Genes and Mechanisms in Arabidopsis Innate Immunity against *Leptosphaeria maculans*

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Abstract

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Leptosphaeria maculans is a hemibiotrophic ascomycete that causes blackleg disease on *Brassica* oilcrops, which globally is a great threat for oilseed production. In order to obtain mechanistic understanding of this devastating pathogen, *Arabidopsis thaliana* was used as a model host. Susceptible genotypes of Arabidopsis facilitated identification of the mechanisms required for resistance. The phytoalexin camalexin was first identified as a quantitative resistance factor; whereas accelerated cell death mutants enabled the pathogen to circumvent the resistance mechanisms by switching to a necrotrophic mode of growth. In addition to this, eleven *Leptosphaeria maculans* susceptible (*lms*) mutants were identified, one susceptible accession (An-1) and a 1:15 loss of resistance in F_2 progenies from the resistant accessions L*er*-0 and Col-0. The transgressive segregation revealed that resistance was dependent on TIR-NB-LRR resistance genes ($RLM1_{Col}$ and $RLM2_{Ler}$), which were independent of signalling components previously associated to all TIR-NB-LRR resistance genes. $RLM1_{Col}$ was found to be responsible for *L. maculans* induced callose depositions. A segregant analysis of the transcriptomes from resistant and susceptible Col-0 x An-1 F_3 lines revealed a region on chromosome 4 with genes significantly more highly expressed in the resistant progenies. T-DNA insertion lines and over expression studies revealed that the N-terminal part of a TIR-NB gene is responsible for resistance to *L. maculans, Alternaria brassicae, A. brassicicola* and *Botrytis cinerea*. In contrast to the other pathogens, *L. maculans* resistance is independent of the phytohormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). In order to establish the physiological mechanisms of Arabidopsis *L. maculans* resistances, characterized mutants defective in other hormone responses were screened. Mutants defective in ABA biosynthesis and signalling were found to impair resistance in both a callose dependent and independent manner. Further analysis of pathogen defence pathways revealed influences from combinations of SA, JA and ET responses on resistance and *L. maculans* mode of growth when the *R* gene and camalexin resistances were disrupted. Taken together, this work describes the establishment of a new model pathosystem with well-characterized pathogen and host organisms, which display both novel mechanisms and features overlapping with biotrophic and necrotrophic pathosystems.

Keywords: *Arabidopsis thaliana*, Blackleg, Innate immunity, *Phoma lingam*

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Plant breeding is not a science or an art, but a technology.
G. C. Buzza (1995)

Cover:
(upper left) Leaf of winter oilseed rape infected with *L. maculans* (snow recently thawed in Kohlstad, Sweden, April 2006), which shows *L. maculans* survival and growth during winter. Photo: Matti Leino.
(upper right) SEM picture of *L. maculans* mycelia growing on the leaf surface. Photo: Christina Dixelius.
(lower left) Arabidopsis *pad3-1* mutant with visible pycnidia, where *RLM1* resistance has been broken by high humidity. Photo: Jens Staal.
(lower right) GFP-tagged *L. maculans* mycelia in the susceptible accession An-1 (confocal microscopy). Photo: Johan Dixelius.

Contents

Appendix

The present thesis is based on the following papers, which will be referred to by their roman numerals:

I. [§]**Bohman, S.,** [§]**Staal, J., Thomma, B.P.H.J., Wang, M. and Dixelius, C.** (2004) Characterisation of an *Arabidopsis – Leptosphaeria maculans* pathosystem: resistance partially requires camalexin biosynthesis and is independent of salicylic acid, ethylene and jasmonic acid signalling. *Plant Journal,* **37**, 9-20.

II. **Staal, J., Kaliff, M., Bohman, S. and Dixelius, C.** (2006) Transgressive segregation reveals two *Arabidopsis* TIR-NB-LRR resistance genes effective against *Leptosphaeria maculans*, causal agent of blackleg disease. *Plant Journal*, **46**, 218-230.

III. **Staal, J., Kaliff, M., Dewaele, E., Persson, M. and Dixelius, C.** (2006) Rapid identification of an Arabidopsis TIR-X alternative transcript involved in innate immunity against necrotrophic fungi. *Plant Journal.* (Submitted)

IV. [§]**Kaliff, M.,** [§]**Staal, J., Myrenås, M. and Dixelius, C.** (2006) ABA is required for *Leptosphaeria maculans* resistance via ABI1 and ABI4 dependent signalling. *Mol. Plant-Microbe Interact.* (Submitted)

V. [§]**Staal, J.,** [§]**Persson, M. and Dixelius, C.** (2006) Genetic dissection of Arabidopsis *Leptosphaeria maculans* responses reveals interactions between host defense signaling and pathogen trophic switch. (In manuscript)

[§] indicates shared first authorship

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Additional publication:

Johansson, A., Staal, J. and Dixelius, C. (2006) Early responses in the *Arabidopsis – Verticillium longisporum* pathosystem are dependent on *NDR1*, JA/ET-associated signals via cytosolic *NPR*1 and *RFO1*. *Mol. Plant-Microbe Interact.* **19**, 958-969.

Introduction

Importance of plant disease resistance

Throughout history, plant diseases have had a severe influence on human society. In many ancient cultures, as with other natural disasters or epidemics, plant diseases and insect invasions have been seen as epic events and a sign from the Gods of their displeasure. Some of the oldest written texts known mention plant diseases as a punishment from God (Old Testament, ~750 B.C.) and the Romans even invented a special god for plant diseases; "the mildew god" Robigus (male) or Robiga (female). According to the roman scholar Varro in *Rerum rusticarum libri III* (Agricultural Topics in Three Books), the Romans had an annual festival (the Robigalia, 25th of April) where wine, incense and entrails from a dog and a sheep were sacrificed in order to save themselves from plant disease (Peck, 1898; Nordquist, G., Department of Archaeology, Uppsala University, personal communication). The concept that plant diseases were a punishment from the Gods was however challenged already in ancient Greece. The Greek philosopher Teophrastus concluded ~300B.C. that the reason plant diseases were more prominent in the lowlands, compared to the highlands, was due to natural phenomena (rain) rather than that the people living in the lowlands were more sinful. Despite numerous superstitions (some still present in some "alternative farming" philosophies), the introduction of disease resistance has been one of the prime objectives of plant breeding throughout history.

Some of the most notable historical events caused by plant diseases are the Irish potato famine 1845-1849 and the Bengal famine 1943. The potato late blight (*Phytophthora infestans*) in Ireland has been estimated to have killed up to a million people and through secondary effects, like a massive emigration to America, decreased the population of Ireland from approximately 8 million to 5 million. In the Bengal region, northern India, rice brown spot disease (*Cochliobolus miyabeanus*) epidemics in 1943 wiped out the staple food, rice, and caused the death of 2 million people due to starvation and malnutrition (Tauger, 2003; Strange and Scott, 2005). There are also more recent examples of catastrophes due to plant disease, where thousands have died due to starvation and malnutrition– especially in poor areas where human nutrition is dependent on a single crop. In 1994, approximately 3000 people died from famine-related causes due to an outbreak of an aggressive strain of African cassava mosaic virus (ACMV) in Uganda (Otim-Nape *et al*., 2002). Reliable food availability is a key component in breaking the 'persistent cycle' of hunger, poverty and ill health (WHO, 2000a, b). The problem of alleviating poverty, a complex of production, distribution and political structures, is one of the great challenges of the near future (Borlaug, 2000; Chrispels, 2000; Machuka, 2001; Potrykus, 2001).

One of the most challenging aspects of plant breeding is biotic (pathogen and insect) resistance, since the biotic stress is an ecologically complex "moving target" where the plant resistance over time is broken by a change in the pathogen or insect population structure. The complex and dynamic stress posed by pathogens may actually have been the driving force for the evolution and maintenance of sexual reproduction and recombination (Kover and Cacedo, 2001). Diseases still

account for a significant part of the yield losses in agricultural production and storage, which particularly is a problem in developing countries and for subsistence farmers, but also the profit potentials in industrialized agriculture suffer from disease-associated yield losses. In the U.S. alone, plant diseases are associated with an estimated annual loss of 33 billion USD (Maor and Shirasu, 2005). A wide range of chemicals are used to control diseases and insect pests, which in addition to their economical costs are associated to costs in terms of health hazards and detrimental environmental impact. Genetic resistance to disease is thus to be preferred both from an economical and environmental perspective (Holub, 2006). The current negative public view of GMOs does unfortunately hamper the development of novel genetic resistances for environmentally friendly agriculture via genetic control of insects (e.g. Bt toxins) and diseases (e.g. virus coat proteins) in Europe and countries dependent on exports to Europe (Strange and Scott, 2005).

Plant innate immunity and pathogen defence strategies

Disease resistance – different resistance definitions

Although plants lack an adaptive immune system, disease is an exception rather than a rule and not all plant-microbe interactions are detrimental for the plant. One of the most challenging tasks for a plant is thus to differentiate between mutualistic partners and parasites (Schulz and Boyle, 2005; Kogel *et al*., 2006), especially since both types of microbes use very similar mechanisms of nutrient acquisition (Paszkowski, 2006). There are several different kinds of plant disease resistance which all are more or less regulated via different genetic frameworks. In addition, there are several different definitions of the forms of resistance, which also have changed over time. The four categories escape, tolerance, resistance and immunity as described by Chahal and Gosal (2002) are fairly descriptive of the various mechanisms that influence the occurrence and severity of disease from a crop yield perspective.

The **escape** mechanism relies on avoidance of contact with the disease agent. Abscission of diseased leaves or growth and flowering early in the season are examples of escape mechanisms. The escape strategy can also be utilized to some extent by agronomical practice, like early or late planting and the use of fertilizers (Barbetti *et al.*, 1975; Chahal and Gosal, 2002). A **tolerant** plant does not suffer any adverse effects from infection, although the plant may even show visible disease symptoms and the pathogen is able to reproduce. A variant of tolerance is **recovery**, where a diseased plant is restored to healthy status by various plant mechanisms. Examples of recovery are woody plants that form new xylem tissue around *Verticillium*-infected tissues (Hiemstra, 1998). The most commonly used trait against diseases in breeding is what is commonly defined as **resistance**, which is a hereditary capability to limit pathogen growth. Resistance does not necessarily imply complete abolishment of pathogen growth. An old distinction of different forms of resistance is the division into **vertical** and **horizontal** resistance (Parlevliet and Zadoks, 1977; Vanderplank, 1984). The two different types of resistance are differentially effective against different pathogens, depending on their life style and reproductive strategies (McDonald and Linde, 2002). So-called **vertical** resistance is the ability of the plant to completely block growth of a pathogen, the determinant of **virulence** of the pathogen. Vertical resistance is also

commonly sub-divided into **race-specific resistance**, where the resistance trait is active against some genotypes (races) of the pathogen, whereas others remain virulent. **Race non-specific resistance** is the ability to block all known isolates of a pathogen, but where some plant genotypes show susceptible phenotype (Hammond-Kosack and Parker, 2003). Vertical resistance can be due to the presence of a **resistance (*R*) gene** according to the **gene-for-gene** resistance model (Flor, 1947) where the plant *R* gene recognize a pathogen **avirulence (*Avr*) gene**, leading to a rapid response and resistance. Vertical resistance, in particular against obligate biotrophs (only feeding on living host tissue) and viruses, can also be due to a lack of a specific host factor required by the pathogen. Present knowledge about resistance mechanisms is primarily based on studies of biotrophic pathogens, which often are inhibited by gene-for-gene type resistance (Dixelius *et al*., 2004; Glazebrook, 2005). There are also so-called **horizontal** resistances, which limit the disease progression of a wide range of pathogen genotypes, the determinant of **aggressiveness** of the pathogen. Horizontal resistance is often inherited as quantitative trait loci (QTLs). This type of resistance can be governed by multiple factors, and is in some cases referred to as 'basal resistance' (Hammond-Kosack and Parker, 2003), which can be confusing since induced resistance due to recognition of non-specific pathogen components like chitin or flagellin often is referred to as 'basal resistance' (de Torres *et al*., 2006). The horizontal ("basal") resistances can, among other things, also be governed through non-induced components like physical characteristics of the plant, toxin resistance and its chemical composition (i.e. the chemical structure of its antimicrobial secondary metabolites, like glucosinolates, phytoalexins, oxylipins etc.). Resistance to necrotrophs (feeding on dead (killed) tissue) has primarily been associated to various forms of horizontal resistances. Horizontal resistances do not **break** like gene-for-gene type resistance, but may **erode** over time.

Finally, not all pathogens are able to attack all plants. The cases where all interactions between all genotypes of a pathogen and all genotypes of a plant are incompatible (= no disease develops) are denoted as **immunity** or **non-host resistance**. There have been many hypotheses about the mechanisms of non-host resistance. One is that the pathogen fails to recognize the plant as a potential host, another hypothesis has been that the plant contains multiple "*R* genes" or "*R* genes" targeting indispensable structures of the pathogen, which makes it virtually impossible for the pathogen to break the induced resistance of the plant (Hammond-Kosack and Parker, 2003; Holub and Cooper, 2004). Other models have proposed that the pathogen lacks the appropriate virulence factors and is thus unable to overcome the basal resistances of the non-host (Holub and Cooper, 2004). Genetic studies have shown that both pre- and post-invasion defences are involved in non-host resistance (Lipka *et al*., 2005), which indicates that many of the proposed mechanisms of non-host resistance could be proven to be correct.

The complexity of disease

Plant disease is a complex interaction of pathogen and host genetics, time, environment and human interference (Zadoks, 1999; Okori, 2004; Figure 1). The nutritional status of the plant has, for example, a significant impact on disease from the necrotrophic fungal maize pathogen *Cercospora zeae-maydis* (Okori, 2004). A

systems biology approach, where models based on combinations of host, pathogen and environment factors, could be a powerful tool to further understand the mechanisms of disease. An example of when human practice broke a genetic resistance was when tomato started to be grown in shorter crop rotations after the discovery of a *Fusarium oxysporum* resistance gene in the 1940s. This led to the emergence of a previously unknown second (virulent) race, which required shorter crop rotations (Vanderplanck, 1984).

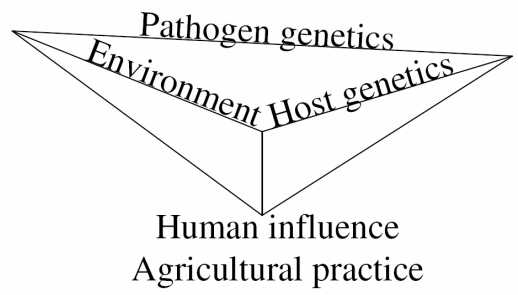


Figure 1. An illustration of the complex interactions influencing plant disease and epidemics.

Many of the disease resistance mechanisms identified under controlled/laboratory conditions have been shown to be less efficient under different or variable/natural environmental conditions. Light quality and stomatal regulation has, for example, been linked to defence components and induction of pathogen responses in Arabidopsis lesion mimic mutants (Mateo *et al.*, 2004). Gene-for-gene type resistance has also been shown to be affected by environmental conditions. *Rlm6* dependent resistance to *Leptosphaeria maculans* in *Brassica napus* has been shown to be broken by high humidity and temperature (Huang *et al*., 2006a). The low reproducibility of some resistance mechanisms between labs and between lab/greenhouse screenings and field conditions could partially be due to this phenomenon. Gene-by-environment (GE) factors are thus important to consider even in cases of resistance that at lab scale appear strictly qualitative (Mendelian), and not only for QTL-type resistances.

Recognition: Specific resistance genes and general pattern receptors

Plants rely on both general recognition of pathogen associated molecular patterns (PAMPs) like plant cell wall degradation products, LPS (lipopolysaccharides), flagellin and chitin (Walton, 1994; Gomez-Gomez and Boller, 2000; Zeidler *et al*., 2004; Ramonell *et al*., 2005) and pathogen-specific gene-for-gene type recognition. The resistance induced from general elicitors is often called ‘basal resistance’ whereas the resistance that relies on specific recognition often is called race specific resistance or race non-specific resistance, depending on the distribution of *Avr* genes in the pathogen population (Hammond-Kosack and Parker, 2003). Alternative denominations are PAMP triggered immunity (PTI) for ‘basal resistance’ and effector triggered immunity (ETI) for gene-for-gene type resistances (Chisholm *et al.*, 2006). To distinguish PTI from other components in ‘basal resistance’, such as different chemical compositions etc, would clarify much of the current confusion of terminology.

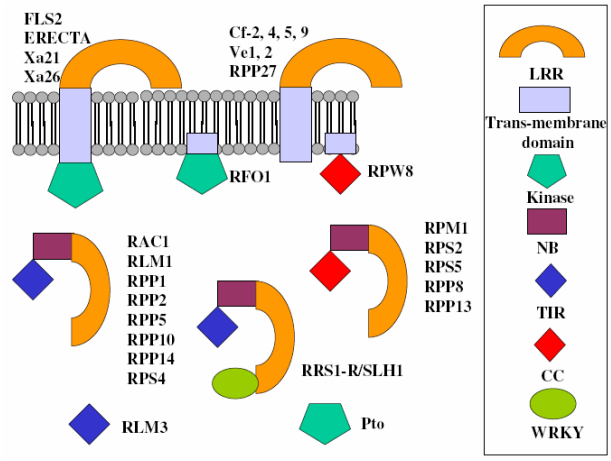


Figure 2. Different classes of known plant disease resistance proteins. Disease resistance proteins are not always directly interacting with the pathogen and may detect secondary effects from the infection attempt. For a more detailed explanation of the different *R* gene classes, see Hammond-Kosack and Parker (2003).

Little is known about PAMP receptors in plants. The flagellin receptor FLS2, for PTI against a wide range of bacteria is of the receptor like kinase (RLK) type and activates defences via MAP kinase signalling cascades (Asai *et al*., 2002; Figure 2). Normally, PTI is induced by recognition of several different PAMPs (Chisholm *et al*., 2006). There are also specific receptors (*R* genes) of the RLK type, like the rice *Xanthomonas* resistance genes *Xa21* and *Xa26* (Meyers *et al*., 2005) and a wall-associated kinase (RFO1) is responsible for race non-specific resistance to *Fusarium oxysporum* and *Verticillium longisporum* (Diener and Ausubel, 2005; Johansson *et al*., 2006a). Another RLK that affects pathogen resistance is *ERECTA*, which together with a heteromeric G protein influences resistance to the necrotrophic generalist fungus *Plectosphaerella cucumerina* (Llorente *et al*., 2005). The G protein dependent pathways have also been shown to influence resistance to *Alternaria brassicicola, Fusarium oxysporum* (Trusov *et al*., 2006) and *Botrytis cinerea* (Llorente *et al*., 2005). Furthermore, the *L. maculans* susceptible phenotype on a Rac GTPase activating protein (RacGap) mutant (Bohman, 2001) may indicate that G protein receptors or the G protein receptor pathway also are involved in PTI to additional fungal pathogens.

R genes have, on the other hand, been extensively studied and are known to be highly variable in sequence due to diversifying selection, possibly an “arms race” (Holub, 2001). Recent principal component analyses (PCA) of a number of evolutionary variables do however challenge an “arms race” mechanism of diversifying selection for most *R* genes (Bakker *et al*., 2006). The *R* genes are, due to their great variability, thought to counter the pathogen population structure on a population scale in natural populations (Dangl and Jones, 2001). An extremely interesting recent finding in this context is the observation of increased homologous recombination in plants after pathogen stress, and that this ability is passed down to the progeny, possibly epigenetic or as an ‘RNA cashe’ (Molinier *et al*., 2006). This could in theory lead to a diversification of the population’s *R* genes upon pathogen challenge. One of the largest classes of *R* genes for pathogen specific resistance in plants is the (nucleotide binding – leucine rich repeat) NB-LRR structural class (Ellis *et al*., 2000), which are clustered in regions of the genome due to tandem duplications of paralogous sequences (Meyers *et al*., 2005).

The NB-LRR class alone comprises of 149 genes in the model plant *Arabidopsis thaliana* and is involved in specific recognition of pathogen avirulence genes. The plant NB-LRR genes are sub-divided into two main classes: the (coiled coil-NB-LRR) CC-NB-LRR (51 genes in Arabidopsis) and the (Toll/Interleukin-1 receptor-NB-LRR) TIR-NB-LRR (92 genes in Arabidopsis) resistance genes (Meyers *et al*., 2003; Figure 2). Since no TIR-NB-LRR genes have been found in monocots (Meyers *et al*., 2005), it was widely assumed that this family of resistance genes evolved after the monocot/dicot split. Findings of TIR-NB-LRR type genes in gymnosperms (Meyers *et al.*, 1999; Liu and Ekramoddoullah, 2003) and the model moss *Physcomitrella patens* (Akita and Valkonen, 2000) does however challenge that conclusion. It is more likely that the monocots lost an important TIR-NB-LRR signalling component, effectively disabling all genes of this family, which would lead to a rapid loss of these genes. Further support of such a mechanism is the finding that the dicot sugar beet (*Beta vulgaris*) is deficient in TIR-NB-LRR type *R* gene –like sequences (Tian *et al*., 2004). The NB domain of TIR-NB-LRR genes is much more conserved than the one found in CC-NB-LRR genes, indicating that the NB domain is under greater functional constraints in this gene family (Cannon *et al*., 2002). An analysis of annotated *R* gene like sequences in the genome database of the primitive unicellular model organism *Chlamydomonas reinhardtii* (www.chlamy.org) revealed several Cf-like and TIR-NB-LRR like genes, but no CC-NB-LRR like genes. The TIR-NB-LRR (TNL) genes annotated as similar to the *C. reinhardtii* sequences (*N, RPS4*, At1g27170, At1g27180, At4g14370, At5g17680 and At5g17890) belong to different TNL sub-families (Meyers *et al*., 2003). This indicates that the NB-LRR type resistance genes existed early in plant evolution (Figure 3) and that the TIR-NB-LRR type may be the ancestral type and previous analyses may have underestimated the age of this group of proteins due to a greater conservation of the NB-ARC domain (normally used for phylogenies of the NB-LRR genes).

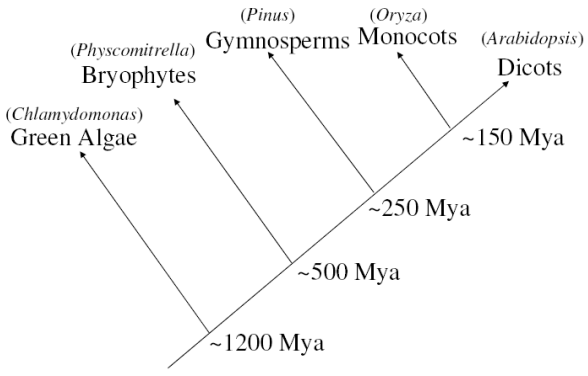


Figure 3. Evolution of plants and green algae and estimated time scales for the different splits. According to annotated database sequences, the TIR-NB-LRR class of proteins preceeds the split between true plants (*Viridiplantae*) and green algae. For a more detailed evolutionary model of photosynthetic eukaryotes, see Yoon *et al*. (2004). Mya = Million years ago.

The remaining 6 genes in Arabidopsis are NB-LRR proteins lacking the N-terminal domain. In addition, there are 58 related genes lacking the LRR domains (Meyers *et al.*, 2002). At least one resistance gene (*RPW8*) belongs to the truncated class of NB-LRR like proteins, encoding a CC-X domain structure. Another variant

of the NB-LRR class of *R* genes is genes where a C-terminal WRKY transcription factor domain and in one case also a protein kinase domain has been fused to the NB-LRR protein (Dangl and Jones, 2001). These protein fusions indicate an evolutionary "signalling short cut" of functionally interacting proteins according to the "Rosetta stone principle" (Lahaye, 2002), and point towards activation of WRKY transcription factors downstream of *R* genes (Nimchuck *et al*., 2003). A general early signalling mechanism for all NB-LRR type plant disease resistance genes and related proteins in other organisms upon elicitation is an ATP/GTP-dependent activation (oligomerization) via the NB domain, which triggers a cell death/immune response (Takken *et al*., 2006). Activation of an *R* gene leads to a rapid oxidative burst and a so-called hypersensitive response (HR), which is efficient against most biotrophic pathogens.

The N terminal part of the NB-LRR proteins determine the downstream signalling requirements of the *R* genes, where the CC-NB-LRR class often require NDR1 and the TIR-NB-LRR class in all known cases but one require the function of the EDS1 (enhanced disease susceptibility 1) and PAD4 (phytoalexin deficient 4) lipase-like proteins (Aarts *et al*., 1998; Figure 4). Some CC-NB-LRR genes (*RPP7* and *RPP8*) are however only weakly influenced when both EDS1 and NDR1 are mutated, indicating at least one additional resistance pathway (McDowell *et al*., 2000). The PAD4/EDS1 dependent resistance appears to be responsible for induction of SA-dependent defences, but recent results also show an SA-independent PAD4/EDS1-dependent response (Bartsch *et al*., 2006). In addition to the differential requirements of signalling components, both classes of *R* genes are influenced by RAR1 and SGT1b, but different *R* genes are influenced differently, where some show synergistic roles of RAR1 and SGT1b and others antagonistic (Holt *et al*., 2005).

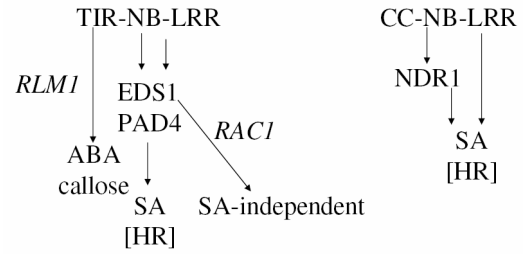


Figure 4. A simplified illustration of the differential requirements for signalling components for the TIR-NB-LRR and CC-NB-LRR class *R* genes. Until recently, all known TIR-NB-LRR genes required both EDS1 and PAD4. For a more detailed description of the different components, see Hammond-Kosack and Parker (2003).

Both RAR1 and SGT1b indicate intriguing links to ubiquitination or ubiquitin-like protein modifications in early *R* gene signalling, since SGT1b is associated to RAR1 and the SCF complexes (Devoto *et al*., 2003). The SGT1b independent *R* genes have been suggested to be independent due to redundant functions of the SGT1a isoform and that the antagonistic roles found between SGT1b and RAR1 are due to a reduced ability for SGT1a to recruit some *R* genes for degradation (Azavedo *et al*., 2006). This hypothesis has been difficult to test until recently, since the *sgt1a/sgt1b* double mutant is embryo lethal. Recent VIGS (virus-induced gene silencing) analysis of *RPS2* signalling in Arabidopsis, where *SGT1b* silencing still remained ineffective, may however indicate that the gene truly is SGT1 independent (Cai *et al*., 2006). The immediate downstream components or the

mechanisms of the plant NB-LRR N terminal domains are however unknown, which may indicate that the interactions are very dynamic or weak. Despite extensive genetic and yeast-two-hybrid studies on some *R* genes, the direct downstream signalling mediators remain elusive, which suggests that new biochemical strategies also must be considered (Belkhadir *et al*., 2004). EDS1 and PAD4 are also together with a third homolog, SAG101 (senescence-associated gene 101), involved in post invasion non-host resistance to pea and grass infecting ascomycetes in Arabidopsis, suggesting that PTI receptors rely on signalling components in common with *R* gene resistance (Feys *et al.*, 2005; Lipka *et al*., 2005).

Other structural classes of *R* genes include cytosolic kinases like Pto and transmembrane receptor-like LRR proteins (RLP), such as the *Cladosporum fulvum* (Cf-), *Verticillium albo-atrum* (Ve-) and *Hyaloperonospora parasitica* (RPP27) resistance proteins (Kawchuck *et al*., 2001; Tör *et al*., 2004). Interestingly, *Cf-4* dependent resistance to *C. fulvum* requires a CC-NB-LRR gene for downstream signalling and HR response (Gabriëls *et al*., 2006). Furthermore, *V. longisporum* resistance in Arabidopsis requires the CC-NB-LRR signalling component NDR1 (Johansson *et al*., 2006a), which may indicate that the transmembrane receptor-like LRR proteins and the NB-LRR proteins represent two different recognition events in the pathogen response. Most genetic analyses currently made have however failed to identify the processes at the immediate infection attempt. New cell imaging techniques have revealed several rapid subcellular localization changes of organelles, the host determining protein MLO and SNARE proteins to the site of penetration (Bhat *et al*., 2005; Koh and Somerville, 2006). Many signalling peptide precursors are located in the cell wall matrix, indicating that responses may be triggered by immediate protein processing, via biophysical events, without need for a signalling cascade (Narváez-Vásquez *et al*., 2005). Interestingly, the PAMP receptor FLS2 is rapidly transported into intracellular compartments upon flagellin challenge, indicating restricted sub-cellular signalling events (Koh and Somerville, 2006). Despite the multitude of unspecific recognition mechanisms of cellular penetration attempts and PAMPs, pathogens are still able to infect plants. Potent responses to PAMPs in susceptible plant genotypes indicate, however, that susceptibility rarely is determined by a failure to detect the pathogen, but rather due to an active suppression of the PTI by the pathogen (Jones and Takemoto, 2004).

Host immuno-suppression and counter measures

How does a plant with a given number of specific receptors withstand challenge from all the possible versions of incompatible pathogens? The guard hypothesis (van der Biezen and Jones, 1998; Dangl and Jones, 2001; Nimchuk *et al.*, 2001) addresses this issue, in which the R proteins are attached to endogenous proteins (as “guards”) that are targets for pathogen virulence proteins (Figure 5). A pathogen lacking a sufficient number of virulence genes (for host immune suppression) would be recognized by the general PAMP receptors and stopped by the PTI. Bacteria, fungi and other pathogenic organisms would then have to secrete effector proteins to disrupt the PAMP-induced defences in order to infect the plant (Alfano and Collmer, 2004; Kim *et al.*, 2005; Rep, 2005; Li *et al*., 2005). A very

appealing aspect of this model is that it can explain how some R proteins can be both race-specific and at the same time confer resistance to several vastly different types of pathogens, like *RPP8/HRT* family which confers resistance to oomycetes and viruses (Cooley *et al*., 2000) and a single CC-NB-LRR gene (*Mi-1*) in tomato confers resistance to potato aphid, root knot nematodes and whitefly (Nombela *et al*., 2003). There are however also recent results that show direct allele-specific interactions between an indispensable *Avr* gene and an *R* gene (Dodds *et al*., 2006), indicating that both the 'guard hypothesis' and 'receptor-ligand' model of pathogen recognition are valid in different host-pathogen contexts (Dangl and McDowell, 2006).

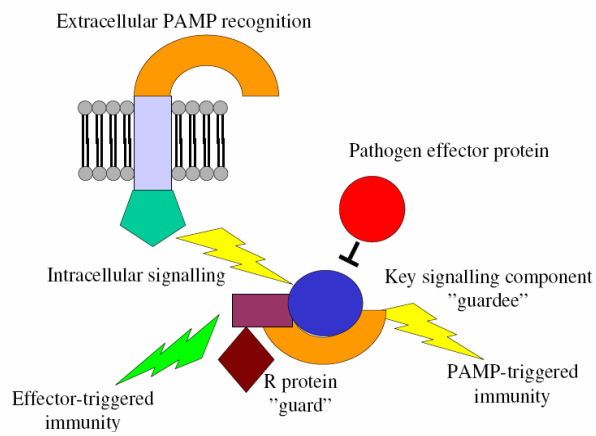


Figure 5. Schematic representation of the 'guard hypothesis'. An immuno-suppression attempt by the pathogen is detected by the plant *R* gene, which then triggers a different type of immunity. Recent analyses have however also demonstrated direct 'receptor-ligand' interactions between R and effector proteins.

The bacterial pathogen *Pseudomonas syringae*, for example, is known to secrete 20-30 effector proteins via the type III secretion system during infection (Chisholm *et al*., 2006). Two of those effectors, AvrPto and AvrPtoB, block early signals upstream of MAP3K (He *et al*., 2006). In contrast to bacteria with type III secretion systems, oomycete and fungal pathogens must employ other mechanisms to distribute their effector proteins into the plant cell (Ellis *et al*., 2006). Some effectors 'hijack' the plant responses by activating the wrong signals in order to suppress responses effective against the pathogen (Maor and Shirasu, 2005). One example is the production of coronatine, a JA precursor, in order to suppress plant SA responses (Kloek *et al*., 2001) or that type III secretion dependent proteins activate auxin and ABA responses, which negatively influence resistance (Thilmony *et al*., 2006). Coronatine also suppresses flagellin-induced expression of *NHO1*, a gene associated to non-host resistance against non-adapted *P. syringae* isolates (Li *et al*., 2005). On the other hand, the flagellin-induced PTI against bacteria seems to suppress auxin sensitivity as part of the defence (Navarro *et al*., 2006), but also TIR-NB-LRR type genes seem to influence 'basal' resistance and auxin responses (Hewezi *et al*., 2006; Holmblad, 2006). Another example of a "hijacking" mechanism is the target protein RIN4, which appears to work as a signalling switch between callose and SA responses, where the effector proteins AvrRpt2 and AvrRpm1 remove the RIN4-dependent suppression of callose responses in order to suppress SA-responses (Kim *et al.*, 2005). Interestingly different modification attempts of RIN4 are detected by different *R* genes (McHale

et al., 2006). Also necrotrophic fungi appear to make use of the plant pathogen responses via their secreted toxins, rather than just killing off the plant cells directly and live as saprophytes (Howlett, 2006)

According to the guard hypothesis, any effector/Avr protein that attacks an important guardee protein (which supposedly has a role in PTI) may activate the *R* gene dependent resistance (Chisholm *et al.,* 2006). The general consensus is that the guard hypothesis is correct and recent results link suppression of PTI and the *R* gene dependent resistance. This indicates that the guardee proteins, as predicted by the hypothesis, do act in the PTI pathway (Kim *et al.*, 2005; Fujikawa *et al*., 2006). The presence of an *R* gene will, in turn, exert a strong selective pressure on the pathogen to evolve to avoid detection, by removing the effector protein that triggers the *R* gene-dependent response (Pitman *et al*., 2005). In theory, such evolution is associated to a fitness cost expressed as reduced aggressiveness (Vera Cruz *et al*., 2000), since a part of the PTI remains active. The relationship between virulence and aggressiveness has also been demonstrated for *AvrLm4/avrLm4* in near isogenic (BC_5) *L. maculans* under controlled lab conditions and relative allele frequencies over a disease cycle in field trials (Huang *et al*., 2006b). Maintaining *R* genes is, however, also associated to a fitness cost for the plant (Tian *et al*., 2003). A complementary hypothesis, in line with older "multi-gene models" for non-host resistance, suggests that a combinatorial effect from several different R proteins gives a large recognition potential. A combinatorial effect from a limited set of receptors, similar to the effect of the olfactory system, could be a mechanism for *R* genes to detect a very large number of pathogens (Fluhr, 2001).

Plant pathogen responses and signalling pathways

Pathogen responses are regulated by complex networks of signalling pathways, which are in turn regulated by a few central common components (Glazebrook, 2001; Glazebrook, 2005; Figure 6). Disease resistance responses need to be tightly regulated due to the high fitness costs associated with inappropriately active resistances (van Hulten *et al*., 2006). The secondary metabolites, primarily in the phenylpropanoid and oxylipin pathways, induced by pathogen stress both act as antimicrobial agents and as signal molecules (hormones) to activate plant responses (Camera *et al*., 2004; Anderson *et al*., 2006). Apart from oxylipins, other lipids and lipid-derived signals are also of central importance for many local/rapid as well as systemic responses to pathogen stress (Shah, 2005; Grant and Lamb, 2006). In addition to the signalling role of secondary metabolites, several (>20) plant peptide hormones have emerged in various physiological processes, where some are important in defense signalling (Navárez-Vásquez *et al*., 2005). Signalling peptides have primarily been described in the tomato system, but similar peptide hormones have not yet been reported in Arabidopsis pathogen responses.

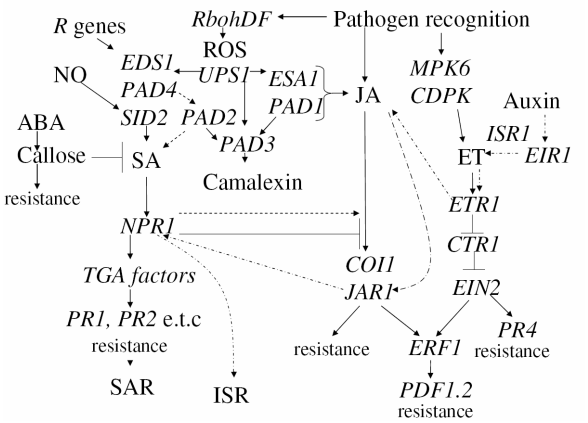


Figure 6. A model over pathways required for induction of resistance against various pathogens with different life styles. Biotrophic pathogens are mainly inhibited by SA-dependent responses, whereas defence to necrotrophs rely on camalexin, JA and ET. For a more detailed review about the mutants involved in the signalling pathways, see Hammond-Kosack and Parker (2003).

A common denominator in plant pathogen defence appears to be the active starvation strategy. Against biotrophic pathogens, the plant tends to respond with a hypersensitive response (HR) which is a localized induced cell death to prevent growth of the pathogen. This response is mediated by reactive oxygen species (ROS) signals and salicylic acid (SA). The HR response is however not very effective against necrotrophic pathogens, which could even be more successful in their infection if the plant is pre-treated with SA (Glazebrook, 2005). The defence against many necrotrophic pathogens is rather dependent on ethylene (ET) and jasmonic acid (JA) derived signals. "JA signalling" is also composed of several other structurally related chemicals (Glazebrook, 2005; Kishimoto *et al*., 2006). JA responses are initiated via SCF^{COI1}–dependent ubiquitinylation, and the *R* gene signalling component SGT1b influences the activity of this complex (Devoto *et al*., 2003; Lorenzo and Solano, 2005). One SCF^{COI1} target important for JA responses is histone deacetylase 19, demonstrating that JA responses are under partial epigenetic control (Lorenzo and Solano, 2005). Ethylene responses might be regulated by early recognition mechanisms, where the pathogen-responsive MAP kinase MPK6 or a calcium-dependent protein kinase (CDPK) modulates the stability of ACC synthase (ACS), which is the committed step in ET biosynthesis. ET will, in turn, be involved in extensive crosstalk and is known to potentiate gene-for-gene, SA and JA responses (Broekaert *et al*., 2006). Defences against necrotrophs can also be regulated either via exclusive JA or ET signalling, or an integrated JA/ET response via ERF1 (Lorenzo *et al*., 2003).

An interesting parallel is that both these hormones are involved in the process of senescence, which is a highly controlled form of cell death where nutrients can be transported to other parts of the plant. Senescence and pathogen defence do have many overlaps in transcriptional profiles (Buchanan-Wollaston *et al*., 2003; Schenk *et al*., 2005). This could work as a "scorched earth strategy". The scorched earth strategy was successfully used throughout history in Russia to fend off invasions from Sweden (1709), France (1812) and Germany (1941). The general idea was to abandon the farms, pull back and destroy all resources on the way to severely weaken the invader before they reached the bigger cities. Analogously, a withdrawal of nutrients and subsequent abscission could be an efficient escape mechanism to fend off necrotrophic pathogens, which then have to rely on

saprophytic (growing on dead/decaying matter) growth. Furthermore, it has been observed that some pathogens suppress senescence and form so-called "green islands" (Hammond-Kosack and Jones, 2000).

In addition to the various forms of cell death induced by SA and JA/ET, both defence hormone pathways induce a number of pathogenesis-related proteins. SA suppresses a large portion of the JA/ET dependent responses, but there are also a sub-class of genes that show synergistic expression (Schenk *et al*., 2000) and a potentiation of JA/ET-dependent resistance by moderate levels of SA and the interactions between the pathways are a complex network (Glazebrook *et al*., 2003; Mur *et al*., 2006; Figure 6). In addition to this, there are genes and processes where ET and JA act antagonistically, e.g. JA via SCF^{COI1} induces the bHLHzip transcription factor AtMYC2/JIN1/JAI1, which deregulates the pathogen response gene expression in favour of wound responsive genes (Lorenzo and Solano, 2005). Interestingly, a human homolog of this transcription factor is regulated via mono-ubiquitination (von der Lehr *et al*., 2003).

NPR1/NIM1 is a central component in the interactions between JA and SA-dependent responses. SA responses are dependent of nuclear localization of a redox-regulated NPR1 (Mou *et al*., 2003) and interactions with TGA transcription factors (Fan and Dong, 2002). Responses downstream of NPR1 also appear to require nuclear trafficking, since the constitutive activation of resistance downstream of NPR1 in the (TIR-NB-LRR) *snc1* mutant requires the function of MOS3, a putative nucleoporin 96 (Zhang and Li, 2005). SA responses will be blocked in both the severely disrupted mutant *npr1-1* and the truncated *npr1-3* mutant, lacking nuclear localization signal. JA responses, on the other hand, partially require cytosolic NPR1 and will only be affected in the *npr1-1* mutant (Glazebrook *et al.*, 2003). Recent results using reverse genetics of *NPR1*-like genes have revealed that *NPR4* also is involved in disease resistance (Liu *et al.*, 2005a).

Another rapid response against pathogen challenge is deposition of callose-rich papillae that limit nutrient leakage from the cell and work as a barrier against pathogens that try to penetrate the cell, which appears to be efficient against both necrotrophs and biotrophs (Flors *et al*., 2005). Callose deposition has, however, a negative influence on SA accumulation which leads to the counter-intuitive result that loss of callose synthase can result in enhanced resistance against some biotrophic pathogens (Vogel and Somerville, 2000). Other modulations of the physical barriers against the pathogen are also known, such as lignification and thickening of the cell wall.

Resistance dependent on SA, JA or ET has been extensively reviewed (Glazebrook, 2005), whereas very little is known about the role of the "abiotic stress" hormone ABA in pathogen responses (Fujita *et al*., 2006). Abscisic acid (ABA), which also has a negative influence on SA accumulation (Ward *et al*., 1989), enhances the ability of the plant to deposit callose in response to pathogens (Ton and Mauch-Mani, 2004). Possibly both these negative interactions to SA are linked, since ABA down-regulates SA-induced beta-glucanases (Rezzonico *et al*., 1998) which act to inhibit callose deposition via degradation (Beffa *et al*., 1996). It is, however, likely that ABA also regulates responses that are antagonistic to the SA response, independently of callose. ABA could possibly also play a role in the senescence-like pathogen response (Park *et al*., 1998), but has also been shown to be mutually antagonistic to JA/ET dependent defences (Anderson *et al.*, 2004).

ROS responses have been suggested to be a primary point of convergence between ABA 'abiotic stress' and SA/JA/ET 'biotic stress' signalling pathways (Fujita *et al*., 2006). This is intriguing, since despite the fact that ABA has a negative influence on both SA and JA/ET dependent defences ABA induces some pathogenesis related genes (Hoth *et al*., 2002). ABA is also required for systemin-induced JA responses in tomato (Peña-Cortés *et al.*, 1995).

ABA causes resistance against necrotrophic fungal pathogens primarily through enhanced callose deposition (Ton and Mauch-Mani, 2004). The pathogen-induced callose deposition may share signalling features with that of incompatible pollen interactions, since both callose responses are enhanced by BABA (β-aminobutyric acid) pre-treatment. Both the named effects require ABA and mutations that disrupt BABA-induced female sterility also disrupt BABA-induced resistance to pathogens (Ton *et al*., 2005). Despite the clear role of the non-protein amino acid BABA in priming physiological responses in plants, and the stress-responsive isoform GABA (γ-aminobutyric acid), BABA has not been found to be produced naturally in plants. In the case of *L. maculans*, the defence induction from ABA and BABA is more complex than only an enhancement of callose, since the *pmr4* mutant experience less *L. maculans* susceptibility if pre-treated with ABA (**IV**).

The various defences differ in timing from rapid (immediate) responses, such as HR and callose depositions, followed by induced defences like camalexin and SA- or JA/ET- induced antimicrobial peptides. A longer lasting resistance is then obtained by the plant, such as systemic acquired resistance (SAR), which basically means that the plant stay alert to defend itself from future attacks (Grant and Lamb, 2006). Grafting studies have shown that SAR requires SA locally. The mobile signal still remains elusive, but is dependent on a lipid transfer protein (Maldolando *et al*., 2002). Another induced resistance requires ET, JA and (cytosolic) NPR1 is referred to as induced systemic resistance (ISR), a long lasting response triggered by non-pathogenic rhizobacteria, which is not associated to elevated levels of pathogenesis-related (PR) proteins (Pieterse *et al*., 2001). ISR is, in many respects, to be regarded as a priming of defences (Verhagen *et al*., 2004), similar to BABA-induced resistance (BABA-IR). BABA-IR is however dependent on the SAR or an ABA-dependent signalling, depending on pathogen (Ton and Mauch-Mani, 2004).

The primed state is a comparably optimal condition under pathogen pressure, since it gives the benefits of rapid responses with limited detrimental effects on fitness, such as those seen when defence responses are constituitively active (van Hulten *et al*., 2006). Despite that fitness costs primarily has been associated to the SAR pathway, SAR can have a beneficial effect under low nutrient conditions, possibly due to the higher costs of pathogen nutrient acquisition under such conditions (Heidel and Dong, 2006). The disease rating was however also significantly higher under low nutrient conditions, despite that the SAR marker *PR1* was significantly higher expressed in low nutrient conditions (Heidel and Dong, 2006). This indicates that the plant determines the level of resistance responses by a highly complex 'cost-benefit' evaluation which still has not undergone sufficient genetic investigations.

The activity of the first line of defence does influence the need and induction of the subsequent defence responses. In incompatible systems, where an active *R*

gene triggers early defence responses, the induction of camalexin is lower than in a compatible interaction (Mert-Türk *et al.*, 2003, Narusaka *et al.*, 2004, **II**). Similar results can be seen when callose deposition is primed by BABA pre-treatment, which then decreases the stress on the plant from invading pathogens and thus decreases the induction of camalexin (Mauch-Mani, B., Laboratory for biochemistry and molecular biology, Neuchâtel university, personal communication).

The dissection of resistance mechanisms in Arabidopsis against biotrophs has primarily been based on studies on gene-for-gene resistance in the oomycete *Hyaloperonospora parasitica* (via *RPP* genes) and the bacteria *Pseudomonas syringae* (via *RPS* genes) and *Pseudomonas syringae* pv. *maculicola* (via *RPM* genes), where NPR1-dependent SA signalling plays a central role. There are however variants of the SA pathway. Gene-for-gene dependent resistance to the hemibiotrophic oomycete *Albugo candida* (via *RAC1*) is SA-independent but require EDS1, indicating differential roles for PAD4 and EDS1 in some resistance responses (Borhan *et al.*, 2004). The hemibiotrophic oomycete *Phytophthora brassicae* only shows enhanced susceptibility in the *pad2* mutant, but resistance does not seem to be dependent on either SA or camalexin, which may indicate additional unknown pathways (Roetschi *et al.*, 2001). Analysis of resistance responses in compatible interactions with the bacterium *Xanthomonas campestris* located ET responses downstream of SA and also showed a parallel dependency of JA and auxin (O'Donnel *et al.*, 2003). The gene-for-gene resistance mechanisms against *X. campestris* (via *RXC* genes) are however still uncharacterized.

The JA, ET and JA/ET resistance mechanisms against necrotrophs, on the other hand, appear to be *R* gene independent and have been focused on the fungi *Alternaria brassicicola*, *Botrytis cinerea* and *Plectosphaerella cucumerina*. Also resistance to the necrotrophic bacterium *Erwinia carotovora* appears to conform to the JA/ET dependent resistance mechanism (Norman-Setterblad *et al.*, 2000). The over-simplified generalizations of one mechanism against biotrophs and a set of others against necrotrophs in Arabidopsis is however getting challenged by additional signalling studies on other pathosystems.

The hemibiotrophic fungus *Leptosphaeria maculans* only showed susceptibility when impaired in camalexin biosynthesis, which appears to be more associated to the JA pathway in this system since the *pad1* and *esa1* mutants also displayed susceptibility (**I**) but appear to primarily rely on a camalexin-independent gene-for-gene (via *RLM* genes) resistance and callose depositions (**II**). Similarly, the hemibiotrophic fungus *Colletotrichum higginsianum* also shows a parallel gene-for-gene (via *RCH1*) and camalexin dependent resistance, whereas SA, JA and ET have no major influence on this resistance (Narusaka *et al.*, 2004).

Most Arabidopsis pathosystems have focused on pathogens infecting the leaves. New models focusing on other parts of the plants are however under development. These include, for example, root-infecting vascular wilt pathogens *Fusarium oxysporum* (Beroca-Lobo and Molina, 2004; Diener and Ausubel, 2005), *Verticillium dahliae* (Veronese *et al.*, 2003), *Verticillium longisporum* (Johansson *et al.*, 2006a) and the clubroot pathogen *Plasmodiophora brassicae* (Ludwig-Müller *et al.*, 1999; Siemens *et al.*, 2006). Taken together, addition of new Arabidopsis pathosystems have revealed novel resistance pathways more or less associated to the characterized SA and JA/ET "standard model" (Figure 6)

pathways but have also shown an influence from the phytohormones ABA, cytokinin and auxin (O'Donnel *et al*., 2003; Veronese *et al*., 2003; Siemens *et al.*, 2006).

Mode of action of pathogen responsive components

The main function of many of the classes of pathogenesis related (PR) proteins (van Loon and van Strien, 1999) is to weaken the cell wall of the pathogen, such as glucanases (PR2), chitinases (PR3, PR4, PR8, PR11), osmotin (PR5; Narasinham, 2003), cyclotides (Kamimori *et al.*, 2005; Svangård, 2005), defensins (PR12; Thomma *et al*., 2002) and thionins (Carrasco *et al*., 1981), or to inhibit their ability to degrade plant tissue via proteinase inhibitor (PR6 and some cyclotides) or α-amylase inhibitor (some defensins) activity. The defensins show target specificity to different types of cell walls and appear to interact with them using electrostatic interactions. The subsequent membrane disruption may however not be the only mode of action of this group of proteins, but rather disruption of RNA, DNA or protein synthesis (Thomma *et al*., 2002).

The definition of PR proteins is however not as clear-cut as it was intended to be, since many PR proteins have been found to be expressed constitutively in some organs and an inconsistent use of the term by the research community (van Loon *et al*., 2006). One of the most enigmatic classes of PR proteins is the PR1 family, which have a completely unknown function but a wide-spread phylogenetic distribution (even vertebrates). Overexpression of pathogen-responsive PR1 class proteins has been shown to have some effects on resistance to some pathogens, but most members of this family have no pathogen-responsive expression (only 1 out of 22 in Arabidopsis) (van Loon *et al.*, 2006). Despite this, *PR1* is the most commonly used marker for SA-associated pathogen responses.

Overexpression analyses of a pea defensin and a pea pathogen-responsive dirigent family (lignan/lignin biosynthesis) protein (DRR206) in *B. napus* background both displayed enhanced resistance to *L. maculans*, illustrating the functional role of these classes of pathogen-responsive proteins in resistance (Wang *et al*., 1999). Further, an ethylene-induced secreted lipase (GLIP) shows a dual role as both an antimicrobial protein against *Alternaria brassicicola* and as a signalling protein for systemic resistance responses (Oh *et al*., 2005). All of the cell wall modulating effects from PR- and other pathogen-responsive proteins are probably also primarily used to actively starve the pathogen via severe nutrient loss. Some of the PR proteins may also act via direct induction of cell death (Narasimhan *et al*., 2001), possibly also as an effect of severe ion leakage over the cell wall. Despite confirmed antimicrobial activities *in vitro*, most PR proteins only give a moderate effect on resistance when overexpressed (van Loon *et al*., 2006).

Another cell wall modulating compound induced in Arabidopsis in response to pathogen stress is the phytoalexin camalexin. Camalexin causes membrane leakage and thus nutrient loss in both fungi and bacteria (Rogers *et al.*, 1996). Camalexin appears to be induced by non-specific signals with overlaps to various pathogen response pathways (Kliebenstein, 2004). *In vitro* studies of various *Brassica*-derived phytoalexins have revealed a differential efficiency in limiting *L. maculans* growth, linked to detoxification mechanisms (Pedras *et al.*, 2003; Pedras and Montaut, 2003). In addition to phytoalexins, a wide range of secondary

metabolites influence resistance to various pathogens. Multivariate analysis could correlate host factors, like chemical contents, with disease data, as seen in a model made using metabolic profiles in Arabidopsis accessions compared to *Botrytis cinerea* disease (Kliebenstein *et al*., 2005). Overexpression of P450 proteins involved in glucosinolate biosynthesis from cassava in Arabidopsis resulted in an altered glucosinolate profile and enhanced resistance to *Erwinia carotovora*, whereas secondary effects on JA signalling enhanced susceptibility to *Alternaria brassicicola*, illustrating complex interactions between signalling and secondary metabolite structure (Brader *et al*., 2006). Also, camalexin biosynthesis shares a metabolic origin with indole glucosinolates and the plant hormone Auxin (IAA), further emphazising the intricate links between antimicrobial compounds and signalling molecules (Glawischnig *et al*., 2004). Glucosinolates do however not influence the disease progression of *L. maculans* (Wretblad and Dixelius, 2000; Andréasson *et al.*, 2001), whereas *in vitro* studies have established growth inhibition on *L. maculans* and *A. brassicae* from some forms of oxylipins found in *B. napus* (Granér, 2002).

The multitude of structural variation in secondary metabolites and their interaction with signalling pathways adds another level of complexity to the genetics of plant pathogen resistance. The natural variation among pathogen-induced components is an important source of novel modes of action and correlations between phylogenetic and phytochemical properties may be an effective way for rational selections and screening for compounds with a specific set of properties (Staal, 2001; Larsson, 2004; Simonsen *et al*., 2005; Schulz and Boyle, 2005). A mechanistic understanding of pathogen-induced components in plants may enable us to engineer more durable resistances, but will also lead to the discovery of new lead compounds that could potentially be used in medicine as cytotoxic substances against cancers or as novel antibiotics (Samuelsson, 1999; Thomma *et al*., 2003).

Plant pathogen defence shows many similarities to animal innate immunity

Plants and animals show some remarkable similarities in their innate immunity systems. In contrast to plants, vertebrates defend themselves both via an inherited (innate, unspecific) immune system and a slower adaptive (specific) immunity. The innate immunity is conserved between vertebrates and invertebrates (which lack the adaptive immune system) and the emerging picture from research on the innate immune systems shows that there are many similarities between this innate immunity and plant pathogen defence. The long list (Table 1) of similarities between the two systems indicates that there are interactions between the innate immunities and some central components in the cellular machinery, which either has conserved ancient features or has driven the two systems into convergent solutions (Nürnberger and Brunner, 2002; Nürnberger *et al*., 2004; Ausubel, 2005; Inohara *et al*., 2005; Zipfel and Felix, 2005).

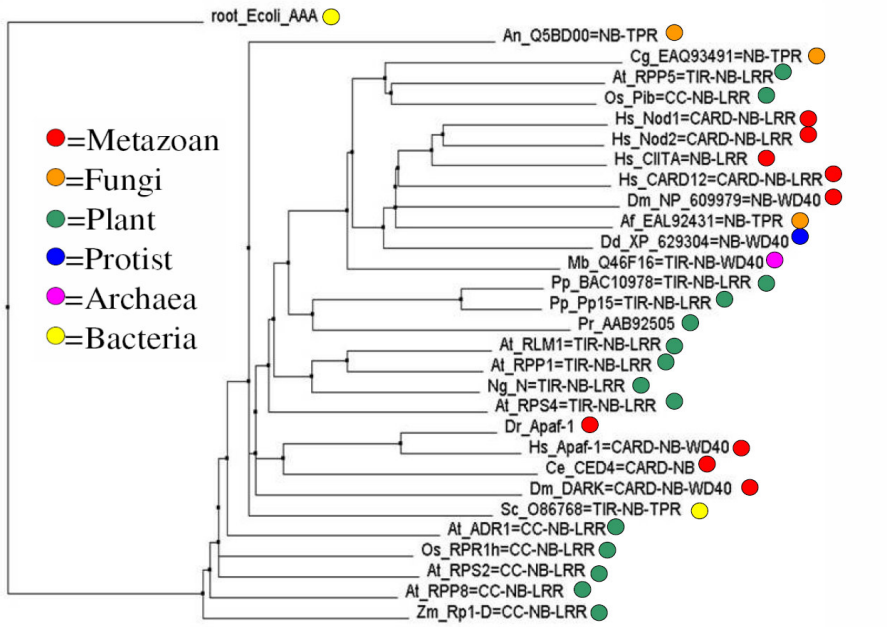
Table 1: List of components in the plant and animal innate immunities with documented similarities.

Feature	Plant	Animal	Function	References
NB-LRR	(NB-LRR) R	Nod family	pathogen receptors	Inohara & Nunez, 2003
HSP90	HSP90	HSP90	NB-LRR partner	Hahn, 2005
CHORD-domains	RAR1	Chp1	NB-LRR partner	Hahn, 2005
CS-domain	SGT1	Chp1	NB-LRR partner	Hahn, 2005
Protein phosphatase 5	PP5	PP5	NB-LRR partner	Hahn, 2005
TIR-domain	(TIR-NB-LRR) R genes	TLR family	pathogen receptors	Beutler and Rehli, 2002
DEATH-TIR	sRPS4 (RPS4-specific?)	MyD88	TIR signalling	Janssens & Beyaert, 2002 Zhang & Gassman, 2003
pathogen induced caspase-like	metacaspase	caspase-1 caspase-11	Cell death / immune response	Hoeberichts *et al*., 2003 Schauvliege *et al*., 2002
evolutionary connections	metacaspase	paracaspase (MALT1)	Cell death / immune response	Uren *et al*., 2000
~ myosin-like	BECLIN-1	ATG6/VPS30/ beclin 1	Autophagy regulation/HR	Liu *et al*., 2005b Patel *et al*., 2006
extracellular LRR	FLS2	TLR5	Flagellin receptors	Zipfel & Felix, 2005
TF interactor	NPR1/NIM1	I kappa B alpha	Cell death / immune response	Ryals *et al*., 1997
Protein kinases	Xa21, Pto, Erecta, PBS1, ACIK1	Pelle, IRAK	Signalling/ perception	Rowland *et al*., 2004
E3 ligase	RIN2, RIN3	autocrine motility factor receptor	cytokine receptor (animals)	Kawasaki *et al*., 2005
Lipase-like	EDS1, PAD4	?	Signalling	Falk *et al*., 1999 Martin *et al*., 2003
NADPH oxidase	RbohD, RbohF	gp91	ROS generation	Torres & Dangl, 2005
iNOS	AtNOS1	iNOS	NO synthesis	Zeidler *et al*., 2004
GSNOR	AtGSNOR1	GSNOR	protein redox	Feechan *et al*., 2005
Lipid hormone	Jasmonic acid	Prostaglandins	hormone	Bergey *et al*., 1996
chitinases	PR3,4,8,11	phagocyte-derived chitotriosidase	antimicrobial proteins	van Eijk *et al*., 2005
Defensins	AFP1.	Drosomycin	antimicrobial proteins	Michaut *et al*., 1996 Thomma *et al*., 2002
PR1-like proteins	NtPR-1a, 1b, 1c	CRISP-3	unknown	Pfisterer *et al*., 1996

Several features, such as the caspase superfamily, TIR- and NB-ARC domains in cell death and immunity in both plants and animals, can be found already in prokaryotes. An evolutionary hypothesis is that these proteins originate from early eukaryote evolution, when the prokaryote organelles still were independent parasites/endosymbionts that when appropriate could kill their host cells (Koonin and Aravind, 2002). One of the most remarkable similarities is the NB-LRR class of plant *R* genes (McHale *et al*., 2006). Also animals have the NB-ARC domain (van der Biezen and Jones, 1998b) in proteins of the NB-LRR class (NODs), which are believed to be involved in the recognition of general pathogen patterns and the regulation of cell death (Inohara and Nunez, 2003). The human

genome contain 25 genes of the NB-LRR class and both human and plant NB-LRR protein function have intimate connections to cell death (Inohara and Nuñez, 2003; Liu *et al*., 2005b). There is, however, no NF-κB in plants, which is a commonly induced cell death associated component in animal innate immunity, but the transcription factor inhibitor I-κB homolog NIM1/NPR1 has a central importance in many disease responses in plants (Ryals *et al*., 1997).

Figure 7. Neighbour-joining tree made from a selection of amino acid sequences of the Nod/NB-ARC/NACHT (NB) domain. No fool-proof correlation between NB-ARC structure and kingdom or domain partners could be seen. Further (parsimony) analysis should be made on more sequences, a more relevant root sequence, and a greater selection of organisms to determine the actual relationships and the evolutionary events leading to domain-swaps.



An = *Aspergillus nidulans* (fungi), Af = *Aspergillus fumigatus* (fungi), At = *Arabidopsis thaliana* (dicot), Ce = *Caenorhabditis elegans* (nematode), Cg = *Chaetomium globosum* (fungi), Cr = *Chlamydomonas reinhardtii* (algae), Dd = *Dictyostelium discoideum* (protist), Dm = *Drosophila melanogaster* (insect), Dr = *Danio rerio* (vertebrate) Hs = *Homo sapiens* (vertebrate), Mb = *Methanosarcina barkeri* (archaea), Pp = *Physcomitrella patens* (moss), , Pr = *Pinus radiata* (gymnosperm), Os = *Oryza sativa* (monocot), Sc = *Streptomyces coelicolor* (bacteria), Zm = *Zea mays* (monocot)

In addition to having similar domain structures, the animal and plant NB-LRR proteins share interacting partners and are both associated to RAR1/CHP1, Hsp90, PP5 and the CS domain found in SGT1 in plants and CHP1 in animals (Hahn, 2005). The fusion of the CS domain from plant SGT1 into animal CHP1 indicates conserved functions of this particular protein-protein interaction (Marcotte *et al*., 1999). Furthermore, rapid elicitation-induced oligomerization of plant NB-LRR proteins, similar to the animal NODs, has recently been demonstrated (Mestre and Baulcombe, 2005). The many similarities between the plant and animal NB-LRR proteins indicate a common function for this class of proteins. Unfortunately, repeating the BLAST analysis using human Apaf-1 NB-ARC sequence (van der

Biezen and Jones, 1998b) against the completely sequenced model protist *Dictyostelium discoideum* and fungal genomes did however not show any obvious NB-LRR motifs in their genomes. *D. discoideum* diverged prior to the split between plants/green algae and metazoans/fungi and would have provided an interesting 'missing link' (Dacks and Doolittle, 2001).

The NB-ARC domain can however even be found in combination with an N terminal TIR domain as far back in evolution as *Archaea* (*Methanosarcina barkeri*) and *Bacteria* (*Streptomyces coelicolor*), which may indicate that this domain combination pre-dates the evolution of eukaryotes. Interestingly, the *Archaea* gene also contains C-terminal WD-40 repeats, which are also found in the animal NB-ARC domain containing cell death regulator Apaf-1. The NB-LRR motif found in proteins involved in innate immunity in both plants and animals is not found in intermediate species or even as high up as *C. elegans* and *D. melanogaster*, which may indicate that the LRR domains have associated to the NB-ARC domain in two independent events (Ausubel, 2005).

In the case of plants, the model algae *Chlamydomonas reinhardtii* shows proteins similar to both TIR-NB-LRR, as found in plants, and NB-TPR, as found in fungi, indicating that the LRR domain existed early in the plant lineage and it can not be excluded that this family has been lost in the intermediate (protist, fungi, invertebrate) species assessed in this analysis, but it seems unlikely. Another interesting observation is the fungal and algae NB-ARC proteins that contain a C-terminal TPR (tetratricopeptide repeat) domain. This domain is found in SGT1b and PP5, which both interact with NB-LRR proteins via the TPR domain in both plants and animals (Hahn, 2005; de la Fuente van Bentem *et al*., 2005). The different protein interaction domains flanking the NB-ARC domain could be examples of evolutionary domain-swaps between interacting proteins (Marcotte *et al*., 1999).

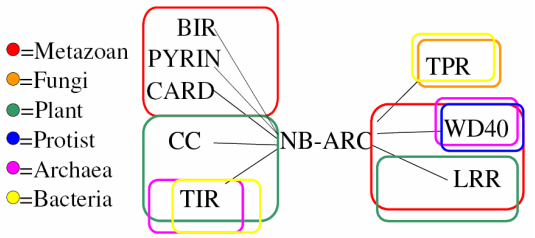


Figure 8. Schematic representation of the domain compositions of NB-ARC proteins in different kingdoms. A domain structure overview of the 'NOD' superfamily can be found in Inohara and Nuñez (2003).

Analyses made on the NB-LRR family in animal systems suggest that these proteins act as direct receptors of certain PAMP motifs, based on overexpression in cell cultures and subsequent challenge with peptidoglycans (Girardin *et al*., 2003). An alternative hypothesis could be that the animal NB-LRR proteins, as proposed by the guard hypothesis for plant NB-LRR proteins, interact with proteins in the TLR-dependent pathways and thus indirectly influence PAMP sensitivity. One indication of this is that the NODs interact extensively with TLR-dependent responses (Strober *et al*., 2006). Alternatively, the NOD proteins could be signalling components downstream of other receptors. For example, NOD1 influences TLR4-dependent LPS recognition (Fritz *et al*., 2005). Analogous results from the plant system would be the recent discovery of a CC-NB-LRR protein

acting as a signalling component downstream of the transmembrane pathogen receptor Cf-4 (Gabriëls *et al*., 2006). If this is the case, the force of conservation between plant and animal innate immunities may be driven by the immuno-suppression mechanisms of the pathogens. There are, however, other indications that favour the concept of direct interaction between pathogen components and the NB-LRR proteins in plants. The strong positive selection on many plant *R* genes indicates a rapid evolution and an "arms race" between pathogen *Avr* and plant *R* genes (Holub, 2001), which is difficult to explain if the interaction is indirect as proposed by the guard hypothesis (Maor and Shirasu, 2005).

Like in plants, viral and bacterial pathogens attacking animals have a wide array of immuno-suppressing mechanisms that enable them to circumvent the innate and adaptive immunity of their hosts (Finlay and McFadden, 2006). Microbial immuno-suppression in bacteria able to infect both plants and animals, such as *Bukholderia cepacia*, *Enterococcus faecalis*, *Erwinia* spp., *Pseudomonas aeruginosa* and *Staphylococcus aureus,* are to a large extent dependent on the same virulence factors during infection of both plant and animal hosts (Hammond-Kosack and Jones, 2000; Cao *et al*., 2001; He *et al.*, 2004; Jha *et al.*, 2005; Prithiviraj *et al*., 2005), indicating conservation of targets of the effector proteins and possibly also of required host factors (Panstruga and Schulze-Lefert, 2003). The plant pathogen *Erwinia carotovora* is also able to suppress LPS responses in cell cultures of *Drosophila melanogaster* (Lindmark *et al*., 2001). Furthermore, the effector protein YopT from the human pathogen *Yersinia pestis* and the avirulence protein AvrPphB from the plant pathogen *Pseudomonas syringae* belong to the same family of cysteine proteases (Shao *et al*., 2002), indicating conserved functions in immuno-suppression. Finally, some bacterial pathogens that cause disease in animals, i.e. *Salmonella enterica* and *Escherichia coli*, are able to spread and proliferate as endophytes over the entire plant and even inside the seeds (Cooley *et al*., 2003), indicating the presence of plant immuno-suppression mechanisms also in those pathogens (Rosenblueth and Martínez-Romero, 2006). Analysis of bacterial and plant mutants has confirmed that the regulation of plant pathogen responses are important for colonization by *S. enterica* in plant tissues (Inguez *et al*., 2005).

In addition, some fungal pathogens, like *Fusarium oxysporum*, can infect both animals and plants (Ortonedra *et al*., 2004). There are even rare cases of *Phoma* sp. and *Leptosphaeria* sp. (phylogenetic relationship unknown) infections in immuno-compromized humans (Mahe *et al*., 1996; Kahtri *et al*., 2002; Everett *et al*., 2003). The mechanisms of immuno-suppression from fungi are however less known in both animal and plant systems. The signal peptide motif of effector proteins from plant pathogenic oomycetes are however surprisingly similar to those found in the malaria parasite *Plasmodium fulciparum* (Ellis *et al*., 2006). The constantly expanding list of characterized effector proteins and refined structural analyses will however facilitate greater understanding of the complex interactions between pathogen and host (Winnenburg *et al*., 2006; Desveaux *et al*., 2006).

Due to the high similarities in virulence determinants of pathogens, irrespective of hosts, a number of genetic host models for pathogenesis are viable options (Pradel and Ewbank, 2004). Taken together, pathogens appear to modulate the defences of plants and animals in a similar manner. This may be a reason for evolutionary conservation or convergence of the plant and animal innate

immunities, in particular the NB-LRR proteins and their interacting partners if they act as guards against such modulations.

The N terminal part of the NB-LRR proteins differs between plants and animals (Inohara and Nuñez, 2003; Inohara *et al*., 2005). The two NB-LRR proteins, Nod1 and Nod2, known to be involved in innate immunity both contain an N-terminal caspase recruitment domain (CARD). Caspases and paracaspase are important signal mediators in animal innate immunity. A role for plant metacaspases has not yet been established in pathogen resistance, but several metacaspases are upregulated by biotic stress. The largest group of Arabidopsis *R* genes is the TIR-NB-LRR class (Meyers *et al*., 2003). The TIR domain is yet another fascinating common component in plant and animal innate immunity. This domain can be found among genes involved in innate immunity in animals, such as the TLR (TOLL like receptors) receptors and the DEATH-TIR adaptor protein MyD88 (Janssens and Beyaert, 2002; Beutler *et al*., 2004). The TIR domain has also predominantly been found in proteins involved in plant innate immunity, which indicates that this domain has some unique features which makes it especially suitable in that context. It is also possible that it is indicative of a similar signalling role in both animal and plant systems.

A TIR adaptor protein like the MyD88 has not been found in plants, but an alternate transcript of the Arabidopsis *R* gene against *Pseudomonas syringae*, *RPS4,* has been shown to be required for the function of full-length (TIR-NB-LRR) *R* gene. This short transcript shows a putative DEATH-TIR domain structure, similar to MyD88. In contrast, a short alternative transcript, *RLM3*, involved in resistance to a large number of necrotrophic fungi in Arabidopsis, only shows a TIR as an obvious domain (**III**). The TIR adaptor protein MyD88 in animal systems has been studied extensively and still is a key component in the understanding of TIR-dependent signalling in animal innate immunity.

The TLR receptors are responsible for most PAMP recognition in animal innate immunity. TLRs can either be expressed extracellularly (TLR1, 2, 4, 5 and 6) or intracellularly (TLR3, 7, 8 and 9) and one TLR is often involved in recognition of several different PAMPs, sometimes in cooperation with another TLR (Akira *et al*., 2006). MyD88 interacts with some of the TLR receptors via a TIR::TIR dimer and recruits kinases (IRAKs, RIPs etc.), depending on TLR, via a DEATH::DEATH dimer (Janssens and Beyaert, 2002). The different TLRs activate different responses, which pathogenic fungi (analogously to plant pathogens) can exploit. By 'hijacking' the immune response – for example by stimulation of TLR2, some fungi can repress the more efficient TLR4 responses (Netea *et al*., 2006). The IRAKs recruited to the TLR receptors via MyD88 are subsequently activating defence responses via MAP3K. The plant genomes contain many IRAK-like kinases and activation of defence responses is in many cases, similar to animal innate immunity, dependent of MAP3K (Dardick and Ronald, 2006).

Why a similar adaptor still is elusive in the plant system is difficult to speculate about, but perhaps it is a tightly regulated interaction which only works after certain modifications. Another reason could be that most plant PAMP receptors, analogously to the flagellin receptor FLS2 (Gomez-Gomez and Boller, 2000) and the rice *Xanthomonas oryzae R* gene *Xa21*, contain a kinase domain whereas the TLR receptors recruit their kinase via TIR adaptor proteins. This does

however not explain what makes the TIR domain especially useful in innate immunity and its role in the NB-LRR type resistance genes in plants. An N-terminal domain specific recruitment of different signalling components to the different classes of NB-LRR R proteins in plants could explain the differential requirement of downstream signalling components identified via genetic screenings (Aarts *et al*., 1998). Based on the ancient evolutionary association between the TIR and NB-ARC domains (Figure 7 and 8), it is very likely that the TIR domain originates from a TIR-NB type protein and has been translocated to a transmembrane-LRR type receptor at a relatively late stage (early animals) to generate the TLR receptor family.

The lipase-like proteins EDS1 and PAD4, which are of central importance to most TIR-NB-LRR *R* gene dependent resistances via SA, show similarity to lipases involved in animal innate immunity signalling (Martin *et al*., 2003). JA-dependent defences also show some similarities to animal immunity. Prostaglandins are hormones involved in animal inflammation responses and are chemically similar to JA. Yet another fascinating parallel is that salicylic acid will inhibit both prostaglandin responses in animals and jasmonic acid responses in plants (Bergey *et al*., 1996), but the two inhibitions occur at different steps since SA blocks COX1/COX2-dependent prostaglandin biosynthesis in animals whereas inhibition of JA responses by SA is mediated via NPR1.

Despite many similarities between plant and animal innate immunity, some may be coincidental or an effect of convergent evolution (Ausubel, 2005). In conclusion, there are many independent, striking similarities between plant and metazoan innate immunity and future comparative studies of the fundamental mechanisms in innate immunity and its relationship to other cellular mechanisms may unveil central components in the pathogen responses among eukaryotes. Since our current knowledge about the processes involved in innate immunity within both plants and animals are incomplete – comparative studies may also help to fill in some gaps in both systems (Hunter, 2005). In particular, it would be interesting with an "evo-devo" approach on the evolution of the superfamilies of NB-ARC containing proteins, caspase-like proteins and TIR-containing proteins (Koonin and Aravind, 2002), their protein interaction domains, and their roles in cell death and immunity in plants, animals and more primitive organisms.

Brassica crops

Origin of *Brassica* crops

Brassica crops are a very diverse group of plants, which ranges from brussels sprouts and broccoli to turnip, mustard and oilseed rape. *Brassica* oil crops were first cultivated in Asia and the Mediterranean for cooking and lamp oil and were first mentioned in Sanskrit literature ~1500 B.C. Despite this great variation in phenotypes and uses among *Brassica* crops, most derive from three diploid parental species (Figure 9): *B. nigra* (black mustard), *B. oleracea* (cabbage) and *B. rapa* (turnip rape) and their interspecific hybrids *B. napus* (ssp. *oleifera*: oilseed rape and ssp. *rapifera*: swede), *B. juncea* (Indian/Brown mustard) and *B. carinata* (Abyssinian mustard).

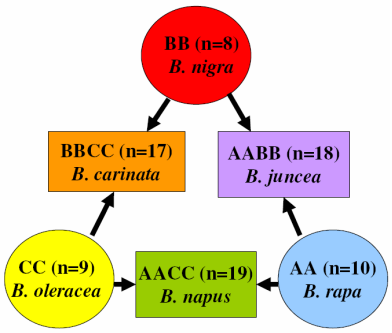


Figure 9. The phylogenetic relationship between the allotetraploid and diploid *Brassica* species as proposed by U (1935).

There are also other cultivated species within *Brassicaceae* in use for agriculture, e.g. *Brassica tournefortii* (Asian mustard), *Eruca sativa* (arugula), *Raphanus sativus* (radish), *Sinapsis alba* (white mustard) and *Wasabi japonica* (wasabi).

Use and importance of *Brassica* crops

Among the *Brassica* crops, the oilseed species are the most valuable on the world market. Modern versions of *Brassica* oils are often of the Canola quality, which means that they contain low levels of erucic acid and glucosinolates. In North America "canola" is synonymous with oilseed *Brassica* crops, irrespective of species. *Brassica napus* has the highest yield potential under favourable conditions, depending on agricultural practice and geographical region. Due to unfavourable conditions, such as low temperatures, drought and short growth seasons, other *Brassica* species are used as oil crops. In Sweden, oilseed *Brassica* crops are primarily *B. rapa* and *B. napus*, whereas parts of Canada also use *B. juncea* for its drought hardiness.

Brassica oils are generally rich in long multi-unsaturated omega 3 fatty acids (Vles and Gottenbos, 1989) and are the only bio-oils that reach the technical standards as alternative fuel within the European Union (EU) due to its cold hardiness and water stability (Ulf Lindgren, Lantmännen Energi, Personal communication; Körbitz, 1995). In addition to oils, they also contribute with a protein rich oilseed cake for animal feed (Bell, 1995) and are ideal crops for crop rotation to reduce soilborne pathogens that challenge economically important crops as wheat (Smith *et al*., 2004) and strawberry (Lazzeri *et al.*, 2003) and other effects due to allelopathy (Marquard and Walker, 1995). There is also a correlation between the consumption of cruciferous vegetables and cabbage, but not other vegetables and fruits, and a decreased risk of developing pancreatic cancer (Larsson *et al*., 2006). The future prospects of *Brassica* oil crops could be engineered oils with enhanced health properties (Domergue *et al*., 2005) and proactive vegetables against cancer as "functional foods" or engineered oils for technical purposes as lubricants, plastics and detergents (Murphy and Mithen, 1995; Poirier, 2001).

Diseases on *Brassica* oil crops world wide

Brassica oil crops are heavily challenged by various fungal pathogens and insects, and to some extent oomycetes, whereas bacterial and viral diseases have little

effect on yield. The relative importance of various pathogens varies between geographical locations and agricultural practices. For reference, there are excellent descriptions and beautiful illustrations of the various diseases and pests on oilseed rape in Paul (2003).

The most devastating fungal diseases for *Brassica* oilseed production are **Verticillium wilt** (*Verticillium dahliae* and *Verticillium longisporum*), **Blackleg** (*Leptosphaeria maculans* (anamorph: *Phoma lingam*)), **black spot** (*Alternaria brassicae* and *A. brassicicola*), **light leaf spot** (*Pyrenopeziza brassicae* (anamorph: *Cylindrosporum concentricum*)) and **stem rot** (*Sclerotinia sclerotiorum*) (Rimmer and Buchwaldt, 1995; Dixelius *et al*., 2004). In addition to these fungi, the oomycete **white rust** (*Albugo candida*) disease affects *B. rapa* and *B. juncea* oilseed crops. Also, **club root** (*Plasmodiophora brassicae*), **grey mold** (*Botrytis cinerea*), **white leaf spot** (*Mycosphaerella capsellae*) and **downy mildew** (*Hyaloperonospera parasitica*) are found on oilseed *Brassica* crops and cause problems in some regions (Rimmer and Buchwaldt, 1995; Terwari and Mithen, 1999).

Bacterial diseases affecting *Brassica* crops are **black rot** (*Xanthomonas campestris* pv. *campestris*), **soft rot** (*Erwinia carotovora*) and **bacterial leaf spot** (*Pseudomonas syringae* pv. *maculicola*). There are approximately 11 species of viruses affecting *Brassica* crops, and some cause significant yield damage in regions of China (Rimmer and Buchwaldt, 1995). In addition to fungal diseases, insects are a big problem for *Brassica* oil crops. The insect pests most devastating for oilseed production are usually crucifer specialists. A special feature of the crucifer specialists is that they use specific glucosinolate compounds or degradation products present in various *Brassicaceae* as attractants, whereas the glucosinolates usually are deterrents and toxic for generalist insects (Ekbom, 1995).

The fungal pathogen *Leptosphaeria maculans*

L. maculans importance

L. maculans causes the devastating blackleg disease on *Brassica* crops with a nearly world-wide distribution (Fitt *et al*., 2006a), which can lead to stem cankers and severe yield loss. Yield losses up to 100% due to *L. maculans* have been recorded. The onset of *L. maculans* epidemics is usually associated to an expansion of the area of cultivated *Brassica* crops (Rimmer and Buchwaldt, 1995). The oilseed rape industry of Australia was completely wiped out due to *L. maculans* in the 1970s, where the planted oilseed rape declined from 49 000 ha in 1972 to 2000 ha in 1974 (Barbetti *et al.*, 1975; Bokor *et al.*, 1975). At approximately the same time, highly virulent *L. maculans* emerged in Canada (Petrie, 1978), Germany (Krüger, 1982; Seidel *et al*., 1984) and Kenya (Piening *et al*., 1975). The disease is also currently a big problem in Australia, Canada and Europe, but not in China and India. The reason for this may be that stubble is removed from the fields in China and India, and that oilseed production in India mainly is cultivated (drought resistant) *B. juncea*, which due to the B genome is resistant to blackleg (Rimmer and Buchwaldt, 1995). Chinese *Brassica* cultivars are generally very susceptible to *L. maculans*, which has raised concerns about maintaining hygiene for materials

transferred to China since many Chinese subsistence farmers rely on *Brassica* crops (Fitt *et al*., 2006a).

Field scorings of *L. maculans* infection are often complicated under Swedish field conditions, since the leaf symptoms often are confused with black spot (*Alternaria brassicae/A. brassicicola*) and the stem symptoms are often confused with Verticillium wilt (Dixelius, 2003). *L. maculans* may also in combination with Verticillium wilt cause "*pieds sec*", premature senescence of *B. napus* (Rimmer and Buchwaldt, 1995). Multi-virulent isolates of *L. maculans* were also recently confirmed to be present in Sweden (Kuusk *et al*., 2002; Stachowiak *et al*., 2006). The rapid adaptation of the pathogen enables it to break most major gene resistances within 2-3 years of commercial introduction, making it a particularly difficult pathogen to control (Sprague *et al.*, 2006).

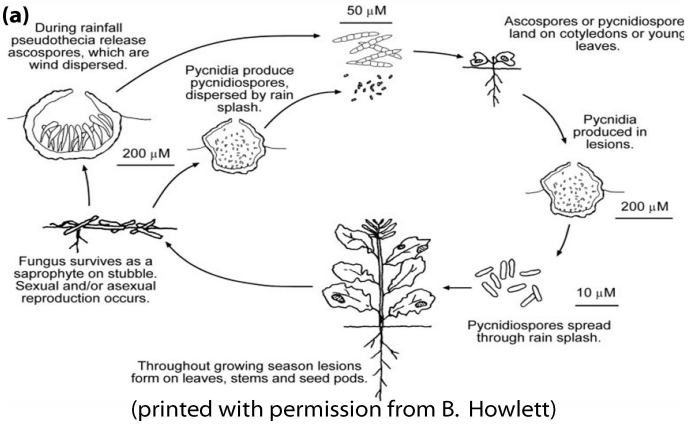
The annual costs of blackleg (*L. maculans*) are estimated to range between 11.3 and 30.1 million € in Australia, 36.8 and 147 million € in France and 14 – 56 million € in the U.K (Fitt *et al*., 2006a). As a comparison (the years between 1987 and 2002), light leaf spot (*Pyrenopeziza brassicae*), considered the most serious *Brassica* disease in the U.K. (Rimmer and Buchwaldt, 1995), accounted for an estimated annual loss of 28 million €, and black spot (*A. brassicae*) only between 0.4 and 2 million € in the U.K. (Fitt *et al*., 2006a).

In order to encourage research on this devastating pathogen, the international blackleg on crucifers network (IBCN) was founded in 1994, managing a collection of approximately 90 isolates with variable properties (Howlett *et al.*, 2001). Due to the well-characterized gene-for-gene interactions between *Brassica* and *L. maculans* and the possibilities of sexual crosses and fungal genetics (Williams, 1992; Kuhn *et al*., 2006), *L. maculans* is becoming an excellent model system for studying the genetics of host-pathogen interactions (Fitt *et al*., 2006b). Significant resources are invested in *L. maculans* research and this devastating pathogen is about to get a fully sequenced genome in an INRA-PMDV and UNI-Melbourne initiative (Rouxel and Balesdent, 2005; Xu *et al*., 2006). See also (http://www.cns.fr/externe/English/Projets/Projet_DM/organisme_DM.html).

L. maculans biology

L. maculans (anamorph: *Phoma lingam*) is probably composed of several morphologically similar species, where the "group A" type is most devastating on *Brassica* oilseeds (Howlett *et al.*, 2001). An early and commonly used isolate structure division of the *L. maculans* "A group" into 3 pathogenicity groups (PG2-PG4; Koch *et al*., 1991) relies on their virulence on the *B. napus* cultivars Westar, Quinta and Glacier. The non aggressive (NA) "B group" (PG1) is further genetically defined as sub-categories NA1 (now *L. biglobosa*), NA2 (chemical and morphological relationship to *Phoma wasabie*) and NA3 (only one isolate), but there are also here difficulties to discriminate between the virulence patterns based on genetic markers (Howlett *et al.*, 2001). Genetic analysis could clearly distinguish the three NA groups in the "B group", but all "A group" isolates clustered together (Koch *et al*., 1991). Recent analyses have investigated the evolution of *L. biglobosa* (B group NA1) as a consequence of coexistence with *L. maculans* in different niches (Fitt *et al*., 2006c).

Extensive race structure studies of the "A group" show that the PG division was flawed and had very little correspondence with genetic relationships between isolates and response patterns to other cultivars than the ones originally used for the PG division (Balesdent *et al.*, 2005). In *L. maculans* (A group), 9 different *Avr* genes, corresponding to different *B. napus R* genes, were found to more satisfyingly describe the race structure (Balesdent *et al*., 2005). *L. maculans* has a sexual stage in its life cycle (Figure 10), which enables it to circumvent resistance in *Brassica* crops by recombination. The reproductive strategy of *L. maculans* makes it a "very high risk" pathogen for breakdown of resistance (McDonald and Linde, 2002). This highlights the importance of knowing the race structure of the pathogen in order to efficiently manage the disease by new breeding strategies and agricultural practices.



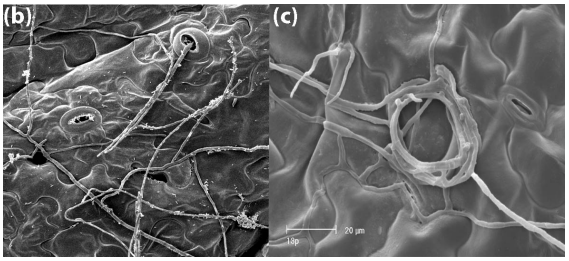


Figure 10. (a) Life cycle. *L. maculans* is spread either via wind (ascospores) or rain splash (ascospores and conidia) and grows initially as a biotroph before switching to a necrotrophic phase late in its infection cycle to generate pycnidia. (b) Mycelia growing through stomata. (c) Possibly initiation of pycnidia formation on the leaf surface. At a macroscopic level, however, pycnidia appear to be formed below the surface of the leaf, emerging from faint black pots that turn purple as they mature.

L. maculans enters the plant tissue preferably via stomata or wounds, then grows in the intercellular spaces of the mesophyll layer and enters the vascular tissue, primarily the xylem, for spreading into systemic leaves and the stem (Hammond *et al*., 1985; Hammond and Lewis, 1987). Pycnidia are formed around the necrotic lesions and disperse conidia for secondary infections (Williams, 1992). Ascospores are wind dispersed and are clearly more aggressive than conidia. In laboratory conditions *B. napus* inoculated with conidia need 10^6-10^7 conidia/ml and wounding to enable entry, whereas ascospores can be applied as a droplet of 10^3 ascospores/ml without wounding (Huang *et al*., 2006b). Ascospores can also effectively be applied as a "shower" by putting agar plates with pseudotheica (that release ascospores) above the plants to be inoculated (Huang *et al*., 2006b). *L.*

maculans is primarily seed borne, but spores are viable for a long time in the soil and can even survive passage through farm animals (OEPP/EPPO, 1994).

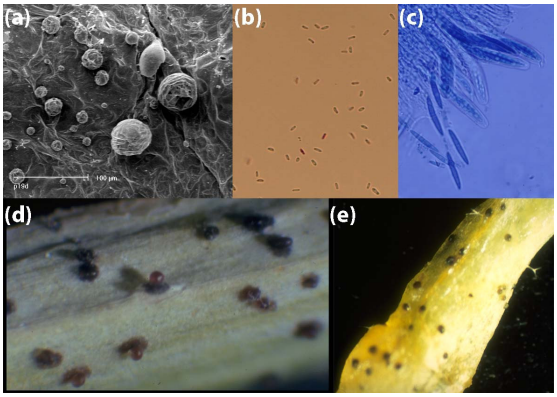


Figure 11. *L. maculans*. (a) SEM picture of pycnidia that contains asexual spores (conidia), (b) conidia, (c) sexual spores (ascospores), (d) pycnidia on a *B. napus* stem, (e) pycnidia on a susceptible (*lms1*) Arabidopsis leaf.

Furthermore, disease develops much more rapidly on *B. napus* inoculated with ascospores, compared to conidia. When the infection reaches the cortical cells of the stem, a blackened canker is formed, hence the name blackleg (Figure 12). The stem cankering is the major cause of yield loss in *Brassica* associated to blackleg disease, since the plants lodge and die without producing seeds.

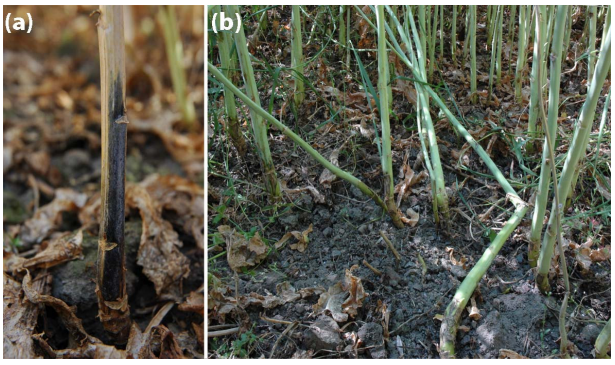


Figure 12. Field symptoms of *L. maculans* (a) blackleg and (b) stem cankering on *B. napus*. Hammarlöv, Sweden, 2006.

Photo: Matti Leino.

Despite its intercellular growth, *L. maculans* harbour cell wall degrading enzymes, but they do not seem to be of central importance for pathogenesis (Sexton *et al*., 2000). Isocitrate lyase, on the other hand, was found to be essential for pathogenicity. This indicates that the glyoxylate pathway, analogously to other pathogenic fungi and bacteria on plants and animals, is important for *L. maculans* pathogenesis (Irdum and Howlett, 2002).

A recent survey of *L. maculans* isolates in southern Sweden showed that there were relatively high levels of multi-virulent isolates present, compared to most parts of Europe (Stachowiak *et al*., 2006). The authors assumed that the high levels of multi-virulent isolates in Sweden was due to former use of old *Brassica* cultivars in Sweden and possibly also cultivation of swedes and that they may contain many of the *R* genes used in modern commercial *Brassica* breeding. An alternative possibility could be that the *R* gene structure in wild *Brassica* hosts may differ

between geographical locations and thus maintain virulent strains in the different populations. Considering the rapid adaptation of the *L. maculans* population and the extensive use of international *Brassica* cultivars in Sweden, this second alternative appears more likely. The use of a "trap cultivar" lacking all known *R* genes for evaluations of race structure (Balesdent *et al*., 2006; Stachowiak *et al*., 2006) may however give a biased output favouring avirulent isolates, since virulence and aggressiveness (fitness) generally are negatively correlated. In the case of *AvrLm4*, a strong selective advantage for the avirulent allele was found in the absence of *Rlm4* (Huang *et al*., 2006b), but considering the frequency of virulent races found already prior to the introduction of an *R* gene in commercial agriculture it is expected that most virulence alleles are associated to a small cost or are completely neutral (Aubertot *et al*., 2006; Balesdent *et al*., 2006), alternatively they are maintained on wild hosts harbouring the corresponding *R* gene.

The first *L. maculans Avr* (*AvrLm1*) gene was recently identified and cloned (Gout *et al.*, 2006). In contrast to bacterial *Avr* genes, little is known about the mechanisms and functions of fungal *Avr* genes. Most fungal *Avr* genes encode small proteins, which seem to be secreted (Rep, 2005), which may indicate that they are involved in defence suppression, as seen for *C. fulvum Avr2* (Rooney *et al*., 2005). The genetics of the 9 currently known *AvrLm* genes indicates that they are inherited as two clusters, *AvrLm1-2-6* and *AvrLm3-4-7-9* (Rouxel and Balesdent, 2005). The *AvrLm1* gene is a constitutively expressed gene encoding a small secreted protein with no close homologs among sequenced fungi and no obvious protein domains except for the secretory signal (Gout *et al.*, 2006).

Complex interactions between fungal stresses and climate on *Brassica* oil crops in Sweden and their influence on yield

Fungal diseases pose a great threat to Swedish oilseed production, but the quantitative influence from different pathogens has been difficult to assess, due to the complex interactions between different diseases and abiotic stresses. Multivariate analyses of climatological variables (monthly precipitation and temperature) and biotic stresses versus yield in southern Sweden (Skåne) between 1988 and 2004 using PLS in SIMCA (Umetrics, Umeå, Sweden) revealed a major role for Verticillium wilt for winter oilseed rape yield (Johansson, 2006), whereas blackleg (*L. maculans*) had a moderate influence and stem rot (*S. sclerotiorum*) no detectable effects on yield (not shown). An additional biotic stress not included in the data with possible significant influence on Swedish oilseed yield is black spot (*A. brassicae*). No models with satisfactory descriptive and predictive qualities could be generated for spring oilseed rape (not shown), indicating that the yield of this crop is determined by other variables not included in this analysis, possibly pollen beetles.

The major impact of Verticillium wilt is in agreement with previous observations and breeding attempts to introduce multigenic resistance traits from both *B. oleracea* and *B. rapa* into *B. napus* are currently being made (Dixelius *et al*., 2005). In contrast to *V. dahliae*, *V. longisporum* has a clear preference for *Brassicaceae* hosts and is the species primarily found in Swedish oilseed rape affected by Verticillium wilt (Johansson *et al.*, 2006b). The major problems with

Verticillium in Swedish soils probably derive from a history of prolonged *Brassica* monocultures with limited crop rotation (Johansson, 2006). Winter oilseed rape yield could be explained by temperature in September and May together with Verticillium wilt incidence with a relatively high descriptive (R^2 = 0.839) and predictive (Q^2 = 0.832) power in a model with two principal components (Algotsson, 2004; Johansson, 2006; Staal, unpublished results).

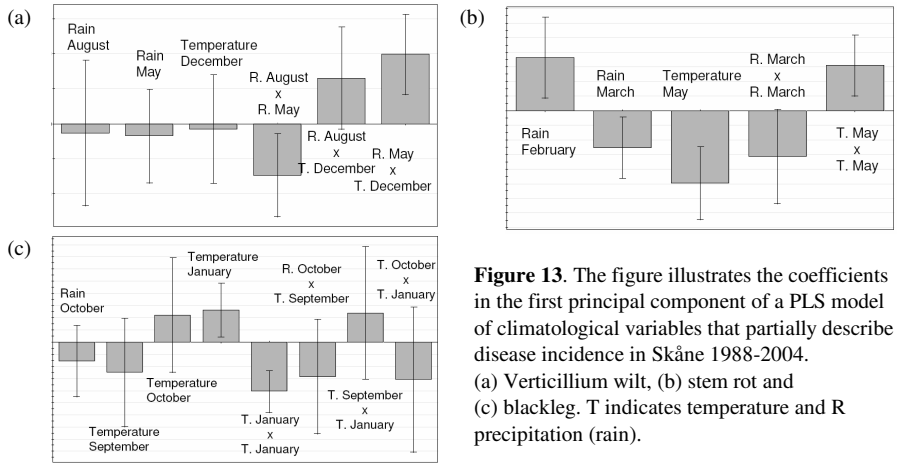


Figure 13. The figure illustrates the coefficients in the first principal component of a PLS model of climatological variables that partially describe disease incidence in Skåne 1988-2004. (a) Verticillium wilt, (b) stem rot and (c) blackleg. T indicates temperature and R precipitation (rain).

Correlations between climatological variables and disease incidence showed that Verticillium wilt (R^2 = 0.878, Q^2 = 0.737) and stem rot (R^2 = 0.887, Q^2 = 0.798) incidence could be reasonably well explained by a few climatological variables, whereas blackleg could not (Figure 13). The extremely complex models to explain blackleg incidence and its low descriptive (R^2 = 0.761) and predictive (Q^2 = 0.353) qualities suggest that the variation in climatic factors found in this region during the years assessed have had a minor influence on this pathogen. It does however not exclude that greater climatic variations may have a profound effect on the disease. This raises the issue of a changing climate, which may also affect the incidence of this pathogen. It is likely that such a scenario is valid since blackleg poses a great threat to *Brassica* production in areas with a warmer climate (e.g. France). Furthermore, controlled experiments have shown that single gene resistances against *L. maculans* are broken by high temperatures and humidity (Huang *et al*., 2006a; **V**).

Taken together, these gene-by-environment relationships indicate that *L. maculans* has a potential to become a more serious threat in Sweden as a consequence of global warming, since the consensus of various meteorological models predict an increase in both temperature and precipitation in Northern Europe (Déqué *et al*., 2005). Predictions of plant disease incidence based on such climatological models are however difficult to make (Garret *et al*., 2006). Controlled field experiments in the UK have previously determined that *L. maculans* operates under a wide range of temperatures and that climatic factors primarily influence the incidence of stem cankers by affecting the early phases of infection (Hammond and Lewis, 1986). Climatological variables, agricultural practice and disease incidence the previous year are variables used for an *L. maculans* disease incidence forecast in UK (http://phoma.csl.gov.uk).

The low impact of *L. maculans* on yield and the low correlation between blackleg and climate in the Swedish data may however partly be due to difficulties scoring blackleg disease incidence in a consistent manner over the years and that the data represents average values over a large region. None of the diseases showed any significant influence from the disease incidence of the previous year when only climatological variables were included. Interestingly, when the other biotic stresses were included in the model, *L. maculans* incidence could partly be described (R^2 = 0.769, Q^2 = 0.518) with a positive influence from *Verticillium*, precipitation in February, the incidence of *L. maculans* the previous year and a negative influence from *Sclerotinia*. Of the four variables, the previous year's *L. maculans* incidence was the least significant and *Verticillium* the most. Further, the reciprocal relationship was also true for *Verticillium* incidence, where *L. maculans* incidence and the previous year's incidence had a major influence. *Sclerotinia* incidence, on the other hand, was exclusively affected by climatological variables. The great overlap between *L. maculans* and *Verticillium* in Sweden could also cause an under-estimation of the influence from blackleg on Swedish yield.

Systems biology studies of the interactions between environmental factors, biotic stresses and host responses in well-defined model systems under designed experimental conditions may give us novel data for refined models and some insight in the biology of field disease resistance mechanisms. For example, the genetic and environmental factors influencing the well-known interaction (in field conditions) between *L. maculans* and *Verticillium* and the development of '*pieds sec*' (Rimmer and Buchwaldt, 1995) could be studied using the Arabidopsis system. Preliminary results in Arabidopsis confirm that there is an interaction between the two pathogens.

L. maculans resistance genes and resistance breeding in *B. napus*

B. napus responds to *L. maculans* infection via necrosis of guard cells near arrested hyphae, phytoalexins, callose deposition and lignin production, accumulation of pectin in the lumen of xylem vessels and induction of PR proteins (Chen and Howlett, 1996; Roussel *et al*., 1999; Howlett *et al*., 2001). Proteomic analysis has however not established any *R* gene dependent PR protein expression (Subramanian *et al.*, 2005). Most *L. maculans* resistance identified has been a gene-for-gene type of resistance, but there are also examples of multigenic QTL-type resistances (Delourme *et al*., 2004).

The type of gene-for-gene resistance is of importance for the efficiency of the response. An experiment using the tomato *Cf9* - *C. fulvum Avr9* induced response in *B. napus – L. maculans* interactions delayed, but could not block, *L. maculans* infection (Hennin *et al*., 2001). One possible explanation for this could be that *C. fulvum* primarily relies on biotrophic growth, whereas *L. maculans* opportunistically can switch to necrotrophic or saprophytic growth also early during infection. Defences against a pathogen with multiple life styles pose a particular challenge for the plant, due to the devastating potential of *L. maculans*, blackleg resistance is one breeding goal of the utmost importance for *Brassica* crops (Becker *et al*., 1999).

Great effort has been put into the identification of the single resistance loci regulating *L. maculans* resistance (Li and Cowling, 2003; Mayerhofer *et al.*, 2005;

Saal and Struss, 2005; Yu *et al*., 2005; Christiansson *et al*., 2006), but also resistance QTLs limiting *L. maculans* disease severity (Pang and Halloran, 1996; Yu *et al*., 2005) in various *Brassica* oil crop species. One breeding strategy has been to introduce resistance genes into *B. napus* from wild collections or old accessions of the parental species *B. rapa* and *B. oleracea*, either via re-synthesis or backcrossing. Screenings of the two parental species have revealed that *L. maculans* resistance genes reside in the A genome (*B. rapa*) and are distributed over several sub-species (*chinensis, japonica, oleifera, parachinensis, pekinensis, periviridis, rapifera, sylvestris, trolocularis*), whereas the C genome (*B. oleracea*) completely lacked *L. maculans* resistance (Delourme *et al*., 2006). A close relative to *B. oleracea*, *B. insularis* (also n=18), does however harbour novel resistance genes which could be used for resistance breeding (Delourme *et al*., 2006).

One commercial example of the introduction of resistance from wild *B. rapa* is the highly resistant cultivar Surpass 400. Surpass 400 contains a single dominant locus, derived from a resistant *B. rapa* ssp *sylvestris* and stabilized by back crosses to *B. napus*. The *R* gene *LepR3* in Surpass 400 regulates both seedling and adult leaf resistance (Li and Cowling, 2003). Analogously to other described *L. maculans R* genes in *B. napus* and Arabidopsis, *LepR3* is associated to *L. maculans* induced callose depositions (**IV**). Introgression of resistance traits from other species than the direct parental species is another possible breeding strategy. One problem with back-crossing wild *Brassica* species with commercial *B. napus* cultivars is that the crosses loose their canola quality, which has to be re-established in the new cultivar.

Beating breakdown of resistance: Strategies for durable *L. maculans* resistance

In order to generate stabile resistance against a pathogen with a sexual stage in its life cycle, we need to find novel resistance genes or resistance mechanisms. Currently available single dominant resistance genes are rapidly broken in areas with frequent sexual recombination, like Australia, which has led to the suggestion that resistance breeding against *L. maculans* should focus on multigenic (QTL-type) resistances and altered agronomical practices to decrease the inoculum latent in debris between the disease cycles (Sivasithamparam *et al*., 2005).

The ability to resist *L. maculans* toxins, such as sirodesmin, is a “basal” resistance trait that can limit fungal growth (Sjödin and Glimelius, 1989). Sirosdesmin resistance could in theory be an interesting broad-range resistance, since similar toxins are produced by a wide range of pathogens (Gardiner *et al*., 2004). Another potentially interesting resistance mechanism is to modify the phytoalexin biosynthesis of the plant (Pedras *et al.*, 2002), but due to the complex interactions between secondary metabolism and disease resistance signalling – this might not be a viable option. An alternative is to design specific inhibitors of phytoalexin detoxification enzymes to use as fungicides (Pedras and Jha, 2006). The traditional method is however to introduce resistance genes from more distant relatives in order to generate a more durable resistance. This can be done by sexual crosses between *Brassica* species or somatic hybridization (Glimelius *et al*., 1991), which has been successful for introduction and mapping of resistance components from the *B. nigra* and *B. juncea* B genome (Sjödin and Glimelius, 1989; Dixelius,

1999), *Sinapsis arvensis* (Hu *et al*., 2002a) and Arabidopsis (Forsberg *et al*., 1994; Bohman *et al.*, 2002) into *Brassica napus.* Other *Brassicaceae* species used for identification of novel *L. maculans* resistance traits are *Coincya monensis, Diplotaxis muralis, Diplotaxis tenuifolia* and *Raphanus raphanistrum* (Delourme *et al*., 2006).

A problem with interspecific hybrids is that the recombination between genomes is very low and the introduced traits often are inherited in a non-Mendelian, instable manner, which partly can be addressed by using asymmetric somatic hybrids (Dixelius, 1999; Bohman *et al*., 1999; Hu *et al*., 2002b). Resistance genes (*Rlm6, r$_j$lm2*) have however been introgressed from the *Brassica* B genome, but appear as breakable as genes already in use and virulent alleles were already present in the *L. maculans* population structure before introduction (Balesdent *et al*., 2006; Sprague *et al*., 2006). An alternative to mapping in B-genome derived resistances in *B. napus* background is to map them directly in the *B. juncea* background, which addresses the issue of the low recombination found in the introgressed segments (Christianson *et al*., 2006). The B genome contains multiple resistance traits and field experiments have shown that an addition line containing a whole B genome chromosome was more durable than the single gene introgression (Delourme *et al*., 2006).

In recent years canola quality *B. juncea* has also been grown in dry areas of Australia with a yield equal to high performance *B. napus* cultivars (Norton *et al.*, 2004). The reason for this is however just the higher drought hardiness of *B. juncea*, and the huge impact of blackleg on Australian *B. napus* yields. An interesting alternative to introgression of single B genome components into *B. napus* (higher-yielding under favourable conditions) may be to generate a canola quality allohexaploid (AABBCC genome) *Brassica* oil crop, combining the strengths of *B. napus* and *B. juncea*. A theoretical additional advantage of such a crop would be that it would have three parental species (and additional closely related species) from which new traits could be introgressed via backcrosses or re-synthesis, as is currently done in *B. napus* breeding programmes. There may however be agronomical reasons or issues of genome stability as arguments against such a crop. The three genomes may also amount to a chromosome number above the optimal number of chromosomes for *Brassica* crops (*prof. eremitus* Olsson, G., Svalöv, personal communication). Another potential problem is that polyploidization and hybrid *Brassica* genomes cause multiple novel gene and protein expression patterns not found in the parental genomes (Albertin *et al*., 2006), which potentially could cause unexpected and unwanted phenotypes.

Both gene-for-gene type and QTL-type resistances are a viable option for novel *L. maculans* resistance. QTL-type resistance does not “break” like gene-for-gene type resistance, but may erode over time due to genetic adaptation of the pathogen population (McDonald and Linde, 2002). Erosion of *L. maculans* resistance does however not generate as severe results as seen when *R* genes are broken (Delourme *et al*., 2006). It is however very attractive to find single genes that give a strong and durable resistance to *L. maculans* and that can rapidly be introduced into existing high-performance cultivars to challenge the threat of blackleg disease. A gene-for-gene resistance may be useful against a pathogen with sexual reproduction if the corresponding avirulence gene either is present in all isolates (race non-specific resistance; Hammond-Kosack and Parker, 2003), the

lack of the avirulence gene occurs in a very low frequency or the loss of the avirulence gene is associated to a significant cost/decreased aggressiveness for the pathogen (Mac Key, 1981; Vera Cruz *et al*., 2000). The durability ("usefulness time") and fate of resistance is a function of the fitness (aggressiveness) cost of virulence and the proportion of the crops used that contain the *R* gene (Vera Cruz *et al*., 2000; Pietravalle *et al*., 2006). Furthermore, if the novel avirulence gene is present in all current strains of *L. maculans* the resistance is expected to be more durable, since *L. maculans* then needs to rely on mutation rather than sexual recombination to break the resistance, which is a slower process.

By growing mixed lines or synthetic varieties, compromising of several different *R* genes, the outbreak of epidemics could also be limited (Dangl and Jones, 2001). Segregating resistances does however also pose a threat when challenged by a pathogen with sexual recombination, since susceptible or partially susceptible individuals will ensure constant contact with resistant material and thus encourage the evolution of virulence. Segregating populations is also not always an option, since homogenous material often is needed for agronomical and quality control reasons. Another strategy to control the breakdown of resistance is pyramiding (Chalal and Gosal, 2002), which means that several resistance genes are introduced to the cultivar. This approach may be broken if there are multi-virulent isolates present in the population or if the genes also are used as single genes in commercial crops – favouring development of multi-virulent isolates (Aubertot *et al*., 2006). The high level of recombination between *AvrLm* loci seen in detailed studies of the race structure does however question the usefulness of pyramiding as an effective strategy to control *L. maculans* (Balesdent *et al*., 2006). Combining a novel resistance trait with a number of others does however decrease the risk that a randomly occurring (mutated) strain of *L. maculans* can overcome the novel form of resistance.

Yet another aspect of stacking resistances is the opposing roles of virulence and aggressiveness, where we can expect a multi-virulent strain to be less aggressive and thus we can get a quantitative effect from stacking multiple *R* genes in our crop (Mac Key, 1981). Such strategies may however be hampered by a cost of resistance for the plant (Tian *et al*., 2003). Novel resistance traits can also be more efficiently used by well-planned integrated disease management (IDM) strategies by taking the dynamics of the pathogen population structure into consideration (Okori, 2004). For *L. maculans*, it is very attractive to decrease the pathogen population and maintain the control possibilities that the *Avr* alleles offers via an integrated avirulence management (IAM) strategy. IAM would focus on spatially and temporally alternating *R* genes (and chemical treatments) and thus decrease the number of virulent isolates at the start of each disease cycle (Aubertot *et al*., 2006). This kind of management will however require a great deal of coordination (Gladders *et al*., 2006).

The Arabidopsis model system – novel genetic and genomic tools for disease resistance research

The model organism *Arabidopsis thaliana* is closely related to the *Brassica* genus, but Arabidopsis has several advantages compared to *Brassica* species for genetic analyses. The favourable biology, such as a large progeny, inbreeding reproductive

strategy and short generation time makes it an ideal genetic model organism. The chromosome number n=5 was determined already in 1907 by von Laibach and *Arabidopsis thaliana* was initially proposed as a model plant over 60 years ago (von Laibach, 1943). Arabidopsis has since undergone extensive investigations (Somerville and Koornneef, 2002) and was the first plant to get a completely sequenced genome (The Arabidopsis Genome Initiative, 2000).

The availability of well-characterized mapping populations (Lister and Dean, 1993; Alonso-Blanco *et al.*, 1998), the large number of characterized mutants and T-DNA insertion mutants in practically every gene (Alonso *et al.*, 2003) are all invaluable resources for gene identification and characterizations of physiological mechanisms. In particular, the recombinant inbred line (RIL) populations have unlocked a great potential for gene identification by segregation analysis (Koornneef *et al.*, 2004). European and American initiatives are currently generating a large number of new RIL populations. In recent years, there has also been a great interest in haplotyping of a large number of accessions for linkage disequilibrium (LD) high resolution mapping and identification of QTLs as an alternative to segregant analysis.

The inbreeding reproductive strategy of Arabidopsis makes this an ideal organism for LD mapping approaches. A number of techniques have been developed for high throughput haplotyping, like Affymetrix array-based single feature polymorphisms (Borevitz and Nordborg, 2003) and ecotilling (Comai *et al.*, 2004). As a proof of concept, LD mapping was able to identify previously characterized genes for flowering time and pathogen resistance from a population of 95 haplotyped accessions (Arazana *et al.*, 2006). Within a year, data on over 250,000 SNPs will be available for over 1000 Arabidopsis accessions, which will give a great boost to the LD approach on mapping genes (Nordborg, M., Department of Molecular and Computational Biology, University of Southern Califonia, personal communication).

Another technique for functional analysis of the natural variation in specific traits is eQTL analysis, where microarray transcription profiles are treated as quantitative traits and compared to a genetic map to find markers associated to gene expression regulatory loci. Particularly very specific microarray comparison conditions and well-defined QTL assessments can benefit from such analyses. The eQTLs are especially informative when phenotypic QTLs are known, since the eQTL gives information about potential downstream genes of the phenotypic QTL (DeCook *et al.*, 2006). Arabidopsis accessions show great differences in transcriptional responses after pathogen or SA treatments (Kliebenstein *et al.*, 2006). Despite this, no large scale attempts have yet been published to associate major pathogen response differences by segregant analysis or by associations to specific haplotype patterns in an “eLD” analysis.

Another pathogen response component suitable for genetic analysis is camalexin induction, which shows clear accession differences after pathogen and abiotic challenges, but different stresses give different camalexin response patterns in different accessions (Kagan and Hammerschmidt, 2002; Denby *et al.*, 2004; Schuhegger *et al.*, 2006). The low correlation in relative camalexin induction levels between treatments and the observation that camalexin biosynthetic genes are differentially regulated in different accessions indicates that the natural

variation is due to differences in stress perception and signalling among the accessions rather than biosynthetic efficiency (Schuhegger *et al*., 2006).

Aims of the study

The overall aim was to use the model organism *Arabidopsis thaliana* to study its high level of resistance to the *Brassica* blackleg pathogen *Leptosphaeria maculans*. Specific aims were to:

- Characterize the Arabidopsis – *Leptosphaeria maculans* pathosystem.
- Identfy resistance genes segregating in crosses between the Arabidopsis accessions Landsberg *erecta* and Columbia.
- Identify the natural mutation in Arabidopsis accession Antwerpen (An-1) that cause susceptibility to *Leptosphaeria maculans*.
- Identify and characterize *Leptosphaeria maculans* resistance mechanisms based on assessments of Arabidopsis mutants.

Results and discussion

Characterization of the Arabidopsis-*L. maculans* pathosystem

Screening of Arabidopsis accessions revealed a widespread distribution of *L. maculans* resistance. Only one accession, An-1, out of 168 tested displayed a clear susceptible response (**I**). An-1 was found to be susceptible against a wide array of pathogens, indicating that the susceptibility observed was not directly linked to *L. maculans* pathogen-host relations (**III**). Despite the almost complete resistance found in Arabidopsis, several susceptible mutants were identified and resistance traits were found to segregate in crosses between resistant accessions (**I**, **II**).

Analysis of resistance pathways revealed that the SA and JA/ET pathways were dispensable for *L. maculans* resistance. The *pad3-1* mutant, impaired in camalexin biosynthesis, and the mutants *esa1* and *pad1-1*, impaired in both camalexin and JA responses, were found to be susceptible to *L. maculans* (**I**). This indicates that a part of *L. maculans* resistance is governed by the camalexin inducing pathway with overlaps with the JA responses. Camalexin was also found to be an important component which influenced the timing and expression of disease symptoms. The Col-0 and L*er*-0 backgrounds were found to induce different levels of camalexin after *L. maculans* challenge and allelic mutants in the two backgrounds displayed distinctly different lesion phenotypes (**II**).

An *R gene* dependent resistance and an *R* gene independent induction of camalexin were found to be the major determinants of resistance to *L. maculans* (**II**). Both of these resistances could however be by-passed by accelerated cell death, as shown with the *acd1-20* and *ran1-1* mutants (**I**). Enhanced susceptibility due to accelerated cell death is however probably more complex than first anticipated, since the *rcd1* mutant (Overmeyer *et al*., 2000) remain resistant whereas *vad1* (Lorrain *et al*., 2004) is clearly susceptible (**V**). The *lms1* mutant shows an enhanced sensitivity to ethylene in the shoot and reduced ethylene

sensitivity in the root, indicating interactions with an unknown signalling pathway. The *lms1* mutant was mapped to a locus on chromosome 2, but the gene remains to be cloned.

Whether *lms1* is susceptible due to premature senescence and cell death is however still not clear, since disease symptoms develop relatively late compared to the other accelerated cell death mutants (*acd1-20, vad1*). Phenotypic analysis of all eleven *lms* mutants shows that *lms1, lms2, lms3, lms4* all have similar *L. maculans* lesion phenotypes, enhanced susceptibility to necrotrophs (*Alternaria, Botrytis*) and show earlier flowering time and senescence. The *lms5, lms8* and *lms9* mutants, on the other hand, are *L. maculans* specific and show 'spotted' lesion phenotypes similar to the *RAR1* mutant *rpr2-4* (**II**). The *lms6* and *lms7* mutants also show *L. maculans* specific susceptibility, but lesions spread along leaf edges. The *lms11* mutant shows a photomorphic phenotype with elongated petioles and epinastic, serrated leaves not present in the L*er*-0 background (in particular under high humidity and low light). Taken together, *L. maculans* susceptible genotypes can be divided into two major groups: Those that cause general susceptibility to necrotrophs and those that show an *L. maculans* specific susceptibility. Both groups of mutants seem to show extensive interactions with other physiological and developmental processes.

Identification of *L. maculans* resistance genes with Arabidopsis

Transgressive segregation in crosses between Col-0 and L*er*-0 indicated that there were two dominant resistance loci ($RLM1_{Col}$ and $RLM2_{Ler}$) that regulated resistance in the two different accessions (**I, II**). It was further established that the accessions Ws-0 and Cvi-1 harboured *RLM1* but were lacking *RLM2*, since both generated susceptible progeny with L*er*-0 and the susceptible progeny were confirmed to be allelic with susceptible Col-0 x L*er*-0 progenies (**II**). Additional mutant analysis showed that the resistance genes were independent of the *R* gene signalling components NDR1, EDS1, PAD4 and SGT1b, but required RAR1 and HSP90.1. None of the 9 currently known *AvrLm* genes correspond to Arabidopsis *RLM1/RLM2* resistance (**II**), but a screening of over 60 Australian *L. maculans* isolates revealed 3 potentially Arabidopsis virulent (*avrLmA*) isolates, where 2 were specifically virulent on *RLM1* genotypes and one on both *RLM1* and *RLM2* genotypes (**V**).

Col-4 x L*er*-0 and Cvi-1 x L*er*-2 recombinant inbred lines were used to map the two resistance loci. *RLM1* was found to be located at the lower arm of chromosome 1 and close recombinations limited the number of gene candidates to 76 genes, of which 7 were of the TIR-NB-LRR *R* gene type (**II**). T-DNA insertion mutant screenings in the mapped locus for *RLM1* revealed that a TIR-NB-LRR gene, At1g64070, was mainly responsible for resistance in the Col-0 background, but also mutants in a neighbouring homologous gene, At1g63880, displayed susceptible phenotypes. Sequence analysis of the L*er*-0 sequence of these two genes revealed loss of function of both genes, where At1g64070 is translationally truncated and At1g63880 is completely deleted. Quantitative disease assessments of the two gene knock-outs in camalexin-free (*pad3-1*) background confirmed a role for both genes in *L. maculans* resistance (**V**). Despite this, a complementation with a genomic clone of Col-4 At1g64070 (*RLM1*) including 1.5kb upstream and

500 bp downstream, revealed a complete restoration of resistance in T_1. The reason for this difference between the T-DNA mutant phenotypes and the complementation was difficult to explain, but was believed to be due to a dose-dependent resistance, where At1g64070 somehow also compensated for the loss of At1g63880 (**II**). Later segregation analyses under a higher inoculation pressure and an extended period with high humidity in T_2 did however reveal a 1:2:1 (resistant:intermediate:susceptible) distribution, further supporting our initial theory of a dose-dependent resistance (**V**). A milder inoculation and no initial period of high humidity did however result in a 3:1 (resistant:susceptible) distribution in T_2. The intermediate phenotypes were very similar to *rar1* mutants in L*er*-0 background (**II; V**), further supporting that RAR1 affects *R* gene activity via altered protein stability.

RLM2, on the other hand, was mapped to chromosome 4 with some discrepancies between the Col-4 x L*er*-0 and Cvi-1 x L*er*-0 RIL mapping results. To further confirm this locus, near isogenic lines (NILs) of L*er*-2 background with segments of chromosome 4 with Cvi-1 genotype were screened. The NILs confirmed a locus between the two mapped *RLM2* loci, as determined by the two RIL populations. Furthermore, this locus on chromosome 4 corresponds to a duplication event from a region around *RLM1* on chromosome 1, indicating that *RLM2* is a paralog of *RLM1* (**II**). As further evidence, a 26bp RNAi construct targeting 4 TIR-NB-LRR genes in the *RLM1* locus caused susceptible phenotype in Ler-0, indicating that *RLM2* is dependent on a gene with sequence similarity to *RLM1*. Interestingly, the RNAi in the *RLM1* backgrounds Col-0 or Ws-0 generated very few viable transformants (1 weak in Col-0, 2 that died before setting seeds in Ws-0), indicating that too efficient silencing of this gene family is lethal.

Since many of the *B. napus L. maculans* resistance genes have been associated to an enhanced callose deposition, *RLM1* and *rlm1* genotypes were compared for callose deposition. It was found that *RLM1* indeed was needed for an *L. maculans* induced callose deposition and callose was found to be an important defence response, since the callose synthase mutant *pmr4* and the papilla formation mutant *pen1* were susceptible to *L. maculans*. Lignification is another *L. maculans*-induced response important for resistance, since the lignification defective mutant *irx4* show moderate susceptibility. Lignin was initially observed as a local response in incompatible Arabidopsis-*L. maculans* interactions (Chen and Séguin-Swartz, 1999), but is also induced along the vascular structures after successful infection (**V**). Lignification is lower in *rlm1/rlm2* genotypes compared to *RLM1* or *RLM2* genotypes (**V**). The lignification response has also in the Arabidopsis-*P. syringae* pathosystem been associated to *R-Avr* gene interactions (Lee *et al*., 2001) and is known as a resistance response in multiple pathosystems (Vance *et al*., 1980).

In addition, analysis of *R* gene signalling components has revealed that the *rar1sgt1b* double mutant had a restored resistance, compared to the *rar1-21* parental genotype, as previously observed on *RPS5* (Holt *et al*., 2005). The restoration was however difficult to observe due to Col-0 background (high camalexin) and the dwarfed phenotype of the *rar1sgt1b* mutant. The effect on callose was suggestive of a restoration of *RLM1*, whereas the *sgt1b* background caused a clear enhancement of lignification after *L. maculans* challenge (**V**). Other *R* gene signalling components, such as *mos2* (Zhang *et al*., 2005), *pbs1* and *pbs3* (Warren *et al*., 1999) did not compromise *RLM1* activity (**V**). Knock-outs in the

NB-LRR interacting protein PP5 were also as resistant as wild-type. For more detailed analysis of the *R* gene components, double mutants to the camalexin deficient *pad3-1* mutant would have to be made. Alternatively, knocking out the genes in *pad3* background by using VIGS has a similar result.

Comparison of blackleg resistance in Arabidopsis and *Brassica*

The *Brassica* and Arabidopsis lineages are estimated to have diverged approximately 20 million years ago based on chloroplast sequence (Koch *et al*., 2001) and *Arabidopsis thaliana* and *Brassica napus* share approximately 87% coding domain sequence identity (Cavell *et al.*, 1998). A recent investigation of a chromosome segment in Arabidopsis and its homeologues in *B. oleracea* does however approximate that the *Arabidopsis* and *Brassica* lineages split 33 million years ago (Town *et al*., 2006). Segmental synteny of *Brassica* species to the Arabidopsis genome (Kowalski *et al*., 1994) has enabled cloning of genes regulating flowering time in *B. nigra* (Lagercrantz *et al*., 1996) and will most probably also facilitate cloning of *L. maculans* resistance genes in *B. napus* (Sillito *et al*., 2000; Mayerhofer *et al.*, 2005). Frequent deletions during the evolution of *R* genes may however limit the use of conserved flanking genes to predict the location of an *R* gene (Grant *et al.*, 1998). Many *L. maculans R* genes (*LepR1*, *LmR1, CLmR1, Rlm1, Rlm3, Rlm7* and *Rlm9*), have however primarily been mapped to loci in *B. napus*, which correspond to the lower chromosome segment ('E') on Arabidopsis chromosome 1 (Delourme *et al*., 2004; Mayerhofer *et al.*, 2005; Parkin *et al*., 2005). The same segment of chromosome 1 also contains *RLM1* in Arabidopsis, indicating that all of these genes may share a common evolutionary ancestry (**II**). A similar correspondence between multiple resistance loci in *B. rapa* and a single genomic region in Arabidopsis has been observed for clubroot resistance (Suwabe *et al*., 2006). It is interesting to note that chromosome 1 also was found to co-segregate with cotyledon resistance in Arabidopsis (+) *B. napus* somatic hybrids in BC_1F_1 and BC_1F_2 generations (Bohman *et al*., 2002).

Despite that the duplication event between the Arabidopsis *RLM1* and *RLM2* loci occurred prior to the Arabidopsis/*Brassica* split (Wolfe, K., Smurfit institute of Genetics, University of Dublin, personal communication), no *L. maculans R* genes have been found in *Brassica* that correspond to the Arabidopsis *RLM2* locus, which may indicate that this gene has been lost in the *Brassica* lineage, or that *RLM2* has been inserted in this locus at a later stage. Recent detailed analyses of a duplicated segment shows that the *Brassica* lineage duplicated or triplicated after divergence from the common ancestor with Arabidopsis. Furthermore, it was shown that multiple *R* genes found in the Arabidopsis segment were all absent in all *Brassica* segments, indicating that the *R* genes either has been deleted in all of the *Brassica* segments or, more likely, inserted in the Arabidopsis segment after the split (Town *et al*., 2006). This result challenges also extensive interpretations of a common evolutionary ancestry of *R* genes based on corresponding genetic locations, even if it provides an interesting link and a potential evolutionary story in the case of both *L. maculans* and clubroot resistance. *R* genes under presence/absence polymorphisms are thought to be under balancing selection, which could explain why Arabidopsis accessions either contain the *RLM1* or *RLM2*

locus. This could also explain the lack of positive selection of the genes found in the *RLM1* locus (Shen *et al*., 2006).

Interestingly, the deletion junctions contain no obvious traces of the *R* gene, but *R* genes under presence/absence polymorphism do not have any obvious flanking sequences such as transposon borders, indicating that this kind of polymorphism is regulated by an unknown mechanism (Shen *et al*., 2006). The relatively low sequence divergence has also been confirmed in a larger scale study including 4 of the TNL-H genes in the *RLM1* locus (Bakker *et al*., 2006).

Another interesting observation derived from the mapping of *L. maculans* resistance in *B. juncea* is that resistance usually is associated with one dominant and one recessive resistance gene (Rimmer, 2006). The segregation in F_2 in crosses between L*er*-0 and Col-0 or Ws-0 with ~4.5% susceptible plants indicates a similar pattern (**II**). A further indication of common resistance mechanisms is that the RGA (*R* gene analogue) marker associated to the B-genome derived recessive resistance *r*$_j$*lm2* shows high sequence similarity to the Arabidopsis TIR-NB-LRR genes At5g40910 and At5g41550 (Saal and Struss, 2005), which both are members of the TNL-H sub group and thus closely related to the TIR-NB-LRR genes found in the Arabidopsis *RLM1* locus (**II**).

The high degree of resistance to *L. maculans* observed in Arabidopsis indicates that Arabidopsis contains efficient resistance mechanisms to this pathogen and identification of susceptible genotypes makes it suitable for genetic investigations (**I, II**). The transfer of resistance by somatic hybridization (Forsberg *et al*., 1994; Bohman *et al.*, 2002), translational biology (**IV**) and transformation with Arabidopsis *RLM1* (Staal, J., Leino, M., Glimelius, K., Dixelius, C., unpublished) further emphasises that Arabidopsis can be used as a source of resistance in *B. napus* breeding. An interesting analysis would be to evaluate the 12 currently identified *Brassica L. maculans R* genes (Delourme *et al*., 2006; Rimmer, 2006) with Arabidopsis *RLM1* and Arabidopsis *R* gene signalling components using VIGS (Cai *et al*., 2006). Another interesting analysis would be to study the mechanisms of immuno-suppression from the *LmAvr* genes in an Arabidopsis background.

A potential resistance trait from Arabidopsis encoded by the Arabidopsis chromosome 3 – derived resistance could be biosynthesis of camalexin (or a novel phytoalexin), since the Arabidopsis *PAD3* gene is located within the mapped region for adult leaf resistance in Arabidopsis (+) *B. napus* asymmetric somatic hybrids (Bohman *et al.*, 2002). In vitro studies have however shown that camalexin activates phytoalexin detoxification mechanisms in *L. maculans*, which, counter intuitively, may actually make the fungus more aggressive on a *B. napus* with camalexin (Pedras *et al*., 2005). Microarray analysis of a *B. napus* disomic alien addition line (DAAL) containing Arabidopsis chromosome 3 (Leino *et al.*, 2004) with a normal *B. napus* cytoplasm revealed a number of gene candidates induced by *L. maculans*, and *PAD3* was not one of those (Karlsson, 2006).

Identification of a central component in resistance to necrotrophic fungal pathogens

In contrast to the two most characterized necrotrophic pathogens on Arabidopsis, *Botrytis cinerea* and *Alternaria brassicicola*, *L. maculans* shows a clear gene-for-

gene relationship with its *Brassica* and Arabidopsis hosts (**IV**). This difference may be due to the hemibiotrophic life style of *L. maculans.* The hemibiotrophic life strategy of *L. maculans* is however not dependent of the biotrophic phase for infection, and the fungus will opportunistically switch to a necrotrophic infection strategy also early during infection if possible. Another difference is that *L. maculans*, in contrast to *B. cinerea*, is a crucifer specialist. *B. cinerea*, on the other hand, has a very wide host range – from grape and strawberry to pine trees in tree nurseries (Capieau *et al.*, 2004).

There are however also similarities between the three pathogens, as transcriptional studies of a compatible *A. brassicicola – B. oleracea* interaction has revealed a number of genes potentially important for pathogenesis in *A. brassicicola, B. cinerea* and *L. maculans* (Cramer *et al.*, 2006). *Alternaria* and *Leptosphaeria* are relatively closely related, since both are ascomycetes belonging to the order *Pleosporales*. A common observation in *L. maculans*, *A. brassicicola* and *B. cinerea* interactions with Arabidopsis is a high level of resistance in wild types of the most commonly used accessions. All three pathogens are also, to varying extents, affected by camalexin responses in Arabidopsis (Glazebrook, 2005).

Analysis of resistant and susceptible progenies of the resistant Arabidopsis accession Col-0 and the susceptible accession An-1 (**I**) in a case-control design bulked segregant analysis on microarrays revealed a genomic region associated to higher expressed genes in the resistant progenies (Dewaele, 2004). By pooling progenies of Col-0 x An-1 based on their phenotypes prior to microarray comparisons, a dramatic decrease of the transcriptional complexity, compared to Col-0 vs An-1 comparisons, could be seen. Interestingly, the most significantly differentially expressed genes with higher expression in the resistant pools showed a clear genetic bias towards a locus on chromosome 4, which was also subsequently confirmed with genetic mapping. Although genetically close to *RLM2*, the locus had to be a genetically distinct locus, since the resistant Col-0 contains a non-functional *RLM2* locus (**II**). The locus responsible for resistance in Col-0 compared to An-1 was thus named *RLM3* (**III**).

The most significantly differentially expressed gene in this region was found to be of central importance for *L. maculans* resistance, as well as resistance to *A. brassicicola, A. brassicae* and *B. cinerea* (**III**). Differential disease phenotypes on insertion mutants in this gene suggested that a short alternative transcript was responsible for the resistance. Overexpression of a short transcript, which corresponded to the transcript length of an observed up-regulated splice form (Dewaele, 2004), resulted in enhanced resistance to all four fungal pathogens (**III**). In order to evaluate whether this short alternative transcript somehow influenced the *RLM1*-dependent resistance, callose stainings were performed on Col-0, An-1, *RLM3* over-expressors and *RLM3* T-DNA insertion mutants. The results showed a requirement for *RLM3* in callose induction and overexpression of *RLM3* partially restored resistance in susceptible (*rlm1rlm2*) Col-0 x L*er*-0 progeny. Taken together, this shows that *RLM3* act downstream of *RLM1/RLM2* and that overexpression of a disease-induced alternative transcript activates downstream responses.

Mechanisms of resistance against *L. maculans*

In order to identify novel resistance mechanisms to *L. maculans*, phenotypically and physiologically characterized mutants were screened for *L. maculans* susceptibility. During those screenings, it was found that the ABA biosynthesis mutant *aba1-3* and the ABA insensitive mutant *abi1-1* were clearly susceptible, whereas *abi2-1* and *abi3-1* were resistant to *L. maculans*. In addition to the differential responses in ABA mutants in L*er*-0 background, we found that *abi5-1* (Ws-0 backround), *aba2-1, aba3-1* and *abi4-1* (Col-0 background) were resistant. Due to our prior knowledge of moderate responses in Col-0 background because of its higher camalexin induction (**II**), the ABA mutants in Col-0 background were crossed to the camalexin deficient mutant *pad3-1*. Evaluations of the double mutants revealed a clearly enhanced susceptibility compared to the *pad3-1* background. Further characterization of the ABA-responses revealed that the resistance was independent of NO from nitrate reductase (*nia1nia2*) or nitric oxide synthase (*Atnos1*). A respiratory burst is however required for *L. maculans* resistance, since the NADPH oxidase mutants *rbohF* and *rbohD* both show moderate susceptibility, and the double mutant *rbohDF* shows severe susceptibility (**IV**).

We have seen that the *L. maculans* resistance genes *RLM1* and *RLM2* are involved in callose deposition in response to the pathogen and that the callose synthase mutant *pmr4* and the papilla formation mutant *pen1* is susceptible to *L. maculans* attack (**II**). Since exogenously applied ABA had previously been linked to priming of callose depositions (Ton and Mauch-Mani, 2004), we tested whether endogenous ABA could influence *RLM1*-dependent callose depositions. Several lines of evidence supported this notion, like an induction of ABA after *L. maculans* challenge and lower callose depositions in susceptible ABA mutants. ABA was found to act downstream of *RLM1*, since *L. maculans* resistance in *rlm1* genotypes was partially restored by exogenous ABA application. ABA treatment did however not restore callose depositions in *rlm1* genotypes, demonstrating the need for another *RLM1*-dependent signal for callose induction. Pre-treatment of susceptible *B. napus* with ABA or BABA also results in induced resistance, which is partially associated to the enhancement of callose deposition in this material (**IV**).

We hypothesised that one mechanism that ABA could act in to enhance callose deposition would be to repress beta-glucanases. Similar results have previously been reported from tobacco systems. By using BGL2-GUS reporter lines and analysis of callose deposition after co-inoculation with *L. maculans* and SA (which induces *BGL2*) we found results further supported our hypothesis (Kaliff, M., Staal, J., Dixelius, C., unpublished). Callose deposition is however not sufficient to completely limit the growth of the pathogen, since loss of the induced camalexin defence also results in a loss of resistance. ABA pre-treatment of the camalexin deficient mutant *pad3* can however compensate this loss via an enhancement of callose deposition which then appears to be sufficient to contain the pathogen without the aid of camalexin. Comparisons of the *R* gene and the camalexin deficient double mutant *rlm1pad3* with the callose and camalexin deficient double mutant *pmr4pad3* revealed that there are additional defences induced by *RLM1* in addition to the induction of callose in response to *L. maculans* challenge (**IV**).

The most notable susceptible ABA mutant is *abi1-1*, which shows clear disease phenotypes relatively early – in contrast to the mutant *abi2-1*, which has an identical dominant negative mutation in a homologous gene. Due to the great phenotypic differences between the two mutants, despite their high similarity at the molecular level, we hypothesised that there was a small sub-set of the ABA-regulated transcriptome affected by ABI1 but not ABI2. This sub-set should be where we find the gene(s) responsible for *L. maculans* resistance. In order to identify this sub-set, we made microarray comparisons between the two mutants after *L. maculans* infection. Most genes appear to be stress-induced due to compatible interactions in the *abi1-1* mutant. Interestingly, TIR-NB-LRR genes in the *RLM1* locus were differentially expressed between *abi1-1* and *abi2-1*, indicating that ABI1 is involved in a negative feedback of *RLM1* accumulation. To confirm this, expression of TIR-NB-LRR genes in the *RLM1* locus were also evaluated in *abi4pad3* and *pad3* plants. Contrary to our expectations, the *abi4* mutation caused a greater accumulation of TIR-NB-LRR transcripts. We concluded that this was due to a lower expression of *ABI1* in the *abi4* background (**IV**).

Interactions between host responses and pathogen strategy

L. maculans resistance responses in *Brassica* are characterized by necrosis of guard cells, induction of callose deposition, pectin in xylem vessels, lignification, phytoalexins and PR proteins (reviewed in Howlett *et al*., 2001). Our genetic dissection of *Arabidopsis-L. maculans* interactions showed that HR at hydathodes and guard cells, callose, vascular plug and lignification are responses dependent on *RLM1-AvrLmA* interactions, indicating similar responses in Arabidopsis and *Brassica L. maculans R* genes (**V**). The phytoalexin camalexin and SA and JA/ET – induced PR proteins were however found to be *R* gene independent responses.

In order to evaluate the functional relevance of these factors, the hypersusceptible *rlm1pad3*(*er*), which is deficient in both camalexin and *R* gene resistance, was crossed with a resistant *coi1-16ein2NahG*(*gl1*) triple mutant defect in response to the three major disease resistance hormones SA, JA and ET. Screening in F_2 revealed a number of individuals that were clearly more susceptible than the *rlm1pad3*(*er*) parent and 4 distinct disease phenotypes could be observed: A = normal local purple lesion surrounded by chlorosis and necrotic mid vein for systemic spreading, B = extensive chlorosis that never develops lesions or pycnidia and complete chlorotic death of some plants, C = extreme grey/green local necrosis, leaf anthocyanin coloured, and D = "spotted" leaves covered with small necroses both locally and systemically (**V**). The *pad3* mutation was found to primarily enable local growth around the inoculation site, whereas *rlm1* enables the vascular and systemic spreading of *L. maculans*.

Genetic analyses of progenies that showed different quantitative and qualitative effects on *L. maculans* susceptibility revealed that the genotypes harbouring *rlm1pad3coi1EIN2* (±*NahG*) caused 'B' and *rlm1pad3ein2NahG* caused 'D' phenotypes. A common denominator for genotypes causing 'C' phenotypes was less clear, but many harboured *ein2*(-*NahG*). In cases of multiple combinations, (*coi1ein2NahG*), the lines showed 'B' phenotype. Curiously, all in vitro selected triple (*coi1ein2NahG*) mutants identified as more susceptible than

rlm1pad3 showed *rlm1PAD3* genotype, indicating that the pentuple mutant was missed in the screening due to high lethality after *L. maculans* infection. However, only the D phenotype showed a clearly higher susceptibility, expressed as number of days until visible symptoms. Other *rlm1pad3* genotype combinations were variants of the normal A phenotype, but may show subtle differences that should be investigated further. There is also an issue of penetrance of the different phenotypic classes. There are reasons to believe that at least some of the phenotypes only are expressed under certain conditions, which could explain some of the problems defining C phenotype genotypes. This indicates that the plant responses influence the development of the pathogen and that the interactions between the pathogen and the host are more intricate and complex than previously assumed. In particular, the plant-derived signals blocked by *coi1* and *ein2*, respectively, are required for the pathogen to switch from vegetative (biotrophic/endophytic) to reproductive (necrotrophic) growth is of particular interest. Further investigations of the pathogen gene expression during infection, depending on host genotype, could give great mechanistic understanding of the Arabidopsis - *L. maculans* pathosystem.

Biocontrol experiments do, however, suggest that ISR in combination with toxins produced with the bacteria used may be active against *L. maculans* in *B. napus* (Danielsson, J., Reva, O. and Meijer, J., submitted). This would suggest that additional JA or a priming of other defenses via ISR may contribute somewhat to resistance when callose and phytoalexin defences are lacking. Whether this is valid also in the Arabidopsis model remains to be shown by mutant studies. Studies on *NahG Brassica napus* suggest that SA does not contribute significantly to *L. maculans* resistance (**IV**), since symptom development is only 1 or 2 days earlier in this material compared to the wild type control, which could be due to a catechol-mediated weakening of the cell walls and cell death due to inappropriate ROS production (van Wees and Glazebrook, 2003).

Interestingly, the *er* mutation was found in many of the clearly susceptible plants but no quantitative impact from *er* could be observed after careful investigations of comparable *er* and *ER* materials. We conclude that this distribution is due to a novel digenic (qualitative) resistance where one component ($RLM4_{Col}$) is linked to *ER*. This novel component also explains the segregation patterns previously observed in RIL and F_2 screenings (**II**).

Conclusions

The 3 layers model: A hierarchical view on Arabidopsis-*L. maculans* resistance

In the case of *L. maculans*, we have seen that the SA- and JA/ET induced defences are not required for resistance against this fungal pathogen (**I**). We have also confirmed this via observation of resistant phenotype on a *coi1-16ein2NahG* triple mutant which is completely devoid of SA, JA and ET pathogen defence signalling (**V**). It is thus very likely that the early defence inductions (*R* gene dependent responses [callose, lignin] and camalexin) in Arabidopsis are quite sufficient to

limit its growth. Both SA and JA/ET pathways are however induced following *L. maculans* challenge (**I, IV, V**).

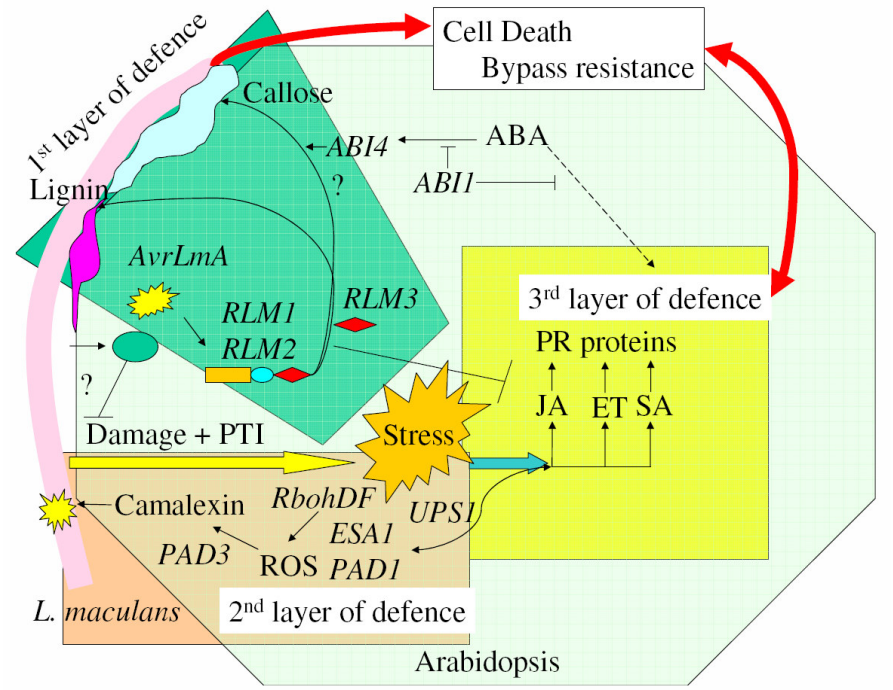


Figure 14. Overview of the different layers of resistance to *L. maculans*. Resistance relies on at least two independent responses – an *R* gene dependent resistance and the antimicrobial secondary metabolite camalexin. ABA is required for efficient *R* gene induced callose but also induces a callose-independent response. The common pathogen response hormones SA, JA and ET quantitatively and qualitatively influence disease development but do not determine resistance.

There is an influence from SA- and JA/ET induced resistances in the development of *L. maculans* disease when *RLM1* and camalexin-dependent resistances are removed (**V**; Figure 14). *L. maculans* is about to get a completely sequenced genome and there are tools available for both forward and reverse genetics, making *L. maculans* one of the most promising plant-fungal models for future Arabidopsis studies. The well-characterized behaviour of *L. maculans* on *Brassica* both in field conditions and lab conditions is yet another advantage for mechanistic studies of Arabidopsis resistance to this pathogen. The economical importance of *L. maculans* also makes the results found in the Arabidopsis system interesting in a *Brassica* breeding perspective. This implies a mutual benefit between the Arabidopsis and *Brassica* models to this pathogen. Taken together, *L. maculans* in Arabidopsis is a new model pathosystem, which reveals new resistance mechanisms but also show overlap with previously described mechanisms against biotrophs or necrotrophs.

References

Aarts, N., Metz, M., Holub, E., Staskawicz, B.J., Daniels, M.J. and Parker, J. E. (1998) Different requirements for *EDS1* and *NDR1* by disease resistance genes define at least two *R* gene-mediated signaling pathways in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA*, **95**, 10306-10311.

Akira, S., **Uematsu, S. and Takeuchi, O.** (2006) Pathogen recognition and innate immunity. *Cell,* **124**, 783-801.

Akita, M. and Valkonen, J. (2000) A novel gene family in moss (*Physcomitrella patens*) shows sequence homology and a phylogenetic relationship with the TIR-NBS class of plant disease resistance genes. *J. Mol. Evol.* **55**, 595-605.

Albertin, W., Balliau, T., Brabant, P., Chèvre, A., Eber, F., Malosse, C. and Thiellement, H. (2006) Numerous and rapid nonstochastic modifications of gene products in newly synthesized *Brassica napus* allotetraploids. *Genetics*, **173**, 1101-1113.

Alonso, J.M., Stepanova, A.N., Leisse, T.J. *et al.* (2003) Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science*, **301**, 653–657.

Alonso-Blanco, C., Peeters, A.J., Koornneef, M., Lister, C., Dean, C., van den Bosch, N., Pot, J. and Kuiper M.T. (1998) Development of an AFLP based linkage map of Ler, Col and Cvi *Arabidopsis thaliana* ecotypes and construction of a Ler/Cvi recombinant inbred line population. *Plant J.* **14**, 259-271.

Alfano, J.R. and Collmer, A. (2004). Type III secretion system effector proteins: Double agents in bacterial disease and plant defence. *Annu. Rev. Phytopathol.* **42**, 385-414.

Algotsson, M. (2004) Impact of seasonal variation and cropping history on the extent of *Verticillium* wilt on oilseed rape. Master's thesis, SLU, Uppsala, Sweden. ISSN 1651-5196 Nr. 68.

Anderson, J.A., Badruzsaufari, E., Schenk, P.M., Manners, J.M., Desmond, O.J., Ehlert, C., Maclean, D.J. and Kazan, K. (2004) Antagonistic interaction between abscisic acid and jasmonate-ethylene signalling pathways modulates defence gene expression and disease resistance in Arabidopsis. *Plant Cell,* **16,** 3460-3479.

Andersson, M., Hamberg, M., Kourtchenko, O., Brunnström, Å., McPhail, K., Gerwick, W., Göbel, C., Feussner, I. and Ellerström, M. (2006) Oxylipin profiling of the hypersensitive response in *Arabidopsis thaliana*: Formation of a novel oxo-phytodienoic acid containing galactolipid arabidopside E. *J. Biol. Chem.* (Electronic publication August 21)

Andréasson, E., **Wretblad, S., Granér, G., Wu, X., Zhang, J., Dixelius, C., Rask, L. and Meijer, J.** (2001) The myrosinase-glucosinolate system in the interaction between *Leptosphaeria maculans* and *Brassica napus*. *Mol. Plant Pathol.* **2**, 281-286.

Arazana, M., **Kim, S., Zhao, K. *et al.*** (2006) Genome-wide association mapping in *Arabidopsis* identifies previously known flowering time and pathogen resistance genes. *PLoS Genetics,* **1**, e60.

Asai, T., **Tena, G., Plotnikova, J., Willmann, M., Chiu, W., Gomez-Gomez, L., Boller, T., Ausubel, F. and Sheen, J.** (2002) MAP kinase signalling cascade in Arabidopsis innate immunity. *Nature,* **415**, 977-983.

Aubertot, J., **West, J., Busset-Vaslin, L., Salam, M., Barbetti, M. and Diggle, A.** (2006) Improved resistance management for durable disease control: A case study of phoma stem canker of oilseed rape (*Brassica napus*). *Eur. J. Plant Pathol.* **114**, 91-106.

Ausubel, F. (2005) Are innate immune signalling pathways in plants and animals conserved? *Nat. Immunology*, **10**, 973-979.

Azavedo, C., Betsuyaku, S., Peart, J., Takahashi, A., Noël, L., Sadanandom, A., Casais, C., Parker, J. and Shirasu, K. (2006) Role of SGT1 in resistance protein accumulation in plant immunity. *EMBO J.* **25**, 2007-2016.

Bakker, E., Toomajian, C., Kreitman, M. and Bergelson, J. (2006) A genome-wide survey of *R* gene polymorphisms in Arabidopsis. *Plant Cell*, **18**, 1803-1818.

Balesdent, M., Barbetti, M., Li, H., Sivasithamparam, K., Gout, L. and Rouxel, T. (2005) Analysis of *Leptosphaeria maculans* race structure in a world wide collection of isolates. *Phythopathology*, **95**, 1061-1071.

Balesdent, M., **Louvard, K., Pinochet, X. and Rouxel, T.** (2006) A large-scale survey of races of *Leptosphaeria maculans* occurring on oilseed rape in France. *Eur. J. Plant Pathol.* **114**, 53-65.

Barbetti, M., Brown, A. and Wood, P. (1975) Prospects for a successful rape crop. *Austr. J. Agric.* **16**, 11.

Bartsch, M., **Gobbato, E., Bednarek, P., Debey, S., Schultze, J., Bautor, J. and Parker, J.** (2006) Salicylic acid-independent ENHANCED DISEASE SUSCEPTIBILITY1 signaling in *Arabidopsis* immunity and cell death is regulated by the monooxygenase *FMO1* and the Nudix hydrolase *NUDT7*. *Plant Cell,* **18**, 1038-1051.

Becker, H., **Löptien, H. and Röbbelen, G**. (1999) Breeding: an overview. In Biology of Brassica Coenospecies. Edited by C. Gomez-Campo. Elsevier Science, Amsterdam, the Netherlands.

Beffa, R., Hofer, R., Thomas, M. and Meins, F. (1996) Decreased susceptibility to viral disease of β-1,3-glucanase-deficient plants generated by antisense transformation. *Plant Cell,* **8**, 1001-1011.

Bell, J. (1995) Meal and by-product utilization in animal nutrition. In *Brassica* Oilseeds Production and Utilization. Edited by D. Kimber, McGregor, D., Wallingford: CAB International.

Belkhadir, Y., **Subramaniam, R. and Dangl, J**. (2004) Plant disease resistance protein signalling: NBS-LRR proteins and their partners. *Curr. Opin. Plant. Biol*. **7**, 391-399.

Bergey, D., Howe, G. and Ryan, C. (1996) Polypeptide signalling for plant defensive genes exhibits analogies to defense signalling in animals. *Proc. Natl. Acad. Sci. USA,* **93**, 12053-12058.

Berrocal-Lobo, M. and Molina, A. (2004) Ethylene response factor 1 mediates *Arabidopsis* resistance to the soilborne fungus *Fusarium oxysporum*. *Mol. Plant-Microbe Interact.* **17**, 763-770.

Beutler, B. and Rehli, M. (2002) Evolution of the TIR, tolls and TLRs: functional inferences from computational biology. *Curr. Top. Microbiol. Immunol*. **270**, 1-21.

Beutler, B., Hoebe, K., Georgel, P., Tabeta, K. and Du, X. (2004) Genetic analysis of innate immunity: TIR adapter proteins in innate and adaptive immune responses. *Microbes and Infection,* **6**, 1374-1381.

Bhat, R., Miklis, M., Schmelzer, E., Schulze-Lefert, P. and Panstruga, R. (2005) Recruitment and interaction dynamics of plant penetration resistance components in a plasma membrane microdomain. *Proc. Natl. Acad. Sci. USA,* **102**, 3135-3140.

Bohman, S., **Forsberg, J., Glimelius, K. and Dixelius, C.** (1999) Inheritance of *Arabidopsis* DNA in offspring from *Brassica napus* and *A. thaliana* somatic hybrids. *Theor. Appl. Genet.* **98**, 99-106.

Bohman, S. (2001) Molecular studies of *Arabidopsis* and *Brassica* with focus on resistance to *Leptosphaeria maculans*. PhD thesis, Agraria 305. SLU Press, Uppsala. Sweden.

Bohman, S., Wang, M. and Dixelius, C. (2002) *Arabidopsis thaliana* derived resistance against *Leptosphaeria maculans* in a *Brassica napus* genomic background. *Theor. Appl. Genet.* **105**, 498-504.

Bokor, A., **Barbetti, M., Brown, A., Mac Nish, G. and Wood, P.** (1975) Blackleg of rapeseed – Unless blackleg can be controlled there is little future for rapeseed as a major commercial crop in W.A. *Austr. J. Agric.* **16**, 7-10.

Borevitz, J. and Nordborg, M. (2003) The impact of genomics on the study of natural variation in Arabidopsis. *Plant Physiol.* **132**, 718-725.

Borhan, M, Holub, E.B., Beynon, J., Rozwadowski, K. and Rimmer, R. (2004) The *Arabidopsis* TIR-NB-LRR gene *RAC1* confers resistance to *Albugo candida* (white rust) and is dependent on *EDS1* but not on *PAD4*. *Mol. Plant-Microbe Interact.* **7**, 711-719.

Borlaug, N. (2000) Ending world hunger. The promise of biotechnology and the threat of antiscience zealotry. *Plant Physiol.* **124**, 487-490.

Brader, G., **Daalgard Michelsen, M., Halkier, B. and Palva, T.** (2006) Altering glucosinolate profiles modulates disease resistance in plants. *Plant J*. **46**, 758-767.

Broekaert, W., Delauré, S., Bolle, M. and Cammue, B. (2006) The role of ethylene in host-pathogen interactions. *Annu. Rev. Phytopathol.* **44**, (Electronic publication March 6).

Buchanan-Wollaston, V., Earl, S., Harrison, E., Mathas, E., Navabpour, S., Page, T. and Pink, D. (2003) The molecular analysis of leaf senescence – a genomics approach. *Plant Biotech. J*. **1**, 3-22.

Buzza, C. (1995) Plant Breeding. In *Brassica* Oilseeds Production and Utilization. Edited by D. Kimber, McGregor, D., Wallingford: CAB International. pp 153-171.

Cai, X., Xu, Q., Wang, C. and Zheng, Z. (2006) Development of a virus-induced gene-silencing system for functional analysis of the *RPS2*-dependent resistance signalling pathways in *Arabidopsis*. *Plant Mol. Biol*. **62**, 223-232.

Camera, S., Gouzerh, G., Dhondt, S., Hoffman, L., Fritig, B., Legrand, M. and Heitz, T. (2004) Metabolic reprogramming in plant innate immunity: the contributions of phenylpropanoid and oxylipin pathways. *Immunol. Rev.* **198**, 267-284.

Cannon, S., Zhu, H., Baumgarten, A., Spangler, R., May, G., Cook, D. and Young, N. (2002) Diversity, distribution and ancient taxonomic relationships within the TIR and non-TIR NBS-LRR resistance gene subfamilies. *J. Mol. Evol.* **54**, 548-562.

Cao, H., **Baldini, R. and Rahme, L.** (2001) Common mechanisms for pathogens of plants and animals. *Annu. Rev. Phytopathol.* **39**, 259-284.

Capieau, K., Stenlid, J. and Stenström, E. (2004) Potential for biological control of *Botrytis cinerea* in *Pinus sylvestris* seedlings. *Scan. J. Forest Res.* **19**, 312-319.

Carrasco, L., Vázquez, D., Hérnandez-Lucas, C., Carbonero, P. and García-Olmedo, F. (1981) Thionins: plant peptides that modify membrane permeability in cultured mammalian cells. *Eur. J. Biochem.*, **116**, 185-189.

Cavell, A., Lydiate, D., Parkin. I., Dean, C. and Trick, M. (1998) Collinearity between a 30-centimorgan segment of *Arabidopsis thaliana* chromosome 4 and duplicated regions within the *Brassica napus* genome. *Genome,* **41**, 62-69.

Chalal, G. and Gosal, S. (2002) Principles and procedures of plant breeding – biotechnological and conventional approaches. Alpha science international Ltd., Pangbourne, UK. pp 360-390.

Chen, C. and Howlett, B. (1996) Rapid necrosis is associated with the arrest of fungal growth in leaves of indian mustard (*Brassica juncea*) inoculated with avirulent isolates of *Leptosphaeria maculans*. *Phys. Mol. Plant Pathol.* **48**, 73-81.

Chen, C. and Séguin-Swartz , G. (1999) Reaction of wild crucifers to *Leptosphaeria maculans*, the causal agent of blackleg of crucifers. *Can. J. Plant Pathol*., **21**, 361-367.

Chisholm, S., **Coaker, G., Day, B. and Staskawicz, B**. (2006) Host-microbe interactions: shaping the evolution of the plant immune response. *Cell,* **124**, 803-814.

Chrispels, M. (2000) Biotechnology and the poor. *Plant Physiol.* **124**, 3-6.

Christianson, J., **Rimmer, R., Good, A. and Lydiate, D.** (2006) Mapping genes for resistance to *Leptosphaeria maculans* in *Brassica juncea*. *Genome,* **49**, 30-41.

Comai, L., Young, K., Till, B., Reynolds, S., Greene, E., Codomo, C., Enns, L., Johnson, J., Burtner, C., Odden, A. and Heinkoff, S. (2004) Efficient discovery of DNA polymorphisms in natural populations by Ecotilling. *Plant J.* **37**, 778-786.

Cooley, M., Pathirana, S., Wu, H., Kachroo, P. and Klessig, D. (2000) Members of the Arabidopsis HRT/RPP8 family of resistance genes confer resistance to both viral and oomycete pathogens. *Plant Cell*, **12**, 663-676.

Cooley, M., Miller, W. and Mandrell, R. (2003) Colonization of *Arabidopsis thaliana* with *Salmonella enterica* and enterohemorrhagic *Escherichia coli* O157:H7 and competition by *Enterobacter asburiae*. *Appl. Envir. Microbiol.* **69**, 4915-4926.

Cramer, R., **La Rota, C., Cho, Y., Thon, M., Craven, K., Knudson, D., Mitchell, T. and Lawrence, C.** (2006) Bioinformatic analysis of expressed sequence tags derived from a compatible *Alternaria brassicicola – Brassica oleracea* interaction. *Mol. Plant Pathol.* **7**, 113-124.

Dacks, J. and Doolittle, W. (2001) Reconstructing/Deconstructing the earliest eukaryotes: how comparative genomics can help. *Cell*, **107**, 419-425.

Dangl, J. and Jones, J. (2001) Plant pathogens and integrated defence responses to infection. *Nature,* **411**, 826-833.

Dangl, J. and McDowel, J. (2006) Two modes of pathogen recognition by plants. *Proc. Natl. Acad. Sci. USA*, **103**, 8575-8576.

Danielsson, J., Reva, O. and Meijer, J (2006) Induced protection of oilseed rape (*Brassica napus*) towards fungal pathogens by strains of the plant-associated ecotype of *Bacillus amyloliquefaciens*. *Microb. Ecol*. (submitted)

Dardick, C. and Ronald, P. (2006) Plant and animal pathogen recognition receptors signal through non-RD kinases. *PLOS Pathog.* **2**, 14-28.

DeCook, R., Lall, S., Nettleton, D. and Howell, S. (2006) Genetic regulation of gene expression during shoot development in Arabidopsis. *Genetics,* **172**, 1155-1164.

de la Fuente van Bentem, S., Vossen, J., de Vries, K., van Wees, S., Tameling, W., Dekker, H., de Koster, C., Haring, M., Takken, F. and Cornelissen, B. (2005) Heat shock protein 90 and its co-chaperone protein phosphatase 5 interact with distinct regions of the tomato I-2 disease resistance protein. *Plant J.* **43**, 284-298.

Delourme, R., Pilet-Nayel, L., Archipiano, M., Horvais, R., Tanguy, X., Rouxel, T., Brun, H., Renard, M. and Balesdent, M. (2004) A cluster of major specific resistance genes to *Leptosphaeria maculans* in *Brassica napus*. *Phytopathoglogy,* **94**, 578-583.

Delourme, R., **Chévré, A., Brun, H., Rouxel, T., Balesdent, M., Dias, J., Sailsbury, P., Renard, M. and Rimmer, S.** (2006) Major gene and polygenic resistance to *Leptosphaeria maculans* in oilseed rape (*Brassica napus*). *Eur. J. Plant Pathol.* **114**, 41-52.

Denby, K., Kumar, P. and Kliebenstein D. (2004) Identification of *Botrytis cinerea* susceptibility loci in *Arabidopsis thaliana*. *Plant J.* **38**, 473-486.

Déqué, M., Jones, R., Wild, M., Giorgi, F., Christensen, J., Hassel, D., Vidale, P., Rockel, B., Jacob, D., Kjellström, E., Castro, M., Kucharski, F. and van den Hurk, B. (2005) Global high resolution versus Limited Area Model climate change projections over Europe: quantifying confidence level from PRUDENCE results. *Climate Dynamics* **25**, 653-670.

Desveaux, D., **Singer, A. and Dangl, J.** (2006) Type III effector proteins: doppelgangers of bacterial virulence. *Curr. Opin. Plant Biol.* **9**, 376-382.

de Torres, M., Mansfield, J., Grabov, N., Brown, I., Ammouneh, H., Tsiamis, G., Forsyth, A., Robatzek, S., Grant, M. and Boch, J. (2006) *Pseudomonas syringae* effector AvrPtoB suppresses basal defence in Arabidopsis. *Plant J*. **47**, 368-382.

Devoto, A., Muskett, P. and Shirasu, K. (2003) Role of ubiquitination in the regulation of plant defence against pathogens. *Curr. Opin. Plant Biol.* **6**, 307-311.

Dewaele, E. (2004) Identification of an Arabidopsis-*Leptosphaeria maculans* resistance component unsing cDNA arrays on pools of resistant and susceptible Col-0 x An-1 progenies. Bachelor's thesis. SLU, Uppsala, Sweden. ISSN 1651-5196 Nr. 59.

Diener, A.C. and Ausubel, F.M. (2005) Resistance to *Fusarium oxysporum 1*, a dominant *Arabidopsis* resistance gene, is not race specific. *Genetics,* **16**, 3460-3479.

Dixelius, C. (1999) Inheritance of the resistance to *Leptosphaeria maculans* of *Brassica nigra* and *B. juncea* in near-isogenic lines of *B. napus*. *Plant Breed.* **118**, 151-156.

Dixelius, C. (2003) Kransmögel – ett problem i norra Europa. *Svensk Frötidning,* **2**, 12-14.

Dixelius, C., **Bohman, S. and Wretblad, S.** (2004) Disease resistance. In Biotechnology in agriculture and forestry, Brassica. Edited by E.C Pua and C. J. Douglas. Springer Verlag, Berlin, Germany. Vol. 54, pp. 253-271.

Dixelius, C., **Happstadius, I. and Berg, G**. (2005) *Verticillium* wilt on *Brassica* oil seed crops – a Swedish perspective. *J. Swedish Seed Assoc.* **115**, 36-48.

Dodds, P., Lawrence, G., Catanzariti, A., Teh, T., Wang, C., Ayliffe, M., Kobe, B. and Ellis, J. (2006) Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. *Proc. Natl. Acad. Sci. USA*, **103**, 8888-8893.

Domergue, F., **Abbadi, A. and Heinz, E.** (2005) Relief for fish stocks: oceanic fatty acid acids in transgenic oilseeds. *Trends Plant Sci.* **3**, 112-116.

Ekbom, B. (1995) Insect Pests. In *Brassica* Oilseeds Production and Utilization. Edited by D. Kimber, McGregor, D., CAB international, Wallingford pp. 141-152.

Ellis, J., Dodds, P. and Pryor, T. (2000) Structure, function and evolution of plant disease resistance genes. *Curr. Opin. Plant Biol.* **3**, 278-284.

Ellis, J., Catanzariti, A. and Dodds, P. (2006) The problem of how fungal and oomycete avirulence proteins enter plant cells. *Trends Plant Sci.* **11**, 61-63.

Everett, J., **Busick, N., Sielaff, D., Wahoff, D. and Dunn, D.** (2003) A deeply invasive *Phoma* species infection in a renal transplant recipient. *Transplantation Proceedings*, **35**, 1387-1389.

Falk, A., Feys, B.J., Frost, L.N., Jones, J.D.G., Daniels, M.J. and Parker, J.E. (1999) EDS1, an essential component of *R* gene-mediated disease resistance in *Arabidopsis* has homology to eukaryotic lipases. *Proc. Natl. Acad. Sci. USA*, **96**, 3292-3297.

Fan, W. and Dong, X. (2002) In vivo interaction between NPR1 and transcription factor TGA2 leads to salicylic acid-mediated gene activation in Arabidopsis. *Plant Cell,* **14**, 1377-1389.

Feechan, A., Kwon, E., Yun, B., Wang, Y., Pallas, J. and Loake, G. (2005) A central role for S-nitrosothiols in plant disease resistance. *Proc. Natl. Acad. Sci USA,* **102**, 8054-8059.

Feys, B., **Wiemer, M., Bhat, R., Moisan, L., Medina-Escobar, N., Neu, C., Cabral, A. and Parker, J.** (2005) Arabidopsis SENESCENCE-ASSOCIATED GENE101 stabilizes and signals within an ENHANCED DISEASE SUSCEPTIBILITY1 complex in plant innate immunity. *Plant Cell,* **17**, 2601-2613.

Finlay, B. and McFadden, G. (2006) Anti-immunology: Evasion of the host immune system by bacterial and viral pathogens. *Cell,* **124**, 767-782.

Fitt, B., Brun, H., Barbetti, M. and Rimmer, S. (2006a) World-wide importance of phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed rape (*Brassica napus*). *Eur. J. Plant Pathol.* **114**, 3-15

Fitt, B., Evans, N., Howlett, B. and Cooke, M. (2006b) Sustainable strategies for managing *Brassica napus* (oilseed rape) resistance to *Leptosphaeria maculans* (phoma stem canker). *Eur. J. Plant Pathol.* **114**, 1

Fitt, B., **Huang, Y., Bosch, F. and West, J.** (2006c) Coexistence of related pathogen species on arable crops in space and time. *Annu. Rev. Plant Pathol.* **44**, (Electronic publication March 15).

Flor, H. (1947) Host-parasite interactions in flax rust – Its genetics and other implications. *Phytopathol.* **85**, 213-217.

Flors, V., Ton, J., Jakab, G. and Mauch-Mani, B. (2005) Abscisic acid and callose: team players in defence against pathogens? *J.Phytopath.* **153**, 377-383.

Fluhr, R. (2001) Sentinels of Disease. Plant resistance genes. *Plant Physiol*. **127**, 1367-1374.

Forsberg, J., Landgren, M. and Glimelius, K. (1994) Fertile somatic hybrids between *Brassica napus* and *Arabidopsis thaliana*. *Plant Sci.* **95**, 213-223.

Fritz, J., Girardin, S., Fitting, C., Werts, C., Mengin-Lecreulx, D., Caroff, M., Cavallon, J., Philpott, D. and Adlib-Conquv, M. (2005) Synergistic stimulation of human monocytes and dendritic cells by Toll-like receptor 4 and NOD1- and NOD2-activating agonists. *Eur. J. Immunol*. **35**, 2459-2470.

Fujikawa, T., Ishihara, H., Leach, J. and Tsuyumu, S. (2006) Suppession of defense response in plants by the *avrBs3/pthA* gene family of *Xanthomonas* spp. *Mol. Plant-Microbe Interact.* **3**, 342-349.

Fujita, M., Fujita, Y., Noutoshi, Y., Takahashi, F., Narusaka, Y., Yamaguchi-Shinozaki, K. and Shinozaki, K. (2006) Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signalling networks. *Curr. Opin. Plant Biol*. **9**, 436-442.

Gabriëls, S., **Takken, F., Vossen, J., Jong, C., Liu, Q., Turk, S., Wachowski, L., Peters, J., Witsenboer, H., de Wit, P. and Joosten, M.** (2006) cDNA-AFLP combined with functional analysis reveals novel genes involved in the hypersensitive response. *Mol. Plant-Microbe Interact.* **19**, 567-576.

Gardiner, D., **Cozijnsen, A., Wilson, L., Pedras, M. and Howlett. B.** (2004) The sirosdesmin biosynthetic gene cluster of the plant pathogenic fungus *Leptosphaeria maculans*. *Mol. Microbiol.* **53**, 1307-1318.

Garret, K., Dendy, S., Frank, E., Rouse, M. and Travers, S. (2006) Climate change effects on plant disease: genomes to ecosystems. *Annu. Rev. Phytopathol.* **44**, 489-509.

Girardin, S., **Travassos, L., Herve, M., Blanot, D., Boneca, I., Philpott, D., Sanosetti, P. and Mengin-Lecreulx, D.** (2003) Peptidoglycan molecular requirements allowing detection by Nod1 and Nod2. *J. Biol. Chem.* **43**, 41702-41708.

Gladders, P., Evans, N., Marcroft, S. and Pinochet, X. (2006) Dissemination of information about management strategies and changes in farming practices for the exploitation of resistance to *Leptosphaeria maculans* (phoma stem canker) in oilseed rape cultivars. *Eur. J. Plant Pathol.* **114**, 117-126.

Glawischnig, E., Hansen, B., Olsen, C. and Halkier, B. (2004) Camalexin is synthesized from indole-3-acetaldoxime, a key branching point between primary and secondary metabolism in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA,* **101**, 8245-8250.

Glazebrook, J. (2001) Genes controlling expression of defense responses in *Arabidopsis* - 2001 status. *Curr. Opin. Plant Biol*. **4**, 301-308.

Glazebrook, J., Chen, W.J., Estes, B., Chang, H-S., Nawrath, C., Métraux, J.-P., Zhu, T. and Katagiri, F. (2003) Topology of the network integrating salicylate and jasmonate signal transduction derived from global expression phenotyping. *Plant J.* **34**, 217–228.

Glazebrook, J. (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.* **43**, 205-227.

Glimelius, K., Fahleson, J., Landgren, M., Sjödin, C. and Sundberg, E. (1991) Gene transfer via somatic hybridization in plants. *Trends Biotech.* **9**, 24-30.

Gomez-Gomez, L. and Boller, T. (2000) FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. *Mol. Cell,* **5**, 1003-1011.

Gout, L., Fudal, I., Kuhn, M., Blaise, F., Eckert, M., Cattolico, L., Balesdent, M. and Rouxel, T. (2006) Lost in the middle of nowhere: the *AvrLm1* avirulence gene of the dothiomycete *Leptosphaeria maculans*. *Mol. Microbiol.* **66**, 67-80.

Granér, G. (2002) Studies on Fungal Pathogens on Oilseed rape (*Brassica napus*). PhD thesis, Agraria 354. SLU Press, Uppsala. Sweden.

Grant, M., **McDowell, J., Sharpe, A., de Torres Zabala, M., Lydiate, D. and Dangl, J.** (1998) Independent deletions of a pathogen-resistance gene in Brassica and Arabidopsis. *Proc. Natl. Acad. Sci. USA,* **95**, 15843-15848.

Grant, M. and Lamb, C. (2006) Systemic immunity. *Curr. Opin. Plant Biol.* **9**, 414-420.

Hahn, J. (2005) Regulation of Nod1 by Hsp90 chaperone complex. *FEBS Lett*. **579**, 4513-4519.

Hammond, K., Lewis, B. and Musa, T. (1985) A systemic pathway in the infection of oilseed rape plants by *Leptosphaeria maculans*. *Plant Pathol.* **14**, 36-45.

Hammond, K. and Lewis, B. (1986) The timing and sequence of events leading to stem canker disease in populations of *Brassica napus* var. *oleifera* in the field. *Plant Pathol*. **35**, 551-564.

Hammond, K. and Lewis, B. (1987) The establishment of systemic infection in leaves of oilseed rape by *Leptosphaeria maculans*. *Plant Pathol.* **36**, 135-157.

Hammond-Kosack, K. and Jones, J. (2000) Responses to plant pathogens. In Biochemistry and Molecular Biology of Plants, Edited by B. Buchanan, W. Guissen, R. Jones. American society of plant physiologists, Rockville, Maryland, pp. 1102-1157.

Hammond-Kosack, K. and Parker, J. (2003) Deciphering plant-pathogen communication: fresh perspectives for molecular resistance breeding. *Curr. Opin. Biotechnol.* **14**, 177-193.

He, J., **Baldini, R., Déziel, E., Saucier, M., Zhang, Q., Liberati, N., Lee, D., Urbach, J., Goodman, H. and Rahme, L.** (2004) The broad host range pathogen *Pseudomonas aeruginosa* strain PA14 carries two pathogenicity islands harboring plant and animal virulence genes. *Proc. Natl. Acad. Sci. USA,* **101**, 2530-2535.

He, P., Shan, L., Lin, N., Martin, G., Kemmerling, B., Nürnberger, T. and Sheen, J. (2006) Specific bacterial suppressors of MAMP signalling upstream of MAPKKK in *Arabidopsis* innate immunity. *Cell,* **125**, 563-575.

Heidel, A. and Dong, X. (2006) Fitness benefits of systemic acquired resistance during *Hyaloperonospora parasitica* in *Arabidopsis thaliana*. *Genetics*, **173**, 1621-1628.

Hennin, C., **Höfte, M. and Dietrichen, E.** (2001) Functional expression of *Cf9* and *Avr9* genes in *Brassica napus* induces enhanced resistance to *Leptosphaeria maculans*. *Mol. Plant-Microbe Interact.* **14**, 1075-1085.

Hewezi, T., Mouzeyar, S., Thion, L., Rickauer, M., Alibert, G., Nicolas, P. and Kallerhof, J. (2006) Antisense expression of a NBS-LRR sequence in sunflower (*Helianthus annuus* L.) and tobacco (*Nicotiana tabacum* L.): Evidence for a dual role in plant development and fungal resistance. *Transgenic Res.* **15**, 165-180

Hiemstra, J.A (1998) Some general features of Verticillium wilt in trees. Pages 137-139 in: A compendium of Verticillium wilts in tree species. J.A. Hiemstra. and D.C Harris. eds CPRO-DLO/HRI-East Malling, Wageningen, The Netherlands/West Malling, UK.

Hoeberichts, F., ten Have, A. and Woltering, E. (2003) A tomato metacaspase is upregulated during programmed cell death in *Botrytis cinerea*-infected leaves. *Planta*, **217**, 517-522.

Holmblad, J. (2006) Plant defence and impact on development. Master's thesis. SLU, Uppsala, Sweden. ISSN 1651-5196 Nr 82.

Holt, B., **Belkhadir, Y. and Dangl, J.** (2005) Antagonistic control of disease resistance protein stability in the plant immune system. *Science,* **309**, 929-932.

Holub, E. (2001) The arms race is ancient history in Arabidopsis the wildflower. *Nature Genetics* **2**, 516-527

Holub, E. and Cooper, A. (2004) Matrix, reinvention in plants: how genetics is unveiling secrets of non-host disease resistance. *Trends Plant Sci.* **9**, 211-214.

Holub, E. (2006) Evolution of parasitic symbioses between plants and filamentous organisms. *Curr. Opin. Plant Biol*. **9**, 397-405.

Hoth, S., Morgante, M., Sanchez, J., Hanafey, M., Tingey, S. and Chua, N. (2002) Genome-wide expression profiling in *Arabidopsis thaliana* reveals new targets of abscisic acid and largely impaired gene regulation in the *abi1-1* mutant. *J. Cell Science,* **115,** 4891-4900.

Howlett, B., Idnurm,, A. and Pedras, M. (2001) *Leptosphaeria maculans*, the causal agent of blackleg disease of Brassicas. *Fungal Genet. Biol.* **33**, 1-14.

Howlett, B. (2006) Secondary metabolite toxins and nutrition of plant pathogenic fungi. *Curr. Opin. Plant Biol*. **9**, 371-375.

Hu, Q., Andersen, S., Dixelius, C. and Hansen, L. (2002a) Production of fertile intergenic somatic hybrids between *Brassica napus* and *Sinapsis arvensis* for the enrichment of the rapeseed gene pool. *Plant Cell Rep.* **21**, 147-152.

Hu, Q., Hansen, L., Laursen, J., Dixelius, C. and Andersen, S. (2002b) Intergenic hybrids between *Brassica napus* and *Orychophragmus violaceus* containing traits of agronomic importance for oilseed rape breeding. *Theor. Appl. Genet.* **105**, 834-840.

Huang, Y., Evans, N., Zi-Qin, L., Eckert, M., Chèvre, A.-M., Renard, M. and Fitt, B. (2006a) Temperature and leaf wetness duration affect phenotypic expression of *Rlm6*-mediated resistance to *Leptosphaeria maculans* in *Brassica napus*. *New Phytol.* **170**, 129-141

Huang, Y., Li, Z., Evans, N., Rouxel, T., Fitt, B. and Balesdent, M. (2006b) Fitness cost associated with loss of the *AvrLm4* avirulence function in *Leptosphaeria maculans* (phoma stem canker of oilseed rape). *Eur. J. Plant Pathol.* **114**, 77-89.

Hunter, P. (2005) Common defences – comparisons between plant and animal immunity can benefit both research communities. *EMBO*, **6**, 504-507.

Inguez, A., Dong, Y., Carter, H., Ahmer, B., Stone, J. and Triplett, E. (2005) Regulation of enteric endophytic bacterial colonization by plant defenses. *Mol. Plant-Microbe Interact.* **18**, 169-178.

Inohara, N. and Nuñez, G. (2003) NODs: Intracellular proteins involved in inflammation and apoptosis. *Nat. Rev. Immunol.* **3**, 371-381.

Inohara, N., Chamaillard, M., McDonald, C. and Nuñez, G. (2005) NOD-LRR proteins: Role in host-microbial interactions and inflammatory disease. *Annu. Rev. Biochem.* **74**, 355-383.

Irdum, A. and Howlett, B. (2002) Isocitrate lyase is essential for pathogenicity of the fungus *Leptosphaeria maculans* to Canola (*Brassica napus*). *Eukaryot. Cell,* **1**, 719-724.

Janssens, S. and Beyaert, R. (2002) A universal role for MyD88 in TLR/IL-1R-mediated signaling. *Trends Biochem. Sci*. **27**, 474-482.

Jha, A., Bais, H. and Vivaco, J. (2005) *Enterococcus faecalis* mammalian virulence-related factors exhibit potent pathogenicity in the *Arabidopsis thaliana* plant model. *Infection and immunity,* **73**, 464-475.

Johansson, A., Staal, J. and Dixelius, C. (2006a) Early responses in the *Arabidopsis – Verticillium longisporum* pathosystem are dependent on *NDR1*, JA/ET-associated signals via cytosolic *NPR*1 and *RFO1*. *Mol. Plant-Microbe Interact.* **19**, 958-969.

Johansson, A., Goud, J-K.C. and Dixelius, C. (2006b) Plant host range of *Verticillium longisporum* and detection of microsclerotia in Swedish soils. *Eur. J. Plant Pathol.* **114**, 139-149.

Johansson, A. (2006) Verticillium longisporum, Infection, Host Range, Prevalence and Plant Defence Responses. Licentiate thesis, SLU Press, Uppsala. Sweden. ISBN 91-576-7153-2.

Jones, D. and Takemoto, D. (2004) Plant innate immunity – direct and indirect recognition of general and specific pathogen-associated molecules. *Curr. Opin. Immunol.* **16**, 48-62.

Kahtri, M., Al-Halali, H., Fouad Khalid, M., Saif, S. and Vyas, M. (2002) Mycetoma in Yemen: clinicoepidemiologic and histopathologic study. *Int. J. Dermatol.* **41**, 586-593.

Kagan, I.A. and Hammerschmidt, R. (2002) *Arabidopsis* ecotype variability in camalexin production and reaction to infection by *Alternaria brassicicola*. *J. Chem. Ecol.* **28**, 2121-2140.

Kamimori H, Hall K, Craik, D. and Aguilar, M. (2005) Studies on the membrane interactions of the cyclotides kalata B1 and kalata B6 on model membrane systems by surface plasmon resonance. *Anal. Biochem.* **337**, 149-153.

Karlsson, M. (2006) Impact of Arabidopsis chromosome 3 on resistance to *Leptosphaeria maculans* – a microarray study. Master's thesis. SLU, Uppsala, Sweden. ISSN 1665-5196 Nr 76.

Kawasaki, T., Nam, J., Boyes, D., Holt III, B., Hubert, D., Wiig, A. and Dangl, J. (2005) A duplicated pair of Arabidopsis RING-finger E3 ligases contribute to the *RPM1*- and *RPS2*-mediated hypersensitive response. *Plant J*. **44**, 258-270.

Kawchuk, L.M., Hachey, .J, Lynch, D.R., Kulcsar, F., van Rooijen, G., Waterer, D.R., Robertson, A., Kokko, E., Byers, R., Howard, R.J., Fischer, R. and Prüfer, D. (2001) Tomato *Ve* disease resistance genes encode cell surface-like receptors. *Proc. Natl. Acad. Sci. USA,* **98**, 6511-6515.

Kim M., da Cunha L., McFall A., Belkhadir Y., DebRoy S., Dangl J. and Mackey D. (2005) Two Pseudomonas syringae type III effectors inhibit RIN4-regulated basal defense in Arabidopsis. *Cell,* **121**, 749-759.

Kishimoto, K., Matsui, K., Ozawa, R. and Takabayashi, J. (2006) Components of C6-aldehyde-induced resistance in *Arabidopsis thaliana* against a necrotrophic fungal pathogen *Botrytis cinerea*. *Plant Sci.* **170**, 715-723.

Kliebenstein, D. (2004) Secondary metabolites and plant/environment interactions: a view through *Arabidopsis thaliana* tinged glasses. *Plant Cell and Environment*, **27**, 675-684.

Kliebenstein, D., Rowe, H. and Denby, K. (2005) Secondary metabolites influence Arabidopsis/Botrytis interactions: variation in host production and pathogen sensitivity. *Plant J*. **44**, 25-36.

Kliebenstein, D., West, M., Leeuwen, H., Kim, K., Doerge, R., Michelmoore, R. and St. Clair, D. (2006) Genomic survey of gene expression diversity in *Arabidopsis thaliana*. *Genetics,* **172**, 1179-1189.

Kloek, A., **Verbsky, M., Sharma, S., Shoelz, J., Vogel, J., Klessig, D. and Kunkel, B.** (2001) Resistance to *Pseudomonas syringae* conferred by an *Arabidopsis thaliana* coronatine-insensitive (coi1) mutation occurs through two distinct mechanisms. *Plant J.* **26**, 509-522.

Koch, E., Song, K., Osborn, T.C. and Williams, P.H. (1991) Relationship between pathogenicity and phylogeny based on restriction fragment length polymorphism in *Leptosphaeria maculans*. *Mol. Plant Microbe Interact.* **4**, 341-349.

Koch, M., **Haubold, B. and Michel-Olds, T.** (2001) Molecular systematics of the *Brassicaceae*: evidence from coding plastidic matK and nuclear Chs sequences. *Am. J. Bot*. **88**, 534-544.

Koh, S. and Somerville, S. (2006) Show and tell: cell biology of pathogen invasion. *Curr. Opin. Plant Biol.* **9**, 406-413.

Kogel, K-H., Franken, P. and Hückelhoven, R. (2006) Endophyte or parasite – what decides? *Curr. Opin. Plant Biol.* **9**, 358-363.

Koonin, E. and Aravind, L. (2002) Origin and evolution of eukaryotic apoptosis: the bacterial connection. *Cell Death and Differentiation,* **9**, 394-404.

Koornneef, M., Alonso-Blanco, C. and Vreugdenhil, D. (2004) Naturally occurring genetic variation in *Arabidopsis thaliana*. *Annu. Rev. Plant Biol*. **55**, 141-172.

Körbitz, W. (1995) Utilization of oil as biodiesel fuel. In *Brassica* Oilseeds Production and Utilization. Edited by D. Kimber, McGregor, D., CAB International, Wallingford, pp 353-372.

Kover, P. and Cacedo, L. (2001) The genetic architecture of disease resistance in plants and the maintenance of recombination by parasites. *Mol. Ecol.* **10**, 1-16.

Kowalski, S., **Lan, T., Feldman, K. and Paterson, A**. (1994) Comparative mapping of *Arabidopsis thaliana* and *Brassica oleracea* reveals islands of conserved organization. *Genetics,* **138**, 499-510.

Krüger, W. (1982) Die Wurzelhals- und Stengelfäule des Rapses, verursacht durch *Phoma lingam* (stat. gen. *Leptosphaeria maculans*), eine schwer bekämpfbare Krankheit. *Zeitschrift für Pflanzenkrankheiten und Pflazenschutz*, **89**, 498-507.

Kuhn, M., **Gout, L., Howlett, B., Melayah, D., Meyer, M., Balesdent, M. and Rouxel, T.** (2006) Genetic linkage maps and genomic organization in *Leptosphaeria maculans*. *Eur. J. Plant Pathol.* **114**, 17-31.

Kuusk A-K., Happstadius I., Zhou L., Steventon L., Griese, H. and Dixelius, C. (2002) Presence of *Leptosphaeria maculans* group A and group B isolates in Sweden. *J. Phytopathol.* **150**, 349-356.

Lagercrantz, U., **Putterill, J., Coupland, G. and Lydiate, D.** (1996) Comparative mapping in *Arabidopsis* and *Brassica*, fine scale genome collinearity and congruence of genes controlling flowering time. *Plant J.* **9**, 13-20.

Lahaye, T. (2002) The *Arabidopsis RRS1-R* disease resistance gene- uncovering the plant's nucleus as the new battlefield of plant defense? *Trends Plant Sci.* **7**, 425-427.

Larsson, S., **Håkansson, N., Näslund, I., Bergkvist, L. and Wolk, A.** (2006) Fruit and vegetable consumption in relation to pancreatic cancer risk: a prospective study. *Cancer Epidemiol. Biomarkers Prev*. **15**, 301-305.

Larsson, S (2004) Phytochemistry and phylogeny: deeds of decisive data mining. Licentiate's thesis. Uppsala university, Universitetstryckeriet, Uppsala. ISSN: 1400-6200.

Lazzeri, L., Baruzzi, G., Malaguti, L. and Loredana, A. (2003) Replacing methyl bromide in annual strawberry production with glucosinolate-containing green manure crops. *Pest Manag. Sci.* **59**, 983-990.

Lee, S., Sharma, Y., Lee, T., Chang, M. and Davis, K. (2001) Lignification induced by Pseudomonas harboring avirulent genes on Arabidopsis. *Mol. Cells*, **12**, 25-31.

Leino, M., **Thyselius, S., Landgren, M. and Glimelius, K.** (2004) *Arabidopsis thaliana* chromosome III restores fertility in a cytoplasmic male-sterile *Brassica napus* line with *A. thaliana* mitochondrial DNA. *Theor. Appl. Genet*. **109**, 272-279.

Li, C. and Cowling, W. (2003) Identification of a single dominant allele for resistance to blackleg in *Brassica napus* 'Surpass 400'. *Plant Breed.* **122**, 485-488.

Li, X., Lin, H., Zhang, W., Zhou, Y., Zhang, J., Tang, X. and Zhou, J. (2005) Flagellin induces innate immunity in nonhost interactions that is suppressed by *Pseudomonas syringae* effectors. *Proc. Natl. Acad. Sci. USA,* **102**, 12990-12995.

Lindmark, H., Johansson, K., Stöven, S., Hultmark, D., Engström, Y. and Söderhäll, K. (2001) Enteric bacteria counteract lipopolysaccharide induction of antimicrobial peptide genes. *J. Immunol.* **167**, 6920-6923.

Lipka, V., Dittgen, J., Bednarek, P., Bhat, R., Wiemer, M., Stein, M., Landtag, J., Brandt, W., Rosahl, S., Scheel, D., Llorente, F., Molina, A., Parker, J., Somerville, S. and Schulze-Lefert, P. (2005) Pre- and Postinvasion defenses both contribute to nonhost resistance in Arabidopsis. *Science,* **310**, 1180-1183.

Liu, J. and Ekramoddoullah, A. (2003) Isolation, genetic variation and expression of TIR-NBS-LRR resistance gene analogs from western white pine (*Pinus monticola* Dougl. Ex. D. Don.). *Mol. Gen. Genomics,* **270**, 432-441.

Liu, G. Holub, E., Alonso, J., Ecker, J. and Fobert, P. (2005a) An Arabidopsis NPR1-like gene, *NPR4*, is required for disease resistance. *Plant J*. **41**, 304-318.

Liu, Y., Schiff, M., Czymmek, K., Talloczy, Z., Levine, B. and Dinesh-Kumar, SP. (2005b) Autophagy regulates programmed cell death during the plant innate immune response. *Cell,* **121**, 567-577.

Lister, C. and Dean, C. (1993) Recombinant inbred lines for mapping RFLP and phenotypic markers in *Arabidopsis thaliana*. *Plant J.* **4**, 745-750.

Llorente, F., Alfonso-Blanco, C., Sanches-Rodrigues, C., Jorda, L. and Molina, A. (2005) ERECTA receptor-like kinase and heteromeric G protein from Arabidopsis are required for resistance to the necrotrophic fungus *Plectosphaerella cucumerina*. *Plant J*. **43**, 165-180.

Lorrain, S., **Lin, B., Auriac, M., Kroj, T., Saindrenan, P., Nicole, M., Balague, C. and Roby, D.** (2004) Vascular associated death1, a novel GRAM domain-containing protein, is a regulator of cell death and defense responses in vascular tissues. *Plant Cell,* **16**, 2217-2232.

Lorenzo, O., Piqueras, R., Sanches-Serrano, J. and Solano, R. (2003) ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. *Plant Cell,* **15**, 165-178.

Lorenzo, O. and Solano, R. (2005) Molecular players regulationg the jasmonate signalling network. *Curr. Opin. Plant Biol*. **8**, 1-9.

Ludwig-Müller, J., Pieper, K., Ruppel, M., Cohen, J., Epstein, E., Kiddle, G. and Bennett, R. (1999) Indole glucosinolate and auxin biosynthesis in *Arabidopsis thaliana* (L.) Heynh. glucosinolate mutants and the development of clubroot disease. *Planta*, **208**, 409-419.

Machuka, J. (2001) Agricultural biotechnology for Africa. African Scientists and Farmers must feed their own people. *Plant Phys.* **126**, 16-19.

Mac Key, J. (1981) Alternative strategies in fungal race-specific parasitism. *Theor. Appl. Genet*. **59**, 381-390.

Mahe, A., Develoux, M., Lienhardt, C., Keita, S. and Bobin, P. (1996) Mycetomas in Mali: causative agents and geographic distribution. *Am. J. Trop. Med. Hyg*. **54**, 77-79.

Maldonando, A., **Doerner, P., Dixon, R., Lamb, C. and Cameron, R**. (2002) A putative lipid transfer protein involved in systemic resistance signalling in *Arabidopsis*. *Nature,* **419**, 399-403.

Marcotte, E., **Pellegrini, M., Ng, L., Rice, D., Yeates, T. and Eisenberg, D** (1999) Detecting protein function and protein-protein interactions from genome sequences. *Science*, **285**, 751-753.

Maor, R. and Shirasu, K. (2005) The arms race continues: battle strategies between plants and fungal pathogens. *Curr. Opin. Microbiology,* **8**, 399-404.

Marquard, R. and Walker, K. (1995) Environmental impact of rapeseed production. In *Brassica* Oilseeds Production and Utilization. Edited by D. Kimber, McGregor, D., CAB international., Wallingford, pp 195-212.

Martin, G., Bogdanove, A. and Sessa, G. (2003) Understanding the functions of plant disease resistance proteins. *Annu. Rev. Plant Biol.* **54**, 23-61.

Mateo, A., **Mühlenbock, P., Rustérucci, C., Chi-Chen Chang, C., Miszalski, Z., Karpinska, B., Parker, J., Mullineaux, P. and Karpinski, S.** (2004) *LESION SIMULATING DISEASE 1* is required for acclimation to conditions that promote excess excitation energy. *Plant Physiol.* **136**, 2818-2830.

Mayerhofer, R., Wilde, K., Mayerhofer, M., Lydiate, D., Bansal, V., Good, A. and Parkin, I. (2005) Complexities of chromosome landing in a highly duplicated genome: Towards map based cloning of a gene controlling blackleg resistance in *Brassica napus*. *Genetics,* **171**, 1977-1988.

McDonald, B. and Linde, C. (2002) Pathogen population genetics, evolutionary potential, and durable resistance. *Annu. Rev. Phytopathol*. **40**, 349-379.

McDowell, J., Cuzick, A., Can, C., Beynon, J., Dangl, J. and Holub, E. (2000) Downy mildew (*Peronospera parasitica*) resistance genes in Arabidopsis vary in functional requirements for NDR1, EDS1, NPR1 and salicylic acid accumulation. *Plant J*. **22**, 523-529.

McHale, L., Tan, X., Koehl, P. and Michelmore, R. (2006) Plant NBS-LRR proteins: Adaptable guards. *Genome Biol.* **7**, 212.1-212.11

Mert-Türk, F., Bennett, M.H., Mansfield, J.W. and Holub, E.B. (2003) Camalexin accumulation in *Arabidopsis* following abiotic elicitation or inoculation with virulent or avirulent *Hyaloperonospora parasitica*. *Physiol. Mol. Plant Pathol.* **62**, 137-145.

Mestre, P. and Baulcombe, D. (2005) Elicitor-mediated oligomerization of the tobacco N disease resistance protein. *Plant Cell,* **18**, 491-501.

Meyers, B., Dickerman, A., Michelmore, R., Sivaramakeishnan, S., Sobral, B. and Young, N. (1999) Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. *Plant J*. **20**, 317-332.

Meyers, B.C., Morgante, M. and Michelmore, R.W. (2002) TIR-X and TIR-NB proteins: two new families related to disease resistance TIR-NB-LRR proteins encoded in *Arabidopsis* and other plant genomes. *Plant J.* **32**, 77-92.

Meyers, B.C., Kozik, A., Griego, A., Kuang, H. and Michelmore, R.W. (2003) Genome-wide analysis of NB-LRR-encoding genes in Arabidopsis. *Plant Cell*, **15**, 809-834.

Meyers, B., Kaushik, S. and Nandety, R. (2005) Evolving disease resistance genes. *Curr. Opin. Plant Biol.* **8**, 129-134.

Michaut, L., **Fehlbaum, P., Moniatte, M., Van Dorsselaer, A., Reichhart, J. and Bulet, P** (1996) Determination of the disulfide array from the first inducible antifungal peptide from insects: drosomycin from *Drosophila melanogaster*. *FEBS letters,* **395**, 6-10.

Molinier, J., Ries, G., Zipfel, C. And Hohn, B. (2006) Transgeneration memory of stress in plants. *Nature*, **442**, 1046-1049.

Mou, Z., Fan, W. and Dong, X. (2003) Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. *Cell,* **113**, 935-944.

Mur, L., Kenton, P., Atzorn, R., Miersch, O. and Wasternack, C. (2006). The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism and oxidative stress leading to cell death. *Plant Physiol.* **140**, 249-262.

Murphy, D. and Mithen, R. (1995) Biotechnology. In *Brassica* Oilseeds Production and Utilization. Edited by D. Kimber, McGregor, D., CAB international, Wallingford, pp 177-191

Narasimhan, M., Damsz, B., Coca, M., Ibeas, J., Yun, D., Pardo, J., Hasegawa, P. and Bressan, R. (2001) A plant defense response effector induces microbial apoptosis. *Mol. Cell*, **8**, 921-930.

Narasimhan, M., **Lee, H., Damsz, B., Singh, N., Ibeas, N., Matsumuto, T., Woloshuk, C. and Bressan, R.** (2003) Overexpression of a cell wall glycoprotein in *Fusarium oxysporum* increases virulence and resistance to a plant PR5 protein. *Plant J.* **36**, 390-400.

Narusaka, Y., Narusaka, M., Park, P., Kubo, Y., Hiryama, T., Seki, M., Shiraishi, T., Ishida, J., Nakahima, M., Enju, A., Sakurai, T., Satou, M., Kobyashi, M. and Shinozaki, K. (2004) *RCH1*, a locus in *Arabidopsis* that confers resistance to the hemibiotrophic fungal pathogen *Colletotrichum higginsianum*. *Mol. Plant-Microbe Interact*. **17**, 749-762.

Naváez-Vásquez, J., Pearce, G. and Ryan, C. (2005) The plant cell wall matrix harbors a precursor of defense signalling peptides. *Proc. Natl. Acad. Sci.USA,* **102**, 12974-12977.

Navarro, L., **Dunoyer, P., Jay, F., Arnold, B., Dharmasiri, N., Estelle, M., Voinnet, O. and Jones, J.** (2006) A plant miRNA contributes to antibacterial resistance by repressing auxin signalling. *Science,* **312**, 436-439.

Netea, M., Van der Meer, W. and Kullberg, B. (2006) Role of the dual interaction of fungal pathogens with pattern recognition receptors in the activation and modulation of host defence. *Clin. Microbiol. Infect.* **12**, 404-409.

Nimchuk, Z., **Rohmer, L., Chang, J. and Dangl, J.** (2001) Knowing the dancer from the dance: *R gene* products and their interactions with other proteins from host and pathogen. *Curr. Opin. Plant Biol.* **4**, 288-294.

Nimchuk, Z., Eulgem, T., Holt, B. and Dangl, J. (2003) Recognition and response in the plant immune system. *Annu. Rev. Genet.* **37**, 579-609.

Nombela, G., Williamson, V. and Muñiz, M. (2003) The root-knot nematode resistance gene *Mi1.2* of tomato is responsible for resistance against the whitefly *Bemisia tabaci*. *Mol. Plant-Microbe Interact.* **16**, 645-649.

Norman-Setterblad, C., Vidal, S. and Palva, T. (2000) Interacting signal pathways control defense gene expression in response to cell wall degrading enzymes from *Erwinia carotovora*. *Mol. Plant-Microbe Interact.* **13**, 430-438.

Norton, R., Burton, W. and Salisbury, P. (2004) Canola quality *Brassica juncea* for Australia. 4th International Crop Science Congress, Brisbane, Australia, pp. 1275.

Nürnberger, T. and Brunner, F. (2002) Innate immunity in plants and animals: emerging parallels between the recognition of general elicitors and pathogen-associated molecular patterns. *Curr. Opin. Plant Biol.* **5**, 1-7.

Nürnberger, T., Brunner, F., Kemmerling, B. and Piater, L. (2004) Innate immunity in plants and animals: striking similarities and obvious differences. *Immunol. Reviews,* **198**, 249-266.

O'Donnel, P., Schmeiz, E., Moussatche, P., Lund, S., Jones, J. and Klee, H. (2003) Susceptible to intolerance- a range of hormonal actions in a susceptible *Arabidopsis* pathogen response. *Plant J.* **33**, 245-257.

OEPP/EPPO (1994) EPPO Standard PP 2/1(1) Guideline on good plant protection practice: principle of good plant protection practice. *Bulletin OEPP/EPPO Bulletin*, **24**, 233-240. Supplement PP 2/7 (1) Vegetable brassicas (1998).

Oh, S., **Park, A., Bae, M., Kwon, S., Kim, Y., Lee, J., Kang, N., Lee, S., Cheong, H. and Park, O.** (2005) Secretome analysis reveals an *Arabidopsis* lipase involved in defense against *Alternaria brassicicola*. *Plant Cell*, **17**, 2832-2847.

Okori, P. (2004) Population studies of *Cercospora zeae-maydis* and related *Cercospora* fungi. PhD thesis, Agraria 468. SLU Press, Uppsala. Sweden.

Ortoneda, M., **Guarro, J., Madrid, M., Caracuel, Z., Roncero, M., Mayayo, E. and Di Pietro, A.** (2004) *Fusarium oxysporum* as a multihost model for the genetic dissection of fungal virulence in plants and animals. *Infect. Immun.* **72**, 1760-1766.

Otim-Nape, G., Sserubombwe, W., Alicai, T. and Thresh, J. (2002) Plant virus diseases in sub-Saharan Africa: impact, challenges, and the need for global action. In *Plant virology in sub-saharan Africa*. International Institute of Tropical Agriculture. pp. 299-311.

Overmeyer, K., **Touminen, H., Kettunen, R., Betz, C., Langebartels, C., Sandermann, H. and Kangasjärvi, J.** (2000) Ozone-insensitive Arabidopsis *rcd1* mutant reveals opposite roles for ethylene and jasmonate signalling pathways in regulating superoxide-dependent cell death. *Plant Cell,* **12**, 1849-1862.

Pang, E. and Halloran, G. (1996) The genetics of blackleg [*Leptosphaeria maculans* (Desm.) Ces. et De Not.] resistance in rapeseed (*Brassica napus* L.). II. Seedling and adult-plant resistance as quantitative traits. *Theor. Appl. Genet.* **93**, 941-949.

Panstruga, R. and Schulze-Lefert, P. (2003) Corruption of host seven-transmembrane proteins: a common theme in animals and plants? *Microbes and Infection,* **5**, 429-437.

Park, J., Oh, S., Kim, Y., Woo, H. and Nam, H. (1998) Differential expression of senescence-associated mRNAs during leaf senescence induced by different senescence-inducing factors in Arabidopsis. *Plant Mol. Biol.* **37**, 445-454.

Parleviet, J. and Zadoks, J. (1977) The integrated concept of disease resistance: A new view including horizontal and vertical resistance in plants. *Euphytica* **26**, 5-21.

Parkin, I., Gulden, S., Sharpe, A., Lukens, L., Trick, M., Osborn, T. and Lydiate, D. (2005) Segmental structure of the *Brassica napus* genome based on comparative analysis with *Arabidopsis thaliana*. *Genetics,* **171**, 765-781.

Paszkowski, U. (2006) Mutualism and parasitism: The yin and yang of plant symbioses. *Curr. Opin. Plant Biol.* **9**, 364-370.

Patel, S., Caplan, J. and Dinesh-Kumar, S. (2006) Autophagy in the control of programmed cell death. *Curr. Opin. Plant Biol.* **9**, 391-396.

Paul, V. (2003) RAPS Krankheiten Schädlinge Schadpflanzen. AgroConcept GmbH, Bonn, Verlag Th. Mann, Gelsenkirchen.

Peck, H. (1898) *Harpers Dictionary of Classical Antiquities.* Harper and Brothers. New York, at http://www.perseus.tufts.edu.

Pedras, M., **Nycholat, C., Monaut, S., Xu, Y. and Khan, A.** (2002) Chemical defenses of crucifers: elicitation and metabolism of phytoalexins and indole-3-acetonitrile in brown mustard and turnip. *Phytochemistry* **59**, 611-625.

Pedras, M. and Montaut, S. (2003) Probing crucial metabolic pathways in fungal pathogens of crucifers: biotransformation of indole-3-acetaldoxime, 4-hydroxyphenylacetaldoxime, and their metabolites. *Bioorganic & Medicinal Chemistry,* **11**, 3115-3120.

Pedras, M., **Chumala, P. and Suchy, M**. (2003) Phytoalexins from *Thaspi arvense*, a wild crucifer resistant to virulent *Leptosphaeria maculans*: structures, syntheses and antifungal activity. *Phytochemistry,* **64**, 949-956.

Pedras, M., **Jha, M. and Okeola, O**. (2005) Camalexin induces detoxification of the phytoalexin brassinin in the plant pathogen *Leptosphaeria maculans*. *Phytochemistry,* **66**, 2609-2616.

Pedras, M. and Jha, M. (2006) Toward the control of *Leptosphaeria maculans*: Design, syntheses, biological activity, and metabolism of potential detoxification inhibitors of the crucifer phytoalexin brassinin. *Bioorganic & Medicinal Chemistry*, **14**, 4958-4979.

Peña-Cortés, H., Fisahn, J. and Willmitzer, L. (1995) Signals involved in wound-induced proteinase inhibitor II gene expression in tomato and potato plants. *Proc. Natl. Acad. Sci. USA,* **92**, 4106-4113.

Petrie, G. (1978) Occurrence of a highly virulent strain of blackleg (*Leptosphaeria maculans*) on rape in Saskatchewan (1975-77). *Canadian Plant Disease Survey*, **58**, 21-25.

Pfisterer, P., König, H., Hess, J., Lipowsky, G., Haendler, B., Schleuning, W. and Wirth, T. (1996) CRISP-3, a protein with homology to plant defense proteins is expressed in mouse B cells under the control of Oct2. *Mol. Cell Biol.* **16**, 6160-6168.

Piening, L., Okolo, E. and Harder, D. (1975) Blackleg of rapeseed in Kenya. *E. Afr. Agric. For. J.* **41**, 110-113.

Pieterse, C., Van Pelt, J., Ton, J., Parchmann, S., Mueller, M., Buchala, A., Metraux J-P. and Van Loon, L. (2001) Rhizobacteria-medited induced systemic resistance (ISR) in *Arabidopsis* requires sensitivity to jasmonate and ethylene but is not accompanied by an increase in their production. *Physiol. Mol. Plant Pathol.* **57**, 123-134.

Pietravalle, S., Lemarié, S. and van den Bosch, F. (2006) Durability of resistance and cost of virulence. *Eur. J. Plant Pathol.* **114**, 107-116.

Pitman, A., **Jackson, R., Mansfield, J. and Arnold, D.** (2005) Exposure to host resistance mechanisms drives evolution of bacterial virulence in plants. *Curr. Biol*. **15**, 2230-2235.

Poirier, Y. (2001) Production of polyesters in transgenic plants. *Adv. Biochem. Eng. Biotech.* **71**, 209-240.

Potrykus, I. (2001) The "Golden Rice" tale. *In Vitro Cell Dev. Biol. – Plant,* **37**, 93-100.

Pradel, E. and Ewbank, J. (2004) Genetic models in pathogenesis. *Annu. Rev. Genet*. **38**, 347-363.

Prithiviraj, B., Bais, H., Jha, A. and Vivaco, J. (2005) *Staphylococcus aureus* pathogenicity on *Arabidopsis thaliana* is mediated either by a direct effect of salicylic acid on the pathogen or by SA-dependent, NPR1-independent host responses. *Plant J.* **42**, 417-432.

Ramonell, K., Beroca-Lobo, M., Koh, S., Wan, J., Edwards, H., Stacey, G. and Somerville, S. (2005) Loss-of-function mutations in chitin-responsive genes show increased susceptibility to the powdery mildew pathogen *Erysiphe cichoracearum. Plant Phys.* **138**, 1027-1036.

Rep, M. (2005) Small proteins of plant-pathogenic fungi secreted during host colonization. *FEMS Microb. Lett*. **253**, 19-27.

Rezzonico, E., Flury, N., Meinz, F., and Beffa, R. (1998). Transcriptional down-regulation by abscisic acid of pathogenesis-related β-1,3-glucanase genes in tobacco cell cultures. *Plant Physiol.* **117**, 585-592.

Rimmer, S. and van den Berg, C. (1992) Resistance of oilseed Brassica spp. to blackleg caused by *Leptosphaeria maculans*. *Can. J. Plant Pathol.* **14**, 56-66.

Rimmer, S. and Buchwaldt, L. (1995) Diseases. In *Brassica Oilseeds*. Edited by D. Kimber, McGregor, D., CAB international, Wallingford, pp 65-92.

Rimmer, S. (2006) Resistance genes to *Leptosphaeria maculans* in *Brassica napus*. *Can. J. Plant Pathol.* **28**, S288-S297.

Roetschi, A., Azeddine, S., Lassaâd, B., Mauch, F. and Mauch-Mani, B. (2001) Characterization of an *Arabidopsis-Phytophthora* pathosystem: resistance requires a functional *PAD2* gene and is independent of salicylic acid, ethylene and jasmonic acid signaling. *Plant J.* **28**, 293-305.

Rogers, E., Glazebrook, J. and Ausubel, F. (1996) Mode of Action of the Arabidopsis thaliana phytoalexin camalexin and its role in Arabidopsis-pathogen interactions. *Mol. Plant-Microbe Interact.* **9**, 748-757.

Rooney, H., **van't Klooster, J., van der Hoorn, R., Joosten, M., Jones, J. and de Wit, P.** (2005) *Cladosporium* Avr2 inhibits tomato Rcr3 protease required for *Cf-2*-dependent disease resistance. *Science,* **308**, 1783-1786.

Rosenblueth, M. and Martínez-Romero, E. (2006) Bacterial endophytes and their interactions with hosts. *Mol. Plant-Microbe Interact.* **19**, 827-837.

Roussel, S., **Nicole, M., Lopez, F., Renard, M., Chévre, M. and Brun, H.** (1999) Cytological investigations of resistance to *Leptosphaeria maculans* conferred to *Brassica napus* by introgressions originating from *B. juncea* or *B. nigra* B genome. *Phytopathology,* **89**, 1200-1213.

Rouxel, T. and Balesdent, M. H. (2005) The stem canker (blackleg) fungus, *Leptosphaeria maculans*, enters the genomic era. *Mol. Plant Pathol.* **6**, 225-241.

Rowland, O., Ludwig, A., Merrick, C., Baillelieul, F., Tracy, F., Durrant, W., Friz-Laylin, L., Nekrasov, V., Sjolander, K., Yoshioka, H. and Jones, J. (2004) Functional analysis of Avr9/Cf-9 rapidly elicited genes identifies a protein kinase, ACIK1, that is essential for full Cf-9-dependent disease resistance in tomato. *Plant Cell*, **17**, 295-310.

Ryals, J., Weymann, K., Lawton, K., Friedrich, L, Ellis, D., Steiner, H.Y., Johnson, J., Delaney, T.P., Jesse, T., Vos, P. and Uknes, S. (1997) The Arabidopsis NIM1 protein shows homology to the mammalian transcription factor inhibitor I kappa B. *Plant Cell,* **9**, 425-439.

Saal, B., **and Struss, D.** (2005) RGA- and RAPD-derived SCAR markers for *Brassica* B-genome introgression conferring resistance to blackleg in oilseed rape. *Theor. Appl. Genet*. **111**, 281-290.

Samuelsson, G. (1999) Drugs of natural origin. Swedish Pharmaceutical Society, Swedish Pharmaceutical Press, Stockholm, Sweden, pp 15-23.

Schauvliege, R., Vanrobaeys, J., Schotte, P. and Beyaert, R. (2002) Caspase-11 gene expression in response to lipopolysaccharide and interferon-gamma requires nuclear factor-kappa B and signal transducer and activator of transcription (STAT) 1. *J. Biol. Chem.* **277**, 41624-41630.

Schenk, P.M., Kazan, K., Wilson, I., Anderson, J.P. Richmond, T., Somerville, S.C. and Manners, J.M. (2000). Co-ordinated plant defence responses in *Arabidopsis* revealed by microarray analysis. *Proc. Natl. Acad. Sci. USA,* **97**, 11655-11660.

Schenk, P., Kazan, K., Rusu, A., Manners, J. and Maclean, D. (2005) The SEN1 gene of Arabidopsis is regulated by signals that link plant defence responses and senescence. *Plant Physiol. Chem.* **43**, 997-1005.

Schuhegger, R., **Rahut, T. and Glawisching, E.** (2006) Regulatory variability of camalexin biosynthesis. *J. Plant Physiol.* (Electronic publication June 10).

Schulz, B., and Boyle, C. (2005) The endophytic continuum. *Mycol. Res.* **109**, 661-686.

Seidel, D., **Daebeler, F. Amelung, D., Engel, K-H. and Lücke, W.** (1984) Auftreten, Shadwirkung und Bekämpfung von *Phoma lingam* an Winteraps. *Nachrichtenblatt für den Pflanzenschutz in der DDR*, **33**, 120-123.

Sexton, A., **Paulsen, M., Woestemeyer, J. and Howlett, B.** (2000) Cloning, characterization and chromosomal location of three genes encoding host-cell-wall-degrading enzymes in *Leptosphaeria maculans*, a fungal pathogen of *Brassica* spp. *Gene,* **248**, 89-97.

Shah, J. (2005) Lipids, lipases, and lipid-modifying enzymes in plant disease resistance. *Annu. Rev. Phytopathol.* **43**, 229-260.

Shao, F., **Merritt, P., Bao, Z., Innes, R. and Dixon, J.** (2002) A *Yersinia* effector and a *Pseudomonas* avirulence protein define a family of cysteine proteases functioning in bacterial pathogenesis. *Cell* **109**, 575-588.

Shen, J., Araki, H., Chen, L., Chen, J-Q. and Tian, D. (2006) Unique evolutionary mechanism in *R gene*s under the Presence/Absence polymorphism in *Arabidopsis thaliana*. *Genetics,* **172**, 1243-1250.

Siemens, J., Keller, I, Sarx, J., Kunz, S., Schuller, A., Nagel, W., Schmulling, T., Parniske, M. and Ludwig-Müller, J. (2006) Transcriptome analysis of Arabidopsis clubroots indicate a key role for cytokinins in disease development. *Mol. Plant-Microbe Interact.* **19**, 480-494.

Sillito, D., **Parkin, I., Mayerhofer, R., Lydiate, D. and Good, A.** (2000) *Arabidopsis thaliana*: a source of candidate disease-resistance genes for *Brassica napus*. *Genome,* **43**, 452-460.

Simonsen, S., **Sando, L., Ireland, D., Colgrave, M., Bharathim R., Göransson, U. and Craik, D.** (2005) A continent of plant defense peptide diversity: cyclotides in Australian *Hybanthus* (Violaceae). *Plant Cell,* **17**, 3176-3189.

Sivasithamparam, K., Barbetti, M. and Li, H. (2005) Recurring challenges from a necrotrophic fungal plant pathogen: a case study with *Leptosphaeria maculans* (causal agent of blackleg disease in Brassicas) in Western Australia. *Ann. Bot. (Lond.)* **96**, 363-377.

Sjödin, C. and Glimelius, K. (1989) Transfer of resistance against *Phoma lingam* to *Brassica napus* L. by asymmetric somatic hybridisation and toxin selection. *Theor. Appl. Genet.* **78**, 513-520.

Smith, B., **Kirkegaard, J. and Howe, G.** (2004) Impacts of *Brassica* break-crops on soil biology and yield of following wheat crops. *Aust. J. Agric. Res.* **55**, 1-11.

Somerville, C. and Koornneef, M. (2002) A fortunate choice: the history of *Arabidopsis* as a model plant. *Nature Rev. Genetics,* **3**, 883-889.

Sprague, S., **Balesdent, M., Brun, H., Hayden, H., Marcroft, S., Pinochet, X., Rouxel, T. and Howlett, B.** (2006) Major gene resistance in *Brassica napus* (oilseed rape) is overcome by changes in virulence of populations of *Leptosphaeria maculans* in France and Australia. *Eur. J. Plant Pathol.* **114**, 33-40.

Staal, J. (2001) Sequencing the cyclotide genes- method development. Master's thesis. Division of Pharmacognosy, Uppsala University & Institute of Technology, Umeå University. Umeå, Sweden.

Stachowiak, A., **Olechnowicz, J., Jedryczka, M., Rouxel, T., Balesdent, M., Happstadius, I., Gladders, P., Latunde-Dada, A. and Evans, N.** (2006) Frequency of avirulence alleles in field populations of *Leptosphaeria maculans* in Europe. *Eur. J. Plant Pathol.* **114**, 67-75.

Strange, R. and Scott, P. (2005) Plant disease: A threat to global food security. *Annu. Rev. Phytopathol.* **43**, 83-116.

Strober, W., **Murray, P., Kitani, A. and Watanabe, T.** (2006) Signalling pathways and molecular interactions of NOD1 and NOD2. *Nature Reviews Immunology* **6**, 9-20.

Subramanian, B., **Bansal., V. and Kav, N** (2005) Proteome-level investigation of *Brassica carinata*-derived resistance to *Leptosphaeria maculans*. *J. Agric. Food Chem.* **53**, 313-324.

Suwabe, K., Tsukazaki, H., Iketani, H., Hatakeyama, K., Kondo, M., Fujimura, M. Nunome, T., Fukuoka, H., Hirai, M. and Matsumoto, S. (2006) Simple sequence repeat-based comparative genomics between *Brassica rapa* and *Arabidopsis thaliana*: The genetic origin of Clubroot resistance. *Genetics*, **173**, 309-319.

Svangård, E. (2005) Cytotoxic cyclotides - Structure, Activity, and Mode of Action. *Acta universatis uppsaliensis,* Dissertations from faculty of Pharmacy 19, Uppsala University. ISBN: 91-554-6378-9.

Takken, F., Albrecht, M. and Tameling, W. (2006) Resistance proteins: molecular switches of plant defence. *Curr. Opin. Plant Biol.* **9**, 383-390.

Tauger, M. (2003) Entitelment, shortage and the 1943 Bengal Famine: Another look. *J. Peasant Studies* **31**, 45-72.

Terwari, J. and Mithen, R. (1999) Diseases. In Biology of Brassica Coenospecies. Edited by C. Gomez-Campo. Elsevier Science, Amsterdam, the Netherlands, pp 309-411.

The Arabidopsis Genome Initiative. (2000) Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. *Nature*, **408**, 796-815.

Thilmony, R., **Underwood, W. and He, S.** (2006) Genome-wide transcriptional analysis of the *Arabidopsis thaliana* interaction with the plant pathogen *Pseudomonas syringae* pv tomato DC3000 and the human pathogen *Escherichia coli* O157:H7. *Plant J.* **46**, 34-53.

Thomma, B., Cammue, B. and Thevissen, K. (2002) Plant defensins. *Planta* **216**, 193-202

Thomma, B., Cammue, B. and Thevissen, K. (2003) Mode of action of plant defensins suggests therapeutic potential. *Curr. Drug Targets Infect. Disord*. **3**, 1-8.

Tian, D., Traw, B., Chen, J., Kreitman, M. and Bergelson, J. (2003) Fitness costs of R gene-mediated resistance in *Arabidopsis thaliana*. *Nature* **423**, 74-76.

Tian, Y., Fan, L., Thurau, T. and Cai, D. (2004) The absence of TIR-type resistance gene analogues in the sugar beet (*Beta vulgaris* L.) genome. *J. Mol. Evol.* **58**, 40-53.

Ton, J. and Mauch-Mani, B. (2004) β-aminobutyric acid resistance against two necrotrophic pathogens is based on ABA-dependent priming for callose. *Plant J.* **38**, 119-130.

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

Tör, M., **Brown, D., Cooper, A., Woods-Tör, A., Sjölander, K., Jones, J. and Holub, E.** (2004) Arabidopsis downy mildew resistance gene *RPP27* encodes a receptor-like protein similar to *CLAVATA2* and Tomato *Cf-9*. *Plant Physiol.* **135**, 1100-1112.

Torres, M. and Dangl, J. (2005) Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. *Curr. Opin. Plant Biol.* **8**, 397-403.

Town, C., Cheung, F., Maiti, R., Crabtree, J., Haas, B., Wortman, J., Hine, E., Althoff, R., Arbogast, T., Tallon, L., Vigouroux, M., Trick, M. and Bancroft, I. (2006) Comparative genomics of *Brassica oleracea* and *Arabidopsis thaliana* reveal gene loss, fragmentation and dispersal after polyploidy. *Plant Cell,* **18**, 1348-1359.

Trusov, Y., Rookes, J., Chakravorty, D., Armour, D., Schenk, P. and Botella, J. (2006) Heteromeric G proteins facilitate Arabidopsis resistance to necrotrophic pathogens and are involved in jasmonate signalling. *Plant Physiol.* **140**, 210-220.

U, N. (1935) Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. *Japan. J. Bot.* **7**, 389-452.

Uren, A., O'Rourke, K., Aravind, L., Pisabarro, M., Seshagiri, S., Koonin, E. and Dixit, E. (2000) Identification of paracaspases and metacaspases: Two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma. *Mol. Cell,* **6**, 961-967.

Vance, C., Kirk, T. and Sherwood, R. (1980) Lignification as a mechanism of disease resistance. *Ann. Rev. Phytopathol.* **18**, 259-288.

van der Biezen, E. and Jones, J. (1998a) Plant disease resistance proteins and the gene-for-gene concept. *Trends Biochem. Sci.* **23**, 454-456.

van der Biezen, E. and Jones, J. (1998b) The NB-ARC domain: a novel signalling motif shared by plant resistance gene products and regulators of cell death in animals . *Curr. Biol.* **8**, R226-227.

Vanderplank, J. (1984) Disease resistance in plants. Second Edition. Academic Press. Orlando, Florida, pp 57-80.

van Eijk, M., van Roomen ,C.P., Renkema, G.H., Bussink, A.P., Andrews, L., Blommaart, E.F., Sugar, A., Verhoeven, A.J., Boot, R.G. and Aerts, J.M. (2005) Characterization of human phagocyte-derived chitotriosidase, a component of innate immunity. *Int. Immunol*. **17**, 1505-1512.

van Hulten, M., **Pelser, M., van Loon, L., Pieterse, C. and Ton, J.** (2006) Costs and benefits of priming for defense in Arabidopsis. *Proc. Natl. Acad. Sci. USA,* **103**, 5602-5607.

van Loon, L. **and van Strien, E.** (1999) The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiol. Mol. Plant Pathol*. **55**, 85-97.

van Loon, L., Rep, M. and Pieterse, C. (2006) Significance of inducible defense-related proteins in infected plants. *Annu. Rev. Phytopathol.* **44**, (Electronic publication March 3).

Van Wees. S,C. and Glazebrook, J. (2003) Loss of non-host resistance of Arabidopsis *NahG* to *Pseudomonas syringae* pv. *phaseolicola* is due to degradation products of salicylic acid. *Plant J.* **33**, 733-742.

Vera Cruz, C., Bai, J., Oña, I., Leung, H., Nelson, R., Mew, T. and Leach, J. (2000) Predicting durability of a disease resistance gene based on an assessment of the fitness loss and epidemiological consequences of avirulence gene mutation. *Proc. Natl. Acad. Sci. USA,* **97**, 13500-13505.

Verhagen, B., Glazebrook, J., Zhu, T., Chang, H., van Loon, L. and Pieterse, C. (2004) The transcriptome of rhizobacteria-induced systemic resistance in Arabidopsis. *Mol. Plant-Microbe Interact.* **17**, 859-908.

Veronese, P., Narasimhan, M.L., Stevenson, R.A., Zhu, J.K., Weller, S.C., Subbarao, K.V. and Bressan, R.A. (2003) Identification of a locus controlling *Verticillium* disease symptom response in *Arabidopsis thaliana*. *Plant J.* **5**, 574-587.

Vles, R. **and Gottenbos, J.** (1989). Nutritional characteristics and food uses of vegetable oils. In Oil crops of the World. Edited by G. Röbbelen, R. Downey and A. Ashiri. McGraw-Hill Inc. USA, pp 63-87.

Vogel, J. and Somerville, S.C. (2000) Isolation and characterization of powdery mildew-resistant Arabidopsis mutants. *Proc. Natl. Acad, Sci, USA*, **97**, 1897–1902.

von der Lehr, N., Johansson, S., Wu, S., Bahram, F., Castell, A., Cetinkaya, C., Hybring, P., Weidung, I., Nakayama, K., Nakayama, K.I., Soderberg, O., Kerppola, T. and Larsson, L.G. (2003) The F-box protein Skp2 participates in c-Myc proteosomal degradation and acts as cofactor for c-Myc-regulated transcription. *Mol. Cell*, **11**, 1189-1200.

von Laibach, **F**. (1943) *Arabidopsis thaliana* (L.) Heynh. als object für genetische und entwicklungs-physiologische untersuchungen. *Bot. Arch*. **44**, 439-455.

Walton, J. (1994) Deconstucting the cell wall. *Plant Physiol.* **104**, 1113-1118.

Wang, Y., Nowak, G., Culley, D., Hadwiger, L. and Fristensky, B. (1999) Constituitive expression of pea defense gene DRR206 confers resistance to blackleg (*Leptosphaeria maculans*) disease in transgenic canola (*Brassica napus*). *Mol. Plant-Microbe Interact.* **12**, 410-418.

Ward, E.W.B,, Cahill, D.M. and Bhattacharyya, M. (1989) Abscisic acid suppression of phenylalanine amonia lyase activity and mRNA, and resistance of soybeans to *Phytophthora megasperma* f. sp. *glycinea*. *Plant Physiol.* **91**, 23-27.

Warren, R., Merrit, P., Holub, E. and Innes, R. (1999) Identification of three putative signal transduction genes involved in R gene-specified disease resistance in Arabidopsis. *Genetics,* **152**, 401-412.

Williams, P. (1992) Biology of *Leptosphaeria maculans*. *Can. J. Plant Pathol.* **14**, 30-35.

Winnenburg, R., **Baldwin, T., Urban, M., Rawlings, C., Köhler, J. and Hammond-Kosack, K.** (2006) PHI-base: a new database for pathogen host interactions. *Nucleic Acids Res.* **34**, D459-464.

World Health Organization (2000a) NUTRITION for health and development – a global agenda for combating malnutrition. Progress report. WHO/NHD/00.6

World Health Organization (2000b) Turning the tide of malnutrition – responding to the challenge of the 21st century WHO/NHD/00.7

Wretblad, S. and Dixelius, C. (2000) B-genome derived resistance to *Leptosphaeria maculans* in near isogenic *Brassica napus* lines is independent of glucosinolate profile. *Physiol. Plant.* **110**, 461-468.

Wretblad, S (2002) Defence responses in *Brassica nigra* and *B. napus* to the fungal pathogen *Leptosphaeria maculans*. PhD thesis, Agraria 316. SLU Press, Uppsala. Sweden.

Xu, J., Peng, Y., Dickman, M. and Sharon, A. (2006) The dawn of fungal pathogen genomics. *Annu. Rev. Phytopathol.* **44**, (Electronic publication April 20).

Yoon, H., Hackett, J., Ciniglia, C., Pinto, G. And Bhattacharya, D. (2004) A molecular timeline for the origin of photosynthetic eukaryotes. *Mol. Biol. Evol.* **21**, 809-818.

Yu, F., Lydiate, D. and Rimmer, S. (2005) Identification of two novel genes for blackleg resistance in *Brassica napus*. *Theor. Appl. Genet.* **110**, 969-979.

Zadoks, J. (1999) Reflections on space, time and diversity. *Annu. Rev. Phytopathol.* **37**, 1-17.

Zeidler, **D., Zähringer, U., Gerber, I., Dunbery, I., Hartung, T., Bors, W., Hutzler, P and Durner, J.** (2004) Innate immunity in *Arabidopsis thaliana*: Lipopolysaccharides activate nitric oxide synthase (NOS) and induce defense genes. *Proc. Natl. Acad. Sci.USA,* **101**, 15811-15816.

Zhang, X. and Gassmann, W. (2003) *RPS4*-mediated disease resistance requires the combined presence of *RPS4* transcripts with full-length and truncated open reading frames. *Plant Cell*, **15**, 2333-2342.

Zhang, Y., Cheng, Y., Bi, D., Palma, K. and Li, X. (2005) MOS2, a protein containing G-patch and KOW motifs, is essential for innate immunity in *Arabidopsis thaliana*. *Curr. Biol.* **15**, 1936-1942.

Zhang, Y. and Li, X. (2005) A putative nucleoporin 96 is required for both basal defense and constitutive resistance responses mediated by suppressor of *npr1-1*, constituitive 1. *Plant Cell,* **17**, 1306-1316.

Zipfel, C. and Felix, G. (2005) Plants and animals: a different taste for microbes? *Curr. Opin. Plant Biol*. **8**, 353-360.

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