 127(2), 171-183.

Liljeroth, E., Burra, D. & Alexandersson, E. (2012). *Fosfit som tillsats i bladmögelbekämpningen.* Rapport från växtodlings- och växtskyddsdagar i Växjö den 4 och 5 december 2012. Meddelande från Södra jordbruksdistriktet, nr 65, 32:1-32:6. SLU-SJFD

Lin, S.C. (1996). Biosurfactants: recent advances. *Journal of Chemical Technology and Biotechnology* 66(2), 109-120.

Liu, T.W., Jiang, X.W., Shi, W.L., Chen, J., Pei, Z.M. & Zheng, H.L. (2011). Comparative proteomic analysis of differentially expressed proteins in beta-aminobutyric acid enhanced Arabidopsis thaliana tolerance to simulated acid rain. *Proteomics* 11(10), 2079-2094.

Liu, Z., Bos, J.I.B., Armstrong, M., Whisson, S.C., da Cunha, L., Torto-Alalibo, T., Win, J., Avrova, A.O., Wright, F., Birch, P.R.J. & Kamoun, S. (2005). Patterns of Diversifying Selection in the Phytotoxin-like scr74 Gene Family of Phytophthora infestans. *Molecular Biology and Evolution* 22(3), 659-672.

Lozano, R., Ponce, O., Ramirez, M., Mostajo, N. & Orjeda, G. (2012). Genome-Wide Identification and Mapping of NBS-Encoding Resistance Genes in Solanum tuberosum Group Phureja. *PLoS ONE* 7(4), e34775.

Luna, E., Bruce, T.J.A., Roberts, M.R., Flors, V. & Ton, J. (2012). Next-Generation Systemic Acquired Resistance. *Plant Physiology* 158(2), 844-853.

Luna, E., Zhang, Y., van Hulten, M., Gamir, J., Berkowitz, O., Pastor, V., Kegge, W., Acoska, D., Van de Meere, A., Pieterse, C.M.J., Flors, V. & Ton, J. IBI1: Master regulator of ß-amino butyric acid-induced priming. In: *Proceedings of IOBC-WPRS: Induced resistance in plants against insects and diseases.* 2013. pp. 37-39.

Macarisin, D., Wisniewski, M.E., Bassett, C. & Thannhauser, T.W. (2009). Proteomic analysis of β-aminobutyric acid priming and abscisic acid – induction of drought resistance in crabapple (Malus pumila): effect on general metabolism, the phenylpropanoid pathway and cell wall enzymes. *Plant, Cell & Environment* 32(11), 1612-1631.

Makkar, R.S., Cameotra, S.S. & Banat, I.M. (2011). Advances in utilization of renewable substrates for biosurfactant production. *AMB Express* 1(1), 1-19.

Marcucci, E., Aleandri, M.P., Chilosi, G. & Magro, P. (2010). Induced Resistance by β-Aminobutyric Acid in Artichoke against White Mould Caused by Sclerotinia sclerotiorum. *Journal of Phytopathology* 158(10), 659-667.

Mayton, H., Smart, C.D., Moravec, B.C., Mizubuti, E.S.G., Muldoon, A.E. & Fry, W.E. (2000). Oospore Survival and Pathogenicity of Single Oospore

Recombinant Progeny from a Cross Involving US-17 and US-8 Genotypes of Phytophthora infestans. *Plant Disease* 84(11), 1190-1196.
McHale, L., Tan, X., Koehl, P. & Michelmore, R. (2006). Plant NBS-LRR proteins: adaptable guards. *Genome Biology* 7(4), 212.
Mersha, Z., Zhang, S., Fu, Y., Mo, X., Raid, R.N. & Hau, B. (2013). Efficacy of Acibenzolar-S-Methyl and β-Aminobutyric Acid for Control of Downy Mildew in Greenhouse Grown Basil and Peroxidase Activity in Response to Treatment with these Compounds. *Journal of Phytopathology* 161(3), 154-164.
Mikes, V., Milat, M.L., Ponchet, M., Panabieres, F., Ricci, P. & Blein, J.P. (1998). Elicitins, proteinaceous elicitors of plant defense, are a new class of sterol carrier proteins. *Biochem Biophys Res Commun* 245(1), 133-9.
Mulligan, C.N. (2005). Environmental applications for biosurfactants. *Environmental Pollution* 133(2), 183-198.
Mulligan, C.N. & Gibbs, B.F. (2004). Types, production and applications of biosurfactants. *Proceedings- Indian National Science Academy Part B* 70(1), 31-56.
Nandeeshkumar, P., Sarosh, B.R., Kini, K.R., Prakash, H.S. & Shetty, H.S. (2009). Elicitation of resistance and defense related proteins by -amino butyric acid in sunflower against downy mildew pathogen Plasmopara halstedii. *Archives of Phytopathology and Plant Protection* 42(11), 1020-1032.
Návarová, H., Bernsdorff, F., Döring, A.-C. & Zeier, J. (2012). Pipecolic acid, an endogenous mediator of defense amplification and priming, is a critical regulator of inducible plant immunity. *The Plant Cell Online* 24(12), 5123-5141.
Niederhauser, J.S. (1991). *Phytophthora infestans: The Mexican connection*. Lucas, JA, Shattock, RC, Shaw, DS & Cooke, LR (eds.) Phytophthora. Cambridge, UK Cambridge University Press. ISBN 9780521400800.
Oka, Y., Cohen, Y. & Spiegel, Y. (1999). Local and systemic induced resistance to the root-knot nematode in tomato by DL-beta-amino-n-butyric acid. *Phytopathology* 89(12), 1138-1143.
Olivieri, F.P., Lobato, M.C., Gonzalez Altamiranda, E., Daleo, G.R., Huarte, M., Guevara, M.G. & Andreu, A.B. (2009). BABA effects on the behaviour of potato cultivars infected by Phytophthora infestans and Fusarium solani. *European Journal of Plant Pathology* 123(1), 47-56.
Ongena, M., Jourdan, E., Adam, A., Paquot, M., Brans, A., Joris, B., Arpigny, J.L. & Thonart, P. (2007). Surfactin and fengycin lipopeptides of Bacillus subtilis as elicitors of induced systemic resistance in plants. *Environmental Microbiology* 9(4), 1084-1090.
Osvald, H. (1965). *Potatisen: Odlingshistoria och användning*: Stockholm, Natur och Kultur.
Pacwa-Plociniczak, M., Plaza, G.A., Piotrowska-Seget, Z. & Cameotra, S.S. (2011). Environmental applications of biosurfactants: recent advances. *Int J Mol Sci* 12(1), 633-54.

Pajerowska-Mukhtar, K.M., Emerine, D.K. & Mukhtar, M.S. (2013). Tell me more: roles of NPRs in plant immunity. *Trends in plant science*.

Pajerowska-Mukhtar, Karolina M., Wang, W., Tada, Y., Oka, N., Tucker, Chandra L., Fonseca, Jose P. & Dong, X. (2012). The HSF-like Transcription Factor TBF1 Is a Major Molecular Switch for Plant Growth-to-Defense Transition. *Current Biology* 22(2), 103-112.

Papavizas, G. & Davey, C. (1963). Effect of amino compounds and related substances lacking sulfur on Aphanomyces root rot of peas. *Phytopathology* 53, 116-122.

Perfect, S.E. & Green, J.R. (2001). Infection structures of biotrophic and hemibiotrophic fungal plant pathogens. *Molecular Plant Pathology* 2(2), 101-108.

Pfautsch, S., Gessler, A., Adams, M.A. & Rennenberg, H. (2009). Using amino-nitrogen pools and fluxes to identify contributions of understory Acacia spp. to overstory Eucalyptus regnans and stand nitrogen uptake in temperate Australia. *New Phytologist* 183(4), 1097-1113.

Pieterse, C.M.J., van Wees, S.C.M., van Pelt, J.A., Knoester, M., Laan, R., Gerrits, H., Weisbeek, P.J. & van Loon, L.C. (1998). A Novel Signaling Pathway Controlling Induced Systemic Resistance in Arabidopsis. *The Plant Cell Online* 10(9), 1571-1580.

Pinto, K., do Nascimento, L., de Souza Gomes, E., da Silva, H. & dos Reis Miranda, J. (2012). Efficiency of resistance elicitors in the management of grapevine downy mildew Plasmopara viticola: epidemiological, biochemical and economic aspects. *European Journal of Plant Pathology* 134(4), 745-754.

Po-Wen, C., Singh, P. & Zimmerli, L. (2013). Priming of the Arabidopsis pattern-triggered immunity response upon infection by necrotrophic Pectobacterium carotovorum bacteria. *Molecular Plant Pathology* 14(1), 58-70.

Porat, R., Vinokur, V., Weiss, B., Cohen, L., Daus, A., Goldschmidt, E. & Droby, S. (2003). Induction of Resistance to Penicillium digitatum in Grapefruit by β-Aminobutyric Acid. *European Journal of Plant Pathology* 109(9), 901-907.

Prokop, S. & Albert, J. *International Year of the Potato: Potatoes, nutrition and diet*. [online] Available from: http://www.potato2008.org/.

Ramírez, V., López, A., Mauch-Mani, B., Gil, M.J. & Vera, P. (2013). An Extracellular Subtilase Switch for Immune Priming in Arabidopsis. *PLoS Pathog* 9(6), e1003445.

Ray, R., Ghosh, A., Bera, A., Dutta, N., Chattopadhyay, D.J. & Chakrabarti, K. (2011). Analysis of differentially expressed transcripts in jute upon fungal infection and beta-amino butyric acid treatment. *Physiological and Molecular Plant Pathology* 76(1), 59-66.

Reuveni, M., Sheglov, D. & Cohen, Y. (2003). Control of moldy-core decay in apple fruits by β-aminobutyric acids and potassium phosphites. *Plant Disease* 87(8), 933-936.

Reuveni, M., Zahavi, T. & Cohen, Y. (2001). Controlling downy mildew (Plasmopara viticola) in field-grown grapevine with β-aminobutyric acid (BABA). *Phytoparasitica* 29(2), 125-133.

Rios, D., Ghislain, M., Rodriguez, F. & Spooner, D.M. (2007). What is the origin of the European potato? Evidence from Canary Island landraces. *Crop Science* 47(3), 1271-1280.

Ron, E.Z. & Rosenberg, E. (2001). Natural roles of biosurfactants. *Environmental Microbiology* 3(4), 229-236.

Ross, A.F. (1961). Systemic acquired resistance induced by localized virus infections in plants. *Virology* 14((3)), 340-358.

Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.-Y. & Hunt, M.D. (1996). Systemic acquired resistance. *Plant Cell* 8(10), 1809-1819.

Sachdev, D.P. & Cameotra, S.S. (2013). Biosurfactants in agriculture. *Appl Microbiol Biotechnol* 97(3), 1005-16.

Sahebani, N. & Hadavi, N. (2009). Induction of H2O2 and related enzymes in tomato roots infected with root knot nematode (M. javanica) by several chemical and microbial elicitors. *Biocontrol Science and Technology* 19(3), 301-313.

Sahebani, N., Najmeh Sadat, H. & Fahimeh Omran, Z. (2010). The effects of β-amino-butyric acid on resistance of cucumber against root-knot nematode, Meloidogyne javanica. *Acta Physiologiae Plantarum*.

Šašek, V., Nováková, M., Dobrev, P., Valentová, O. & Burketová, L. (2012). β-aminobutyric acid protects Brassica napus plants from infection by Leptosphaeria maculans. Resistance induction or a direct antifungal effect? *European Journal of Plant Pathology* 133(1), 279-289.

SCB *Statistiska meddelanden (2011). Plant protection products in agriculture and horticulture. Use in crops.* [online] Available from: http://www.scb.se/.

SCB *Statistiska meddelanden (2012). Plant protection products in Swedish agriculture. Number of hectare-doses in 2011. Final statistics.* [online] Available from: http://www.scb.se/.

Schornack, S., Huitema, E., Cano, L.M., Bozkurt, T.O., Oliva, R., Van Damme, M., Schwizer, S., Raffaele, S., Chaparro-Garcia, A., Farrer, R., Segretin, M.E., Bos, J., Haas, B.J., Zody, M.C., Nusbaum, C., Win, J.O.E., Thines, M. & Kamoun, S. (2009). Ten things to know about oomycete effectors. *Molecular Plant Pathology* 10(6), 795-803.

Shah, J. & Chaturvedi, R. (2013). Long-Distance Signaling in Systemic Acquired Resistance. In: Baluška, F. (Ed.) *Long-Distance Systemic Signaling and Communication in Plants*. pp. 1-21 Springer Berlin Heidelberg. (Signaling and Communication in Plants; 19). ISBN 978-3-642-36469-3.

Shailasree, S., Ramachandra, K.K. & Shetty, S.H. (2007). β-Amino butyric acid-induced resistance in pearl millet to downy mildew is associated with accumulation of defence-related proteins. *Australasian Plant Pathology* 36(2), 204-211.

Shailasree, S., Sarosh, B.R., Vasanthi, N.S. & Shetty, H.S. (2001). Seed treatment with β-aminobutyric acid protects Pennisetum glaucum systemically from Sclerospora graminicola. *Pest Management Science* 57(8), 721-728.

Sharifi-Sirchi, G., Beheshti, B., Hosseinipour, A. & Mansouri, M. (2011). Priming against Asiatic citrus canker and monitoring of PR genes expression during resistance induction. *African Journal of Biotechnology* 10(19), 3818-3823.

Sharma, K., Butz, A.F. & Finckh, M.R. (2010). Effects of host and pathogen genotypes on inducibility of resistance in tomato (Solanum lycopersicum) to Phytophthora infestans. *Plant Pathology* 59(6), 1062-1071.

Shiu, S.-H. & Bleecker, A.B. (2003). Expansion of the Receptor-Like Kinase/Pelle Gene Family and Receptor-Like Proteins in Arabidopsis. *Plant Physiology* 132(2), 530-543.

Shoresh, M., Harman, G.E. & Mastouri, F. (2010). Induced Systemic Resistance and Plant Responses to Fungal Biocontrol Agents. *Annual Review of Phytopathology* 48(1), 21-43.

Siegrist, J., Orober, M. & Buchenauer, H. (2000). β-Aminobutyric acid-mediated enhancement of resistance in tobacco to tobacco mosaic virus depends on the accumulation of salicylic acid. *Physiological and Molecular Plant Pathology* 56(3), 95-106.

Silué, D., Pajot, E. & Cohen, Y. (2002). Induction of resistance to downy mildew (Peronospora parasitica) in cauliflower by DL-β-amino-n-butanoic acid (BABA). *Plant Pathology* 51(1), 97-102.

Singh, P., Kuo, Y.C., Mishra, S., Tsai, C.H., Chien, C.C., Chen, C.W., Desclos-Theveniau, M., Chu, P.W., Schulze, B., Chinchilla, D., Boller, T. & Zimmerli, L. (2012). The Lectin Receptor Kinase-VI.2 Is Required for Priming and Positively Regulates Arabidopsis Pattern-Triggered Immunity. *Plant Cell* 24(3), 1256-1270.

Singh, P., Wu, C.C. & Zimmerli, L. (2010). beta-aminobutyric acid priming by stress imprinting. *Plant Signal Behav* 5(7), 878-80.

Slaughter, A., Daniel, X., Flors, V., Luna, E., Hohn, B. & Mauch-Mani, B. (2012). Descendants of Primed Arabidopsis Plants Exhibit Resistance to Biotic Stress. *Plant Physiology* 158(2), 835-843.

Slaughter, A.R., Hamiduzzaman, M.M., Gindro, K., Neuhaus, J.-M. & Mauch-Mani, B. (2008). Beta-aminobutyric acid-induced resistance in grapevine against downy mildew: involvement of pterostilbene. *European Journal of Plant Pathology* 122(1), 185-195.

Song, J., Bradeen, J.M., Naess, S.K., Raasch, J.A., Wielgus, S.M., Haberlach, G.T., Liu, J., Kuang, H., Austin-Phillips, S., Buell, C.R., Helgeson, J.P. & Jiang, J. (2003). Gene RB cloned from Solanum bulbocastanum confers broad spectrum resistance to potato late blight. *Proceedings of the National Academy of Sciences* 100(16), 9128-9133.

Song, J., Win, J., Tian, M., Schornack, S., Kaschani, F., Ilyas, M., van der Hoorn, R.A.L. & Kamoun, S. (2009). Apoplastic effectors secreted by two unrelated eukaryotic plant pathogens target the tomato defense protease

Rcr3. *Proceedings of the National Academy of Sciences* 106(5), 1654-1659.

Spoel, S.H. & Dong, X. (2012). How do plants achieve immunity? Defence without specialized immune cells. *Nat Rev Immunol* 12(2), 89-100.

Spooner, D.M., McLean, K., Ramsay, G., Waugh, R. & Bryan, G.J. (2005). A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. *Proceedings of the National Academy of Sciences of the United States of America* 102(41), 14694-14699.

Stanghellini, M.E. & Miller, R.M. (1997). Their identity and potential efficacy in the biological control of zoosporic plant pathogens. *Plant Disease* 81(1), 4-12.

Steward, G.R., J.F, T. & CE, D. (1949). Gamma-Aminobutyric acid: a constituent of the potato tuber? *Science* 110, 439-440.

Sunwoo, J.Y., Lee, Y.K. & Hwang, B.K. (1996). Induced resistance againstPhytophthora capsici in pepper plants in response to DL-β-amino-n-butyric acid. *European Journal of Plant Pathology* 102(7), 663-670.

Tan, M.Y.A., Hutten, R.C.B., Visser, R.G.F. & van Eck, H.J. (2010). The effect of pyramiding Phytophthora infestans resistance genes R (Pi-mcd1) and R (Pi-ber) in potato. *Theoretical and Applied Genetics* 121(1), 117-125.

Tavallali, V., Karimi, S., Mohammadi, S. & Hojati, S. (2008). Effects of-aminobutyric Acid on the Induction of Resistance to Penicillium italicum. *World Applied Sciences Journal* 5(3), 345-351.

Tian, M., Benedetti, B. & Kamoun, S. (2005). A Second Kazal-Like Protease Inhibitor from Phytophthora infestans Inhibits and Interacts with the Apoplastic Pathogenesis-Related Protease P69B of Tomato. *Plant Physiology* 138(3), 1785-1793.

Tian, M., Huitema, E., da Cunha, L., Torto-Alalibo, T. & 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(25), 26370-26377.

Tian, M., Win, J., Song, J., van der Hoorn, R., van der Knaap, E. & Kamoun, S. (2007). A Phytophthora infestans Cystatin-Like Protein Targets a Novel Tomato Papain-Like Apoplastic Protease. *Plant Physiology* 143(1), 364-377.

Tiwari, S., Meyer, W.L. & Stelinski, L.L. (2013). Induced resistance against the Asian citrus psyllid, Diaphorina citri, by beta-aminobutyric acid in citrus. *Bull Entomol Res*, 1-9.

Tjamos, S.E., Flemetakis, E., Paplomatas, E.J. & Katinakis, P. (2005). Induction of Resistance to Verticillium dahliae in Arabidopsis thaliana by the Biocontrol Agent K-165 and Pathogenesis-Related Proteins Gene Expression. *Molecular Plant-Microbe Interactions* 18(6), 555-561.

Ton, J., Jakab, G., Toquin, V., Flors, V., Iavicoli, A., Maeder, M.N., Metraux, J.-P. & Mauch-Mani, B. (2005). Dissecting the beta-aminobutyric acid-induced priming phenomenon in arabidopsis. *Plant Cell* 17(3), 987-999.

Ton, J. & Mauch-Mani, B. (2004). beta-amino-butyric acid-induced resistance against necrotrophic pathogens is based on ABA-dependent priming for callose. *Plant Journal* 38(1), 119-130.

Torto, T.A., Li, S., Styer, A., Huitema, E., Testa, A., Gow, N.A.R., West, P.v. & Kamoun, S. (2003). EST Mining and Functional Expression Assays Identify Extracellular Effector Proteins From the Plant Pathogen Phytophthora. *Genome Research* 13(7), 1675-1685.

Tosi, L., Luigetti, R. & Zazzerini, A. (1998). Induced Resistance Against Plasmopara helianthi in Sunflower Plants by DL-β-Amino-n-butyric acid. *Journal of Phytopathology* 146(5-6), 295-299.

Tosi, L. & Zazzerini, A. (2000). Interactions between Plasmopara helianthi, Glomus mosseae and Two Plant Activators in Sunflower Plants. *European Journal of Plant Pathology* 106(8), 735-744.

Tran, H., Ficke, A., Asiimwe, T., Hoefte, M. & Raaijmakers, J.M. (2007). Role of the cyclic lipopeptide massetolide A in biological control of Phytophthora infestans and in colonization of tomato plants by Pseudomonas fluorescens. *New Phytologist* 175(4), 731-742.

Tucker, S.L. & Talbot, N.J. (2001). Surface attachment and pre-penetration stage development by plant pathogenic fungi. *Annual Review of Phytopathology* 39(1), 385-417.

Umaerus, V. & Umaerus, M. (1994). Inheritance of resistance to late blight. *Potato genetics.*, 365-401.

van Damme, M., Bozkurt, T.O., Cakir, C., Schornack, S., Sklenar, J., Jones, A.M.E. & Kamoun, S. (2012). The Irish Potato Famine Pathogen Phytophthora infestans Translocates the CRN8 Kinase into Host Plant Cells. *PLoS Pathog* 8(8), e1002875.

van de Mortel, J.E., de Vos, R.C.H., Dekkers, E., Pineda, A., Guillod, L., Bouwmeester, K., van Loon, J.J.A., Dicke, M. & Raaijmakers, J.M. (2012). Metabolic and Transcriptomic Changes Induced in Arabidopsis by the Rhizobacterium Pseudomonas fluorescens SS101. *Plant Physiology* 160(4), 2173-2188.

van der Ent, S., Van Hulten, M., Pozo, M.J., Czechowski, T., Udvardi, M.K., Pieterse, C.M.J. & Ton, J. (2009). Priming of plant innate immunity by rhizobacteria and β -aminobutyric acid: differences and similarities in regulation. *New Phytologist* 183(2), 419-431.

van der Plank, J.E. (1963). *Plant diseases: epidemics and control.* Academic Press, New York.

van der Vossen, E., Sikkema, A., Hekkert, B.t.L., Gros, J., Stevens, P., Muskens, M., Wouters, D., Pereira, A., Stiekema, W. & Allefs, S. (2003). An ancient R gene from the wild potato species Solanum bulbocastanum confers broad-spectrum resistance to Phytophthora infestans in cultivated potato and tomato. *The Plant Journal* 36(6), 867-882.

van der Vossen, E.A.G., Gros, J., Sikkema, A., Muskens, M., Wouters, D., Wolters, P., Pereira, A. & Allefs, S. (2005). The Rpi-blb2 gene from Solanum bulbocastanum is an Mi-1 gene homolog conferring broad-

spectrum late blight resistance in potato. *The Plant Journal* 44(2), 208-222.

van Hulten, M., Pelser, M., van Loon, L.C., Pieterse, C.M.J. & Ton, J. (2006). Costs and benefits of priming for defense in Arabidopsis. *Proceedings of the National Academy of Sciences* 103(14), 5602-5607.

van Loon, L.C. (2007). Plant responses to plant growth-promoting rhizobacteria. *European Journal of Plant Pathology* 119(3), 243-254.

van Loon, L.C., Bakker, P.A.H.M. & Pieterse, C.M.J. (1998). Systemic resistance induced by rhizosphere bacteria. *Annual Review of Phytopathology* 36(1), 453-483.

van Oosten, V.R., Bodenhausen, N., Reymond, P., Van Pelt, J.A., Van Loon, L.C., Dicke, M. & Pieterse, C.M.J. (2008). Differential Effectiveness of Microbially Induced Resistance Against Herbivorous Insects in Arabidopsis. *Molecular Plant-Microbe Interactions* 21(7), 919-930.

Varnier, A.-L., Sanchez, L., Vatsa, P., Boudesocque, L., Garcia-Brugger, A., Rabenoelina, F., Sorokin, A., Renault, J.-H., Kauffmann, S., Pugin, A., Clement, C., Baillieul, F. & Dorey, S. (2009). Bacterial rhamnolipids are novel MAMPs conferring resistance to Botrytis cinerea in grapevine. *Plant, Cell & Environment* 32(2), 178-193.

Verhagen, B.W.M., Glazebrook, J., Zhu, T., Chang, H.-S., van Loon, L.C. & Pieterse, C.M.J. (2004). The Transcriptome of Rhizobacteria-Induced Systemic Resistance in Arabidopsis. *Molecular Plant-Microbe Interactions* 17(8), 895-908.

Visker, M.H.P.W., Heilersig, H.J.B., Kodde, L.P., Weg, W.E., Voorrips, R.E., Struik, P.C. & Colon, L.T. (2005). Genetic linkage of QTLs for late blight resistance and foliage maturity type in six related potato progenies. *Euphytica* 143(1-2), 189-199.

Walker, V., Bertrand, C., Bellvert, F., Moënne-Loccoz, Y., Bally, R. & Comte, G. (2011). Host plant secondary metabolite profiling shows a complex, strain-dependent response of maize to plant growth-promoting rhizobacteria of the genus Azospirillum. *The New phytologist* 189(2), 494-506.

Walters, D. & Heil, M. (2007). Costs and trade-offs associated with induced resistance. *Physiological and Molecular Plant Pathology* 71(1–3), 3-17.

Walters, D.R., Havis, N.D., Paterson, L., Taylor, J. & Walsh, D.J. (2011). Cultivar Effects on the Expression of Induced Resistance in Spring Barley. *Plant Disease* 95(5), 595-600.

Walters, D.R., Ratsep, J. & Havis, N.D. (2013). Controlling crop diseases using induced resistance: challenges for the future. *Journal of Experimental Botany*.

Walz, A. & Simon, O. (2009). beta-Aminobutyric Acid-induced Resistance in Cucumber against Biotrophic and Necrotrophic Pathogens. *Journal of Phytopathology (Berlin)* 157(6), 356-361.

Weston, D.J., Pelletier, D.A., Morrell-Falvey, J.L., Tschaplinski, T.J., Jawdy, S.S., Lu, T.-Y., Allen, S.M., Melton, S.J., Martin, M.Z., Schadt, C.W., Karve,

A.A., Chen, J.-G., Yang, X., Doktycz, M.J. & Tuskan, G.A. (2012). Pseudomonas fluorescens Induces Strain-Dependent and Strain-Independent Host Plant Responses in Defense Networks, Primary Metabolism, Photosynthesis, and Fitness. *Molecular Plant-Microbe Interactions* 25(6), 765-778.

Whisson, S.C., Boevink, P.C., Moleleki, L., Avrova, A.O., Morales, J.G., Gilroy, E.M., Armstrong, M.R., Grouffaud, S., van West, P., Chapman, S., Hein, I., Toth, I.K., Pritchard, L. & Birch, P.R.J. (2007). A translocation signal for delivery of oomycete effector proteins into host plant cells. *Nature* 450(7166), 115-118.

Worrall, D., Holroyd, G.H., Moore, J.P., Glowacz, M., Croft, P., Taylor, J.E., Paul, N.D. & Roberts, M.R. (2012). Treating seeds with activators of plant defence generates long-lasting priming of resistance to pests and pathogens. *New Phytologist* 193(3), 770-778.

Wu, C.-C., Singh, P., Chen, M.-C. & Zimmerli, L. (2010). L-Glutamine inhibits beta-aminobutyric acid-induced stress resistance and priming in Arabidopsis. *Journal of Experimental Botany* 61(4), 995-1002.

Yang, J., Kloepper, J.W. & Ryu, C.-M. (2009). Rhizosphere bacteria help plants tolerate abiotic stress. *Trends in plant science* 14(1), 1-4.

Yoshioka, Y., Ichikawa, H., Naznin, H.A., Kogure, A. & Hyakumachi, M. (2012). Systemic resistance induced in Arabidopsis thaliana by Trichoderma asperellum SKT-1, a microbial pesticide of seedborne diseases of rice. *Pest Management Science* 68(1), 60-66.

Zhang, C., Wang, J., Zhang, J., Hou, C. & Wang, G. (2011). Effects of β -aminobutyric acid on control of postharvest blue mould of apple fruit and its possible mechanisms of action. *Postharvest Biology and Technology* 61(2–3), 145-151.

Zhang, S., Schisler, D.A., Boehm, M.J. & Slininger, P.J. (2007). Utilization of chemical inducers of resistance and Cryptococcus flavescensOH 182.9 to reduce Fusarium head blight under greenhouse conditions. *Biological Control* 42(3), 308-315.

Zipfel, C. (2008). Pattern-recognition receptors in plant innate immunity. *Current Opinion in Immunology* 20(1), 10-16.

8 Acknowledgements

After all these years in Alnarp, I have so many happy memories that I always will bring with me. In moments of distress and frustration, I am grateful to have had so many kind-hearted colleagues from whom I always received great support and encouragement.

First of all I would like to thank my main supervisor, *Erland Liljeroth*, for giving me the opportunity to work in this project and for always believing in me. I am grateful for your support in everything from writing to valuable advices, not to forget your help with statistics. You were never afraid of giving an appreciated extra hand in the lab when I was trying to keep up with all my experiments.

I would also like to thank my co-supervisor *Erik Andreasson* for invaluable input when planning and evaluating my experiments as well as in the writing of the manuscripts.

I would like to thank to my co-supervisor *Marit Lenman* for your support and wise input regarding my experiments, especially in the start of my PhD.

I am so grateful to my co-supervisor *Anna Holefors*, for your help in molecular techniques, but also for all the fun and adventure we have shared in Sweden and in Egypt. Especially thanks for giving me the opportunity to carry out laboratory work in "In Vitro Plant-tech" while I was finishing the writing of this thesis (I think I would have gone crazy without some practical work).

Erik Alexandersson, you came to Alnarp during the end of my study, but you quickly became an indispensable co-supervisor to me. I am truly grateful for all

of your technical and theoretical help in the realization of the “omics” manuscript.

I am grateful to the Swedish University of Agricultural Sciences (SLU), the Swedish Research Council Formas, the Swedish Farmers’ Foundation for Agricultural Research (SLF) and the Royal Physiographic Society in Lund, for funding of this work.

I would also like to thank:

Malin Hultberg for great collaboration. I really enjoyed working with you and I like your efficient way of working and attitude towards life and science. Thanks for all discussions and tips regarding everything from science to children and decorating.

Johanna Witzell for great collaboration and valuable help with the HPLC analysis.

Britt Green for being the most brilliant laboratory teacher I could have had in the lab when I started as a biomedical scientist. I wish you could be here to celebrate with me; you will always have a special place in my heart. *Tomas Bryngelsson* and *Arnulf Merker* for giving me the opportunity to start working in your groups and join the H-house “family”. *Ann-Charlotte Strömdahl* for all the fun moments we shared in lab and for the help with sampling and extraction.

Big thanks to people in the Resistance biology group. Svante Resjö and *Estelle Wera* for your contributions to the ¨omics¨ manuscript. *Mia Mogren* for all help in the lab, *Dharani Burra* and *Pelle Mülenbock* for all amusements and for being who you are (I will never trust the two of you completely, you know why!), *Salla Marttila* and *Kerstin Brismar* for excellent help with microscopy techniques and also to *Itziar Frades*, *Firuz Odilbekov, Kibrom Abreha, Maria Strandh, My Bengtsson, Sofia Hydbom and Åsa Lankinen,* for making the group to what it is.

To *Fredrik Levander* and *Marianne Sandin,* in the Department of Immunotechnology, Lund and to *Pete Hedley* and *Jenny Morris* at the James Hutton Institute in Scotland, for their great contribution to the “omics”-manuscript.

Helena Hovmalm Persson, for always listening and cheering! For your advises and endless support and for being such a good friend and office mate. Not to forget for your excellent illustrations. Hopefully you will get a more quietly atmosphere to work in now.

Hélen Lindgren, Annelie Ahlman and *Ann-Sofie Fält* for unstinted support and consideration for everyone around you. There was never a problem that you could not fix. I will really miss you!

Kerstin Olsson and *Nadezhda Zoteyeva* for sharing your knowledge and enthusiasm for the brown tuber with me. *Ulrika Carlson-Nilsson, Fredrik Reslow* and *Ingegerd Nilsson* for the discussions and collaborations and for nice company during lunch breaks. *Anders TS Nilsson* for sharing your experience on late blight and fungicides. Always with great enthusiasm.

Göran Olsson and *Göran Nilsson* for the excellent help I got to conduct experiments in the greenhouse and Biotron, respectively.

Nadire Kilicasan for brightening up the corridors both with your smile and polish.

To all past and present PhD students engaged in the *ASK PhD student council*. Especially to *Gustav Richnau* that left us all with great sorrow all to early. I am truly grateful for our collaboration during the time in the council.

To *Eva Johansson,* for your engagement in the research education and for being a great travel companion during the FUR-trips to Uppsala and Wageningen.

Ashfaq Ali, Phuong Nguyen and *Åsa Grimberg* for all the fun, but also times of hard work that we shared and for being such good friends.

Mira Rur for all the fun and deep talks about life and less serious things over a glass of wine and for being a great friend.

Abigail Walters, Agnese Kolodinska, Alessandro Nicolia, Anders Carlsson, Anders Smolka, Anna Zborowska, Bakhromiddin Khusenov, Bill Newson, Birjan Usubaliev, Björn Salomon, Boel Sandskär, Carlos Loaisiga, Dickson Ng´uni, Emilie Ivarson, Emily Too, Helle Turesson, Isabel Herrera, Inger Åhman, Jenny Lindberg Yilmaz, Johannes Albertsson, Knut Wåhlstedt, Laith

Moushib, Larisa Gustavsson, Lars Mogren, Li-hua Zhu, Linda-Marie, Lotta Holmqvist, Makhbubdzhon Rahmatov, Maksat Amanov, Maria Luisa Prieto-Linde, Mariette Andersson, Marie-Louise Måtegen, Margareta Welander, Marufkul Makhkamov, Mirela Beganovic, Mulatu Geleta, Nils-Ove Bertholdsson, Per Hofvander, Pia Ohlsson, Rodomiro Ortiz, Rui Guan, Sergay Hegay, Shu-Chin Hysing, Svetlana Leonova, Staffan Andersson, Sten Stymne, Susanne Andersson, Tintin Santén, Thuy Doan, Toan Pham, Waheeb Henen and my "witch" fellows *Ida Lager* and *Ramune Kuktaite* and all other past and present undergraduate students, PhD students and postgraduate researchers that I have come to know during my time in Alnarp. Thanks for creating a great atmosphere to work in and for all good times we shared.

The Microhort research school. Thanks to *Anna-Karin Rosberg, Christine Larsson, Justine Sylla, Karl-Johan Bergstrand, Liaqat Ali, Mehboob Alam, Nicolas Gruyer, Srisailam Marupakula* and *Srivathsa Nallanchakravarthula* for great times spent together during the Microhort courses. Especially thanks to *Beatrix Alsanius* and *Sammar Khalil* for great organisation of the research school.

Finally thanks to *Dan-Ola*, my brother *Tommy,* and my *parents* for always supporting me and believing in me. Without you there would not have been any thesis! I am also grateful to my son *Viggo* for making me remember what is most important in life.