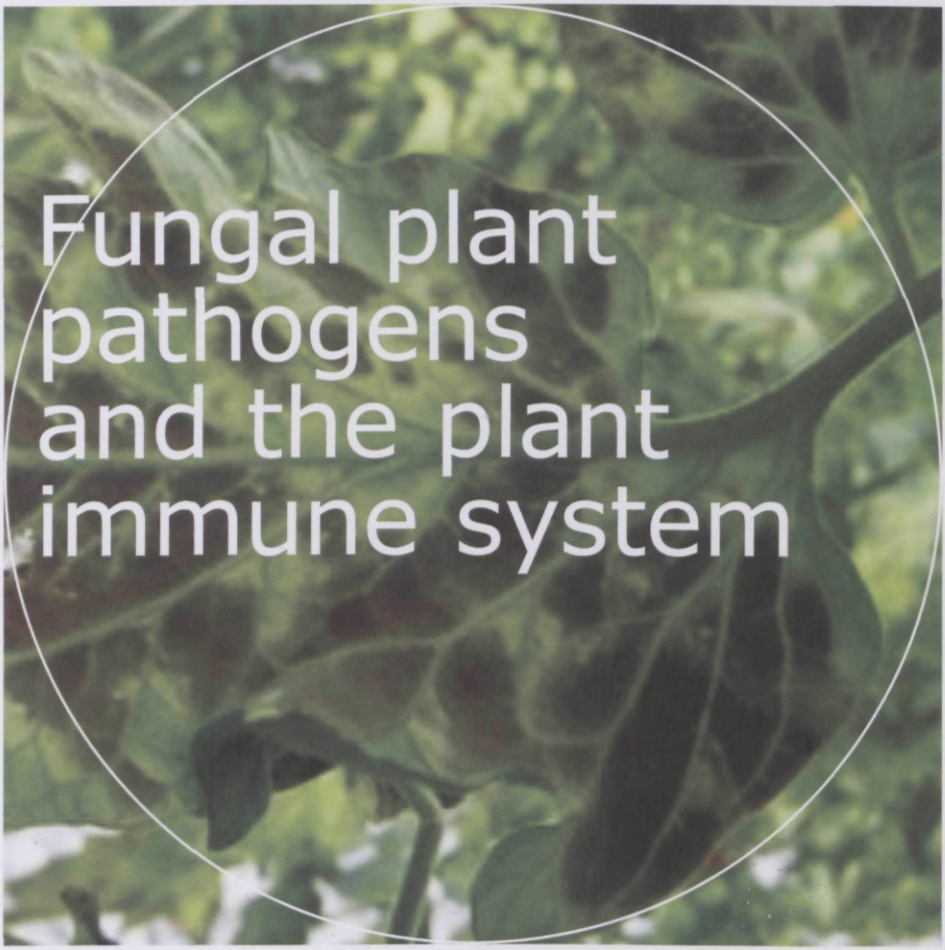

Fungal plant pathogens and the plant immune system

Prof. dr *ir.* Pierre J.G.M. de Wit

Farewell address upon retiring as Professor of Phytopathology
at Wageningen University on 5 June 2014

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Fungal plant pathogens and the plant immune system[1] [2]

Rector Magnificus, relatives, friends, colleagues, ladies and gentlemen,

I was often asked the question by friends and relatives: "Are you still working on tomato and *Cladosporium fulvum*?" When will your work be finished? Then I often responded by comparing my work with that of a medical doctor or immunologist who also works on just one species: *Homo sapiens*.
Human and plant diseases are very complex. Take the flu caused by influenza virus as an example. There are annual outbreaks of epidemics and sometimes pandemics of this virus. Against viruses we can be vaccinated, but new subtypes or new strains will emerge over and over again, year after year, in different countries. In **Figure 1** you see the major flu pandemics in history, including the dramatic Spanish flu of 1918, the year that Wageningen University was founded.

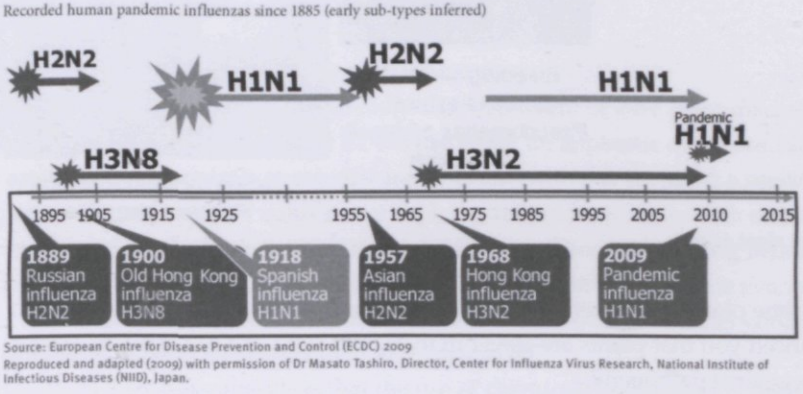


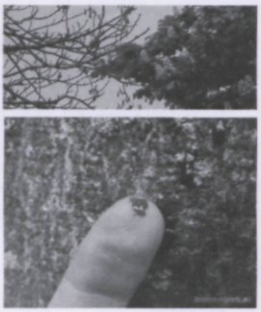
Figure1. Pandemics of influenza virus (flu)

[1] Link to farewell symposium (5-6-2014) Pierre de Wit:
http://wurtv.wur.nl/p2gplayer/Player.aspx?id=dP5AFn

[2] Link to farewell address (5-6-2014) Pierre de Wit:
http://wurtv.wur.nl/p2gplayer/Player.aspx?id=bJBu5T

Similarly, we often get outbreaks of new plant diseases like "sudden oak death" in California caused by *Phytopthora ramorum* (Rizzo et al., 2002), or "bleeding canker of horse chestnut" in The Netherlands caused by *Pseudomonas syringae* pv. *aesculi* (Webber et al., 2008), or leaf rust caused by the Ug99 strain of *Puccinia graminis*, which threatens wheat crops in East Africa and the Middle East (Singh et al., 2011) (**Figure 2**). Plant diseases are a continuous threat to global food production and the work of a phytopathologist never ends, much like my research on *C. fulvum*. This afternoon I would like to share with you my fascination and passion for biology and phytopathology in particular. Please join me on a short journey through the history of phytopathology. It will be followed by a short overview of my own research on the plant immune system at the Laboratory of Phytopathology of Wageningen University. I will make a comparison with our own immune system, and also show some applications of our research.

"Sudden oak death"
Phytophthora ramorum



"Bleeding canker of horse chestnut"
Pseudomonas syringae pv. *aesculi*

"Uganda 99 stem rust strain"
Puccinia graminis

Figure 2. New plant diseases

For a long time plants were seen as passive organisms, but after my talk I hope to have convinced you that plants are clever in their own right and can actively defend themselves against pathogens.

My interest in plants and their pathogens started in my childhood. I grew up on a farm in Limburg (the most southern province of The Netherlands) and as a child I liked to join my father in the fields when he was looking after his crops. I also saw his concern when potato suffered from late blight. This disease is caused by *Phytophthora infestans*, a pathogen that has infected potato in Europe since 1845, when it caused a dramatic famine in Ireland. To protect potato against late blight he sprayed the crop

with chemicals known as fungicides. Another disease you all know is apple scab caused by *Venturia inaequalis*. Also this disease is usually cured with fungicides. The third disease is tomato leaf mould caused by *C. fulvum*, a fungal pathogen that I have studied my whole career (**Figure 3**). Tomato can be perfectly protected against this disease by growing cultivars with resistance genes. No fungicides are required to protect tomato against this disease.

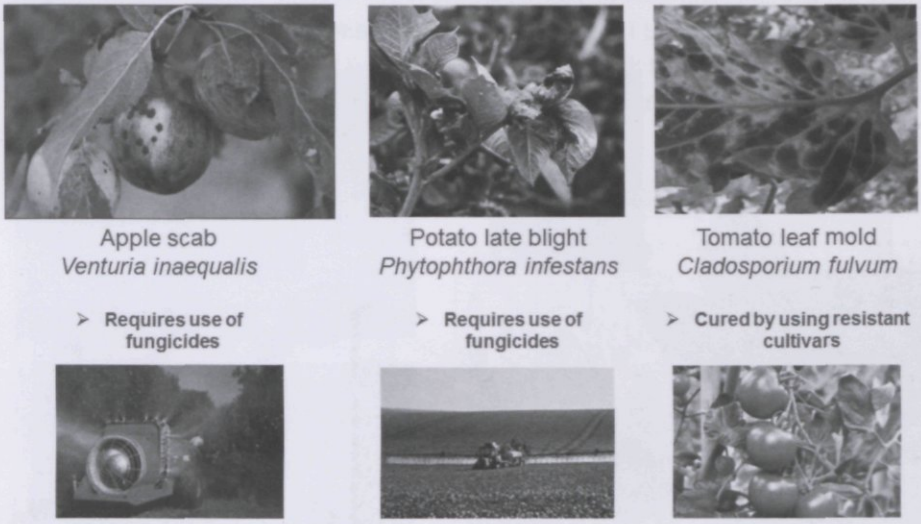


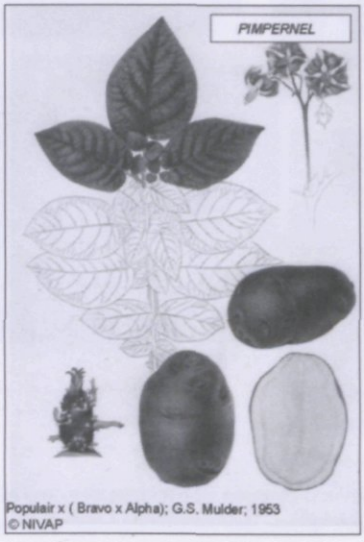
Figure 3. Plant diseases cured by chemicals or disease resistance genes

Control of human and plant diseases

The two measures that farmers usually take to prevent or cure plant diseases are also taken by ourselves when we get ill. We use either an antibiotic or we are vaccinated. Farmers spray chemicals or use disease-resistant cultivars. By using a vaccine or using a disease-resistant cultivar, we exploit our own immune system or that of the plant, respectively. I grew up in "the sixties" when there was a strong belief in the power of chemicals to prevent or cure pests and diseases. There were even daily news bulletins on the radio informing farmers about optimal weather conditions to spray fungicides against late blight and apple scab.

However, it soon became clear that the use of chemicals had undesired effects on human health and the environment. The book "Silent Spring" written by Rachel Carson in 1962 documented the negative effects on human health and the environment (Carson, 1962). It influenced president John F. Kennedy, as he called for testing of the chemicals mentioned in her book. The use of pesticides was not a stimulus to develop and use resistant cultivars, although they were already available for many crops, including potato.

At home in our vegetable garden, my father grew a potato variety that was very resistant against late blight. It was a late season red potato cultivar called "Pimpernel" (**Figure 4**). However, the susceptible cultivar "Bintje" was grown as a crop that needed a lot of chemicals for protection against late blight. Apparently, the potato industry, growers and consumers preferred Bintje, which is still grown to-day. Since my last years at high school I knew I would like to go to Wageningen to learn more about plants and their diseases. I would rather become a plant doctor than a medical doctor. As a child I was afraid of seeing blood when I was vaccinated against infectious diseases, but at the same time I was curious about the mechanism behind vaccination and the human immune system. I wondered whether plants would have an immune system too.



➢ resistant to potato late blight; **does NOT require chemicals**

➢ susceptible to potato late blight; **does require chemicals**

Figure 4. Potato cultivars Pimpernel and Bintje

A short history of phytopathology

Pests and diseases of crops have always been around since man started to domesticate plants for farming around 10.000 years ago. There are reports on pests and diseases in Babylonian, Greek and Roman literature, and in the bible. Pests and diseases were believed to be incited by angry Gods. In Roman times an annual festival called "The Rubigalia" was held on the 25th of April . Red dogs and sheep were offered to please the god Rubigus who had the power to destroy cereals by

inciting disease (Beard et al., 1998). Also from the Middle Ages there are reports of plant diseases causing many victims among the human population. One representative example is Saint Anthony's fire, a disease that caused gangrene after eating flour made from cereals infected by the ergot fungus *Claviceps purpurea*. Prayers were devoted to St. Anthony to become cured from this disease. More information about diseases and practices of crop protection in medieval agriculture can be found in a book written by professor Jan Carel Zadoks, which was published recently (Zadoks, 2013) (**Figure 5**).

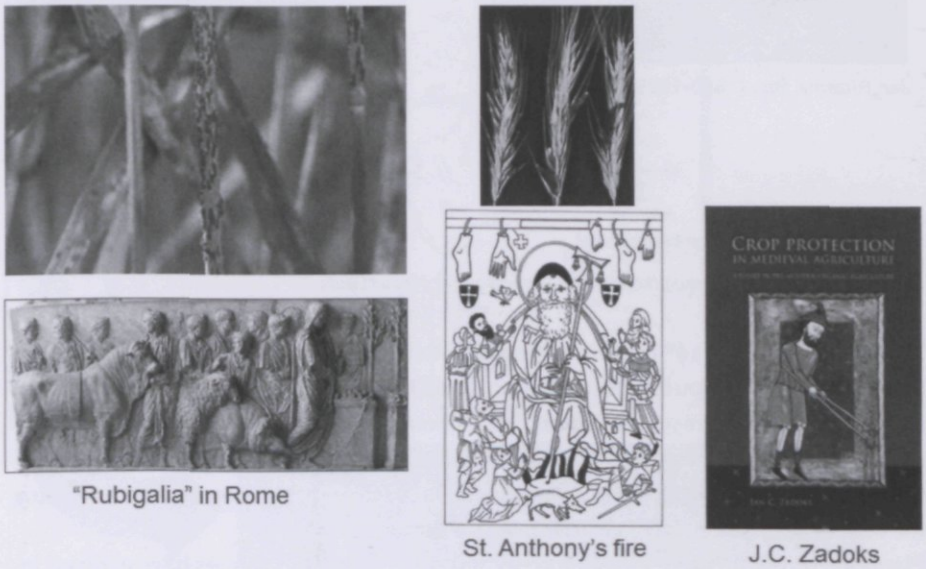


Figure 5. "Curation" of plant diseases in ancient and medieval times

It is hard to believe that it is only in the second half of the nineteenth century that microbes were discovered as causal agents of human and plant diseases. The German surgeon, botanist, microbiologist and mycologist Anton de Bary is seen as the founding father of phytopathology and mycology in Europe. He discovered the causal agents of several plant diseases (De Bary, 1861). The first professor who started to teach phytopathology at Wageningen University was professor Ritzema Bos (Ritzema Bos, 1895). Did you know that the first female Dutch professor was a phytopathologist? Her name was Johanna Westerdijk. She was professor of Phytopathology at Utrecht University and the University of Amsterdam (Faasse, 2012) (**Figure 6**).

Jan Ritzema Bos (1850-1928)

Anton de Bary (1831-1888)

Johanna Westerdijk (1883-1961)

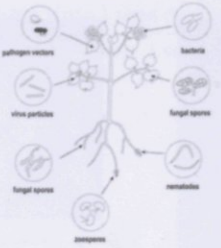
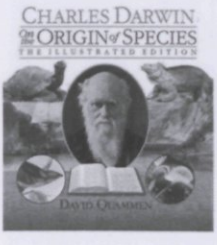


Figure 6. Founders of Phytopathology in Europe and the Netherlands

Two scientists in the 19th century have revolutionized biology. The first is Charles Darwin with his famous publication on: "The Origin of Species", creating the first tree of life (Darwin, 1859) also based on fieldwork of Alfred Russel Wallace presented at the Linnean Society of London in 1858. The second one is the geneticist Gregor Mendel, who discovered the principles of heredity by crossing and analyzing the offspring of pea plants (Mendel, 1865) (**Figure 7**).

Plants appeared on earth long before us and different plants species have evolved in different geographical regions also known as the centers of biodiversity. For example, the potatoes and tomatoes that we eat today evolved from wild relatives in South America (**Figure 8**). There is a high degree of variation among these wild plant species. Plants and their pathogens have co-evolved for millions of years. The wild relatives of our present crops have developed resistance genes against pathogens. When the laws of Mendel were rediscovered around 1900, plant breeders started to introduce resistance genes in crop plants by crossing them with wild relatives. In 1905, Biffen in the UK was the first to discover a disease resistance gene effective against wheat rust (Biffen, 1905) (**Figure 9**). He transformed plant breeding from a 'game of chance' to an exact science. He showed that disease resistance was inherited as a dominant Mendelian factor and created the first rust-resistant wheat variety called "Little Joss". Indirectly, Mendel and his followers are responsible for the fact that The Netherlands have become a major exporter of plant seeds. Did you know that one kilo of tomato seeds costs twice as much as one kilo of gold or more? In 2007 one kilo of tomato seeds was even sold for $350.000 (Cohen, 2007). This is because

Charles Darwin (1809-1882)



Gregor Mendel (1822-1884)

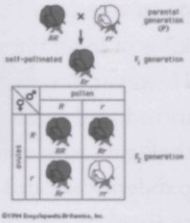


Figure 7. Pioneering evolutionist and geneticist who revolutionized biology

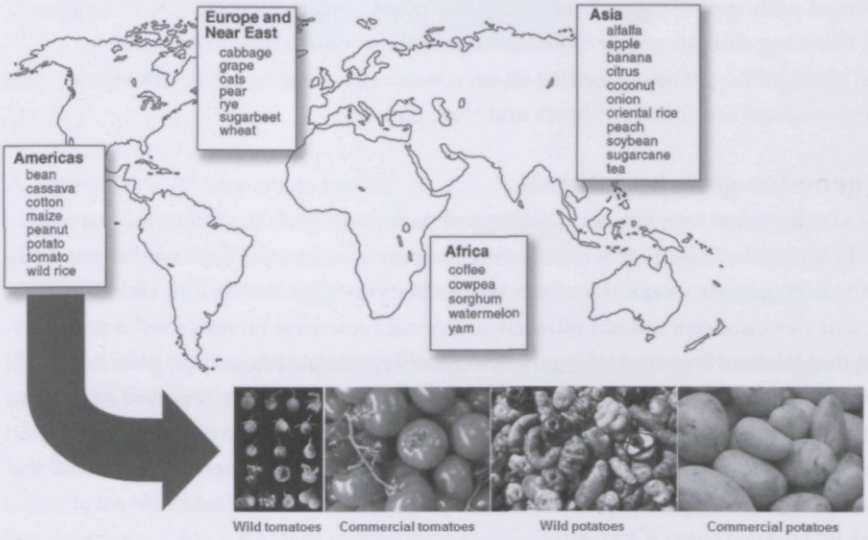


Figure 8. Centres of biodiversity of major crop plants (centres of origin)

- Biffen transformed plant breeding from a '**game of chance**' to an exact science
- Proved that wheat rust resistance is inherited as a dominant Mendelian factor
- Created the first rust-resistant wheat variety "Little Joss"
- United plant genetics with plant pathology

Figure 9. Rowland Harry Biffen created the first rust-resistant wheat variety (1905)

breeders have introduced many useful genes including genes for high yield and disease resistance. One tomato seed generates one tomato plant that can produce 30 kilos of fresh tomatoes.

However, resistance genes do not last forever. Pathogens can overcome an introduced resistance gene. Compare it with the new HN strains of influenza virus that can cause epidemics and pandemics. The H1N1 strain caused the Spanish flu pandemic in 1918 (Taubenberger, 2006) (**Figure 1**). A new variant of that same strain showed up again during the pandemic of 2009. Can we compare disease outbreaks of influenza virus with outbreaks of fungal diseases? The answer is yes. A new strain of influenza virus escapes detection by the human immune system, while a new strain of a fungal pathogen escapes detection by the plant immune system (De Wit et al., 2009). Growing disease-resistant plants imposes a similar selection pressure on a fungal plant pathogen as antibodies do on a strain of influenza virus. There is a continuous arms race between hosts and their pathogens.

The gene-for-gene hypothesis

Studies on the arms race between plants and their pathogens started in the 1940s by Harold Flor in the USA. Flor worked with flax cultivars and the flax rust fungus and described the genetic interactions between them. Some flax rust strains did infect particular flax cultivars but not others. For these interactions he proposed a genetic model that became known as the gene-for-gene hypothesis (Flor, 1942; Flor, 1971). He showed that flax cultivars with a dominant resistance gene, *R*, are resistant against a flax rust strain with the corresponding dominant avirulence gene or *Avr* gene.

Around the same time, Jan Arend Oort in Wageningen, The Netherlands, showed the basis of the gene-for-gene hypothesis in the pathosystem wheat and loose smut caused by *Ustilago tritici* (Oort, 1944). It is a coincidence that last week I was informed by my Canadian colleague Guus Bakkeren that the first *Avr* gene, *UhAvr1*, from *Ustilago hordei* was cloned (Ali et al., 2014). In biochemical terms, the gene-for-gene hypothesis proposes that the product of a dominant *R* gene and the product of a dominant *Avr* gene interact and will cause the activation of immune responses including the hypersensitive response (HR), leading to resistance (De Wit, 1997). The products of a recessive *r* gene and a recessive *avr* gene will not interact and will not induce defence responses, leading to disease (**Figure 10**). When I started my PhD research in 1974, my promotor, the late professor Dekker (Zadoks, 1989), gave me the freedom to choose my own research project. I was torn between *Venturia inaequalis*, the apple scab fungus (Boone, 1971), and *C. fulvum*, the causal agent of tomato leaf mold . Both interactions were supposed to represent a gene-for-gene relationship. Research on C. *fulvum* has a long history in Canada where initially Langford (Langford, 1937), followed by Bailey (Bailey, 1948), and later Verna Higgins (Higgins et al., 1998), studied genetic, structural and physiological aspects of the pathogen.

Fungus: Avirulence gene \ Plant: Resistance gene	*R*	*r*
Avr	Resistance	Disease
avr	Disease	Disease

Biochemical model:

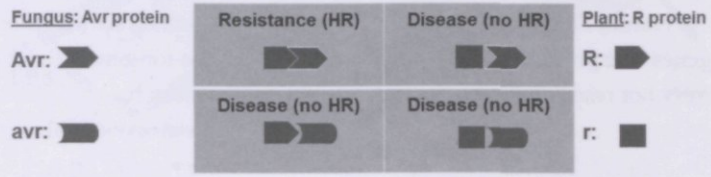


Figure 10. Genetic and biochemical model for Flor's gene-for-gene hypothesis

A summary of my own research

I chose to research the *C. fulvum*-tomato system, as a collection of near-isogenic lines of tomato with major *Cf* resistance genes was available at the former Institute of Horticultural Plant Breeding generated by Ietje Boukema (Boukema, 1977). A collection of *C. fulvum* strains was also available at the former Institute of Phytopathological Research (IPO), initially set up by Hubbeling (Hubbeling, 1971), and continued by Thijs Gerlagh (Lindhout et al., 1989). With those two collections at hand, I hoped to learn more about the immune system of tomato against *C. fulvum*. The near-isogenic tomato lines gave very strong defence responses. The plants are either fully resistant (R) or fully susceptible (S) to strains of the pathogen (**Figure 11**).

Cf genes in near-isogenic tomato lines	Strains of *Cladosporium fulvum*				
	0	2	4	5	2.4
None	S	S	S	S	S
Cf-2	R	S	R	R	S
Cf-4	R	R	S	R	S
Cf-5	S	R	R	S	R
Cf-2/Cf-4	S	R	R	R	S
Cf-9	R	R	R	R	R

S: susceptible

R: resistant

Figure 11. The collections of tomato lines and C. fulvum *strains available at the start of my PhD*

Immediately after entering a stoma, tomato recognizes *C. fulvum* and an HR is induced. The resistant plant sacrifices a few cells and arrests the growth of the biotrophic pathogen (**Figure 12**).

In **Figure 13,** a *C. fulvum* strain is depicted infecting a susceptible tomato plant colonizing the intercellular space surrounding mesophyll cells. The plant does not show an HR and the fungus grows happily. Apparently, in a susceptible plant, the immune system does not recognize the pathogen. Why, is the big question to be answered. Since the pioneering work of Harold Flor, many phytopathologists have searched for the products of the *Avr* genes that provide a biochemical basis for the gene-for-gene hypothesis. It was a very hot research topic when I started my PhD research.

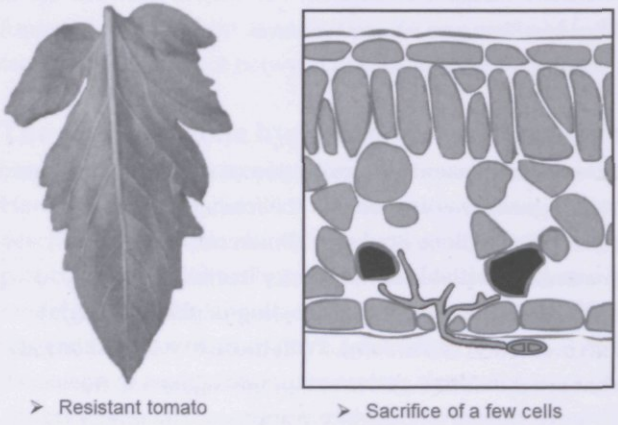


Figure 12. A resistant plant shows a hypersensitive response (HR) to an avirulent fungal strain

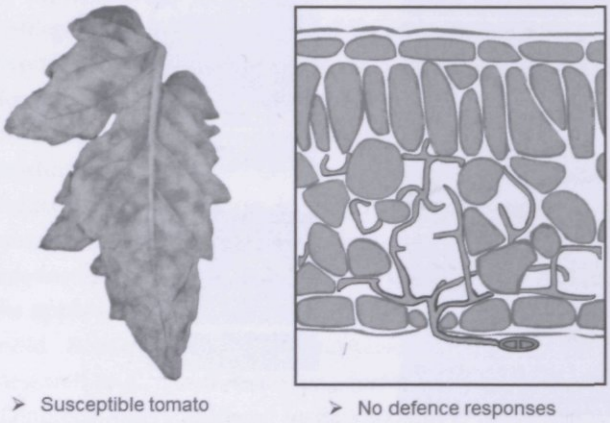


Figure 13. A susceptible plant does not show defence responses to a virulent strain of C. fulvum

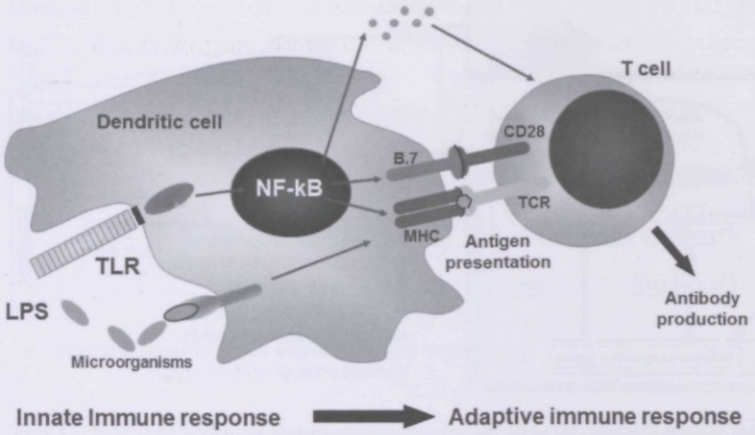


Figure 14. Activation of the innate and adaptive immune system in mammals

How does the immune system work in mammals? In mammals an immune response is usually triggered when the host recognizes conserved molecules from pathogens, the so-called pathogen-associated molecular patterns (PAMPs) (Alexander et al., 2014). These molecules are present in a broad spectrum of micro-organisms, like lipopolysaccharide (LPS) in bacteria. LPS is recognized by a Toll-like receptor (TLR) present in dendritic cells that present antigens to T cells to activate the adaptive immune system, leading to the production of antibodies (**Figure 14**). Plants have only an innate immune system. What are the differences between the innate and the adaptive immune system?

The innate immune system is non-specific, gives an immediate maximal response and does not provide an immunological memory. In contrast, the adaptive immune system is only present in vertebrates. It is antigen-specific and slow, but gives an immunological memory, where antigens of each pathogen are remembered by memory cells (**Table 1**). When the same pathogen infects the host again, these memory cells are activated to quickly attack and eliminate it.

Table 1. Properties of the innate and adaptive immune system

Innate immune system	Adaptive immune system
Response is non-specific	Response is antigen-specific
Immediate maximal response	Slow response
No immunological memory	Immunological memory
Present in plants	**Absent in plants**

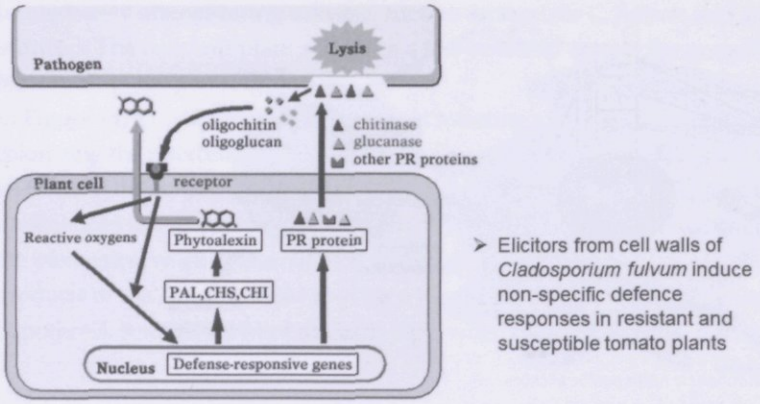


Figure 15. Cell wall-derived elicitors (PAMPs) from fungi induce defence responses

Until the 1980s, virtually nothing was known about the molecular mechanism of the immune system in plants. We could show that defence responses were induced in resistant plants after inoculation with an avirulent strain of a pathogen. Elicitors isolated from those strains could induce similar defence responses. Elicitors were defined as fungal cell wall-derived molecules like oligochitin, oligoglucans and glycoproteins, released by plant chitinases and glucanases . They induce the accumulation of antimicrobial compounds like phytoalexins (antimicrobial plant metabolites induced after infection) and pathogenesis-related (PR) proteins (**Figure 15**), but the encoding genes were unknown (Keen, 1975). The identification of fungal elicitors and their immune receptors in the plant took a long time. Everybody was trying to identify specific elicitors that could specifically induce phytoalexins and an HR in a resistant but not in a susceptible plant. These race-specific elicitors would be the presumed products of the *Avr* genes proposed in Flor's gene-for-gene hypothesis (Flor, 1971). In the 1970s, many elicitors were identified that could induce an HR, but they all appeared to be non-specific. The first years of my PhD study were frustrating as from *C. fulvum* we could only isolate cell wall-derived glycoprotein elicitors, that were non-specific. They elicited the same response in susceptible and resistant cultivars, irrespective of whether they were isolated from a virulent or an avirulent strain of *C. fulvum* (De Wit, 1977; De Wit and Roseboom, 1980; De Wit, 1981; De Wit and Kodde, 1981a, b)

In the general discussion of my PhD thesis defended in 1981, I presented a model that would fit all the observations that we made for *C. fulvum* elicitors and of other fungi until that time (**Figure 16**). The model was published, together with my former colleague Leen Davidse (De Wit and Davidse, 1980), is not much different from present models, but the terminology used is different. Now we call elicitors PAMPs. We proposed that *C. fulvum* needed to suppress the defence responses activated by

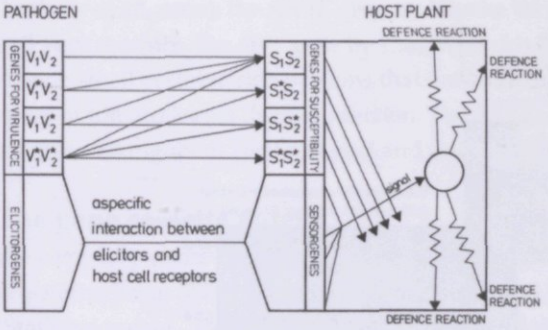


Figure 16. Non-specific elicitor and specific suppressor model for C. fulvum*-tomato interaction (1981)*

elicitors in order to infect tomato. To prove the existence of the proposed specific suppressor molecules that would be the products of virulence genes, we started to search for those molecules in infected susceptible plants as these plants did not show defence responses because they were supposed to be suppressed by these specific suppressors. The model looks like an old version of what is now known as the effector-triggered-susceptibility (ETS) model (Jones and Dangl, 2006). I will come back to ETS later.

The break-through in identifying *Avr* gene products that could specifically induce an HR came from work that I performed together with MSc student Ger Spikman in 1980. When we analyzed apoplastic fluid isolated from *C. fulvum*-infected susceptible tomato plants to find race-specific suppressors, we found evidence for race-specific elicitors instead (De Wit and Spikman, 1982) (**Figure 17**). We could mimic the HR induced by a strain of *C. fulvum* on a resistant plant by injecting apoplastic fluid isolated from susceptible plants that were infected by that same strain. Thus, we could reproduce the gene-for-gene hypothesis using Avr proteins secreted by particular strains of *C. fulvum* during infection of plants. In **Figure 17** (right bottom panel), the necrotic sections in the leaves occurring after injection with intercellular fluids represent the HR induced by Avr proteins present in those fluids. From this experiment we could draw two conclusions: (i) specific compounds are only produced by *C. fulvum* during infection of tomato but not *in vitro* during growth on synthetic media and, (ii) the compounds are race-specific elicitors instead of race-specific suppressors.

However, now we know that race-specific elicitor molecules have dual functions depending on whether a matching *Cf* resistance gene is present or absent in the tomato plant. Thus, both terms are correct. A race-specific elicitor can act as a race-specific suppressor depending on presence or absence of a matching *Cf* resistance gene.

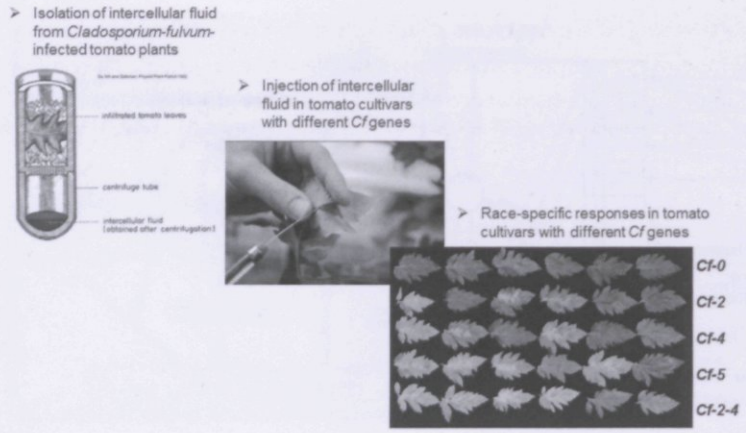


Figure 17. Intercellular fluids of C. fulvum*-infected plants contain race-specific elicitors*

Cloning of *Avr* genes

Although we had evidence that the race-specific elicitors must be the products of *Avr* genes already in the early 1980s, it took us many years to isolate and purify them from apoplastic fluids and to clone the encoding genes. For the cloning we used a reverse genetics approach. This means that one tries to find the encoding genes based on amino acid sequence information obtained from the purified Avr proteins. This can be problematic as there are 64 triplet codons available for 23 amino acids, which means there is a lot of redundancy, but I will not go into further detail. The first fungal *Avr* gene ever cloned was *Avr9*. It was cloned by Guido van den Ackerveken and Jan van Kan in 1991 (Van Kan et al., 1991; Van den Ackerveken et al., 1992). Four additional *Avr* genes have been cloned in our lab since the 1990s. The *Avr4* gene was cloned by Matthieu Joosten (Joosten et al., 1994), the *Avr2* gene by Rianne Luderer

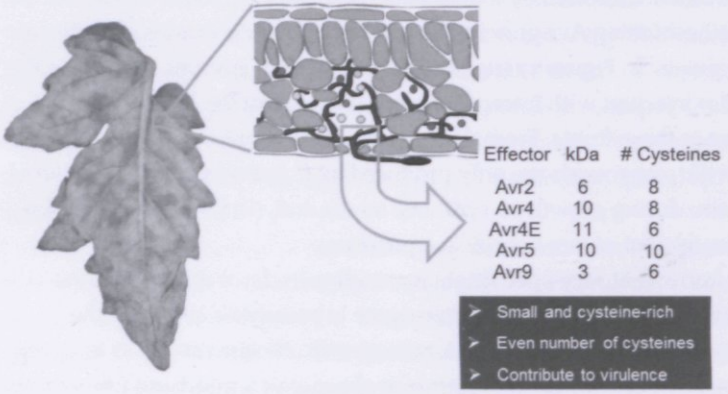
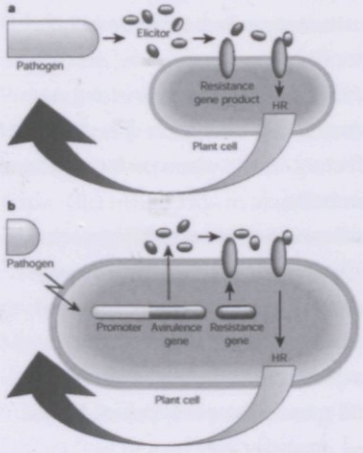


Figure 18. Avr effectors secreted in the apoplast by C. fulvum that are recognized by Cf proteins

(Luderer et al., 2002), the *Avr4E* gene by Nienke Westerink (Westerink et al., 2004) and very recently, the *Avr5* gene by Carl Mesarich (Mesarich et al., 2014). They all encode small cysteine-rich proteins that are secreted by the fungus in the apoplastic space of tomato leaves during infection. The Avr proteins contain many disulfide bridges making them very compact and stable molecules (**Figure 18**).

The gene cassette patent

We were very excited to have cloned the first fungal *Avr* gene in 1991, quite some years after Brian Staskawicz and Noel Keen cloned the first bacterial *Avr* gene (Staskawicz et al., 1984). We filed a patent on exploiting this gene in molecular disease resistance breeding (De Wit, 1990; Honée et al., 1995; Honée et al., 1998; Stuiver and Custers, 2001). It became known as the gene cassette (**Figure 19**). We proposed to transform Cf-9 tomato plants with the *Avr9* gene under the control of a fungus-inducible promoter, but in principle it could be done with any *Avr* gene and its matching resistance gene. When a pathogen would enter such an *Avr9*-transgenic plant the HR would be induced giving broad spectrum resistance against biotrophic pathogens. It was filed together with the former Biotech company Mogen International NV in Leiden (Mogen). We found proof of concept. By selling the patent to Mogen, a few postdocs could be appointed at the Laboratory of Phytopathology, showing that our work was of economic relevance and appreciated by Biotech companies. However, the patent has not been developed further after the merger of Mogen with Zeneca and later with Syngenta, as the company was concerned that the Avr9 protein could cause allergy, and that genetically modified (GM) plants would not be accepted by society.



a. Avr produced by the pathogen itself induces hypersensitive responses (HR)

b. Pathogen-induced secretion of Avr by transgenic plants results in HR and provides broad spectrum resistance

Figure 19. The gene cassette patent, providing broad resistance to pathogens affected by an HR

Primary functions of *Avr* genes

It took a long time to discover the primary functions of the *Avr* genes of *C. fulvum*. As the primary function of their products is to facilitate infection (virulence factors) they are nowadays called effectors instead of elicitors. Of all effector genes identified so far from *C. fulvum*, we now know the primary function of three only. The Avr2 effector inhibits the tomato cysteine protease Rcr3 to prevent it from hydrolyzing fungal proteins required for infection and colonization (Van Esse et al., 2008). As an evolutionary response, tomato has developed the immune receptor Cf-2 that recognizes the Rcr3-Avr2 complex, and subsequently induces an HR and resistance to the fungus (Rooney et al., 2005).

The Avr4 effector is a chitin-binding protein that is secreted by the fungus and binds to chitin present in fungal cell walls. In this way, Avr4 protects fungal cell walls against hydrolysis by plant chitinases (Van den Burg et al., 2003; Van den Burg et al., 2004; Van den Burg et al., 2006; Van Esse et al., 2008). Avr4 does occur in additional fungal plant pathogens (Stergiopoulos et al., 2010). As an evolutionary response, tomato has developed the Cf-4 immune receptor that recognizes the fungal Avr4 protein and induces an HR and resistance (Thomas et al., 1997).

Finding the avirulence function of an effector was easier than finding its virulence function. In the first case, one searches for secreted proteins that induce a Cf-specific HR. An HR induced by an effector is easy to score, but less so its virulence function. Assume that 100 effector molecules are produced by a pathogenic fungus and they all contribute equally to virulence, then the contribution to virulence of one effector is only 1%. A difference in virulence of 1% is difficult to score. However, new technologies like quantitative PCR are more sensitive and by using those we could find a contribution to virulence of many more effector proteins. Looking for structural and functional homologues of effectors in other organisms can be helpful to find their primary function. However, effectors are often species-specific, so there are not many homologues out there, except for a few that we call core effectors, as they are used by different pathogens, or major effectors, when they have a huge effect on virulence. Of course we did not discover effectors in the same order as they appeared in pathogens during co-evolution with their hosts.

In influenza virus mutations in hemagglutinin (H) and neuraminidase (N) proteins that reside at the outside of the virus particle can cause genetic drift leading to new virus subtypes causing seasonal outbreaks, while recombination between different strains of the virus (by genetic shift) leads to new strains causing pandemics. Recombination between viral strains usually occurs when two different strain recombine in a shared host like man, birds or pigs and generate new types of H and N proteins (Noah and Noah, 2013) (**Figure 20**).

Also, new strains of *C. fulvum* can develop mutations in effectors that can overcome recognition by Cf immune receptors. Mutation is followed by selection and

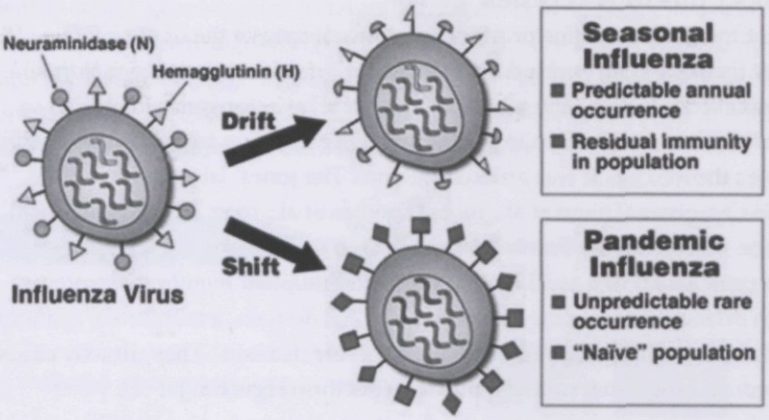


Figure 20. Mutation (drift) in H and N generates subtypes and recombination (shift) generates new types of influenza

multiplication of the selected strain, usually not by recombination as C. *fulvum* is not known to reproduce sexually. However, in other fungi or oomycetes like *Phytophthora* species studied by my colleague Francine Govers in the Laboratory of Phytopathology, both mutations and recombination do occur, which causes quicker development of new strains (Li et al., 2012). Mutations occur continuously and help organisms to adapt to new environments. Mutations and recombination are the driving forces behind evolution. An individual with a lethal mutation will disappear from the population, but when a mutation gives a (small) advantage it will start to dominate the population. We discovered five different types of mutations in C. *fulvum* that enabled new strains to escape recognition by different Cf immune receptors (Van den Ackerveken et al., 1992; Joosten et al., 1994; Luderer et al., 2002; Westerink et al., 2004; Mesarich et al., 2014): (i) mutation in an effector gene causing a stop codon leading to a truncated effector protein, (ii) mutations in an effector gene leading to the production of an effector protein with one or a few different amino acids, (iii) mutations in an effector gene leading to the production of an unstable effector protein, (iv) loss of an effector gene from the genome leading to loss of effector protein production or (v) insertion of a transposon in an effector gene preventing production of a functional effector protein.

Mutation in an effector gene that produces a modified effector protein that is no longer recognized by the corresponding immune receptor will keep its virulence function and C. *fulvum* remains fit. However, loss of an effector gene will make C. *fulvum* less fit and less virulent. Loss of virulence is a problem for C. *fulvum*, but sometimes there is redundancy in effectors and the function can be taken over by a homologue, or, the fungus simply develops a new effector.

Cloning of *Cf* immune receptor genes

The cloning of the *C. fulvum* effector genes sped the cloning of the *Cf* genes. The availability of the *Avr9* gene enabled the research group of Jonathan Jones at the Sainsbury Laboratory to clone the matching *Cf-9* gene by a transposon tagging approach (Jones et al., 1994). I was in his lab when the first *Cf-9* tagged mutants made by David Jones showed up. It was an exciting time. The Jones' lab also cloned the *Cf-2*, *Cf-4* and *Cf-5* genes (Dixon et al., 1996) (Thomas et al., 1997; Dixon et al., 1998). The *Cf-4E* gene was cloned by Frank Takken (Takken et al., 1999). All *Cf* genes encode receptor-like proteins known as RLPs. They are integral plant membrane proteins containing an extracellular leucine-rich repeat or LRR domain, a membrane spanning domain, and a short cytoplasmic tail without signaling domain . They directly or indirectly mediate recognition of the matching effectors (**Figure 21**).

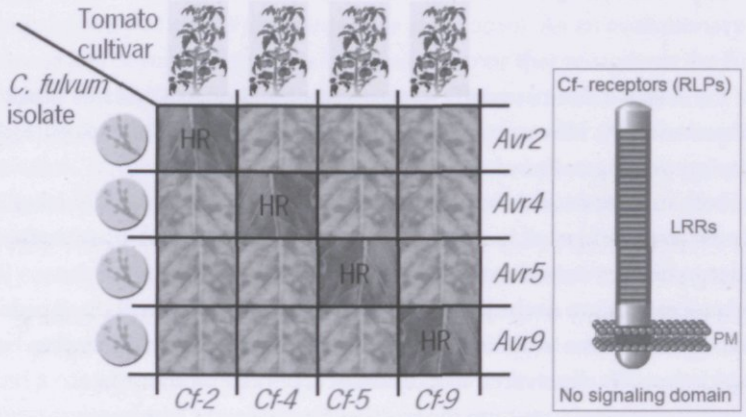


Figure 21. Cf immune receptors are LRR-membrane proteins without a signaling domain

In the late 1990s, all *Cf* genes from the collection of Ietje Boukema were cloned, except the minor *Cf* genes, *Cf-1* and *Cf-3* (Stevens, 1988). We wondered whether we could isolate novel *Cf* genes from wild tomato species by assuming that *C. fulvum* produces many more effectors. The genome of *C. fulvum* was not available at that time, so once again we had to go the hard way from protein to gene. PhD student Richard Laugé set out to isolate and purify additional extracellular effector protein candidates (Ecps). Similar to the approach we used in 1980, he injected them into a collection of wild tomato species, or expressed their encoding genes using the PVX expression system (Laugé et al., 1998). He identified many wild tomato species that responded with an HR. In this way, five additional effector proteins and matching resistance genes that induced a specific HR were identified (Laugé et al., 2000) (**Figure 22**). His idea was soon followed up by many researchers in the research community to

identify new *R* genes against different pathogens in wild crop plant species mediating effector recognition (Torto et al., 2003). Nowadays, with many genome sequences available, high throughput screens can be set up for bacteria, fungi and oomycetes, to test the HR- inducing activity of hundreds of potential effector genes and identify a similar number of immune receptors.

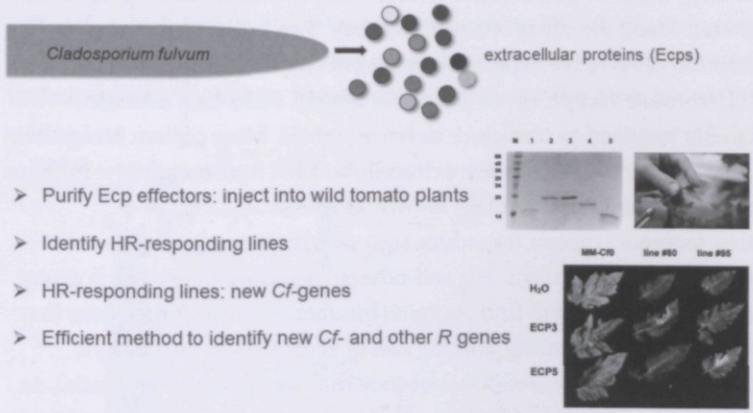


Figure 22. Identification of new Cf *genes from wild tomato species by Ecp effectors causing HR*

Interaction of effectors with Cf immune receptors and downstream defence signaling

We have also tried hard to show whether *C. fulvum* effectors and the corresponding Cf immune receptors interact. The biochemical model of the gene-for-gene system suggests direct interaction. In the *C. fulvum* group, PhD students Miriam Kooman-Gersmann, Renier van der Hoorn, Rianne Luderer, Nienke Westerink, John van 't Klooster, and recently, Mansoor Karimi-Jashni have worked on these and related projects (**Figure 23**). They have tried to prove direct interaction *in vitro* using both

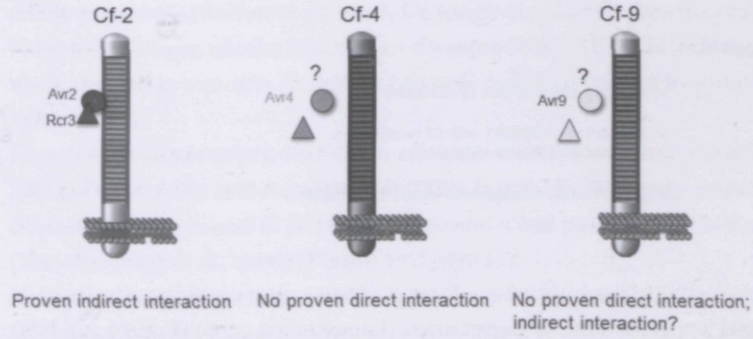


Figure 23. Effectors interact indirectly via virulence targets with Cf immune receptors?

radiolabeled and biotinylated Avr4 and Avr9 effectors and Cf proteins produced in different expression vectors including COS cells, insect cells or yeast cells, but with variable success (Kooman-Gersmann et al., 1996; Luderer et al., 2001); (Van 't Klooster et al., 2011). This is mainly due to the fact that RLPs with so many extracellular, heavily glycosylated LRRs are difficult to produce stably and in sufficient amounts outside the plant. Only indirect interaction between Avr2 and Cf-2 via Rcr3 has been demonstrated, as mentioned earlier (Rooney et al., 2005; Van 't Klooster et al., 2011). Perhaps with exception of Avr4 we expect that most effectors will be indirectly recognized by RLP immune receptors via their host targets. RLPs lack a kinase domain that is usually required to transduce defence signals. Most pattern recognition receptors (PRRs) that recognize PAMPs are extracellular LRR transmembrane proteins with a cytoplasmic kinase signaling domain known as RLKs.

Thus, RLP immune receptors cannot transduce signals without engaging co-receptors like RLKs (Kruijt et al., 2005). We and others, including the research group of Jonathan Jones, have tried hard to find proteins interacting with Cf receptors that are required for downstream signaling making use of yeast two-hybrid assays. Indeed interacting proteins were identified, but they did not appear to be crucial, as silencing of the encoding genes did often only weakly compromise HR and resistance (Rivas et al., 2004; Nekrasov et al., 2006; Van den Burg et al., 2008).

However, recently PhD student Thomas Liebrand in the research group of Matthieu Joosten made great progress in identifying a crucial RLP interactor using a proteomics fishing approach. He identified the RLK called SOBIR1 that interacts with all Cf proteins enabling them to transduce defence signals, eventually leading to HR and resistance (Liebrand et al., 2013; Liebrand et al., 2014) (**Figure 24**). This RLK is crucial for activity and downstream signaling of Cf immune receptors and related RLPs, as silencing does compromise HR and resistance.

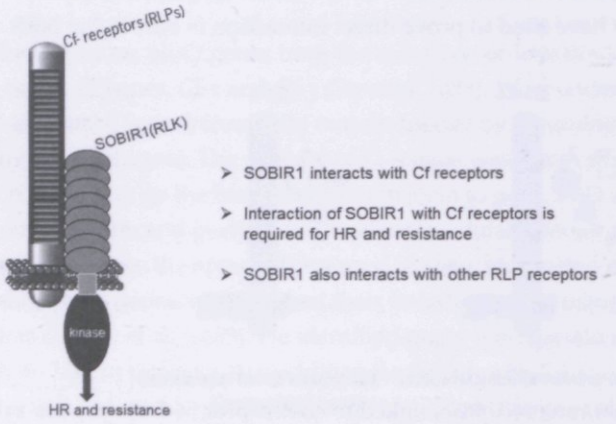


Figure 24. Cf immune receptors interact with SOBIR1 to transduce defence signals leading to HR

An evolutionary scenario of the arms race between host and pathogen

In **Figure 25A, B, C, D,** I give a simple evolutionary scenario that could have occurred during co-evolution between *C. fulvum* and wild tomato species. Of course, evolution does work gradually and not in discrete steps, but for simplicity I assume it does. In **Figure 25A** you see *C. fulvum* dwelling in the apoplastic space. It is not yet a pathogen, as tomato recognizes its PAMP, the chitin fragments released from its cell wall, which induce PTI after being recognized by plant chitin receptors. The PTI response is not strong, but strong enough to keep *C. fulvum* in check. That is unfortunate for *C. fulvum*, but it does not give up. You have to lose before you win.

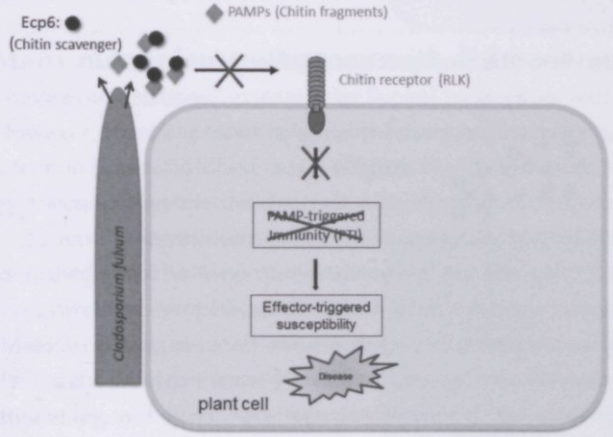


Figure 25A. Chitin fragments from C. fulvum *are recognized by chitin receptor-inducing PAMP-triggered immunity*

During evolution, *C. fulvum* developed a new weapon that helped it to grow a little bit further. *C. fulvum* secretes the Ecp6 effector that binds chitin fragments with a higher affinity than the tomato chitin receptor, preventing the induction of PTI by chitin fragments (Bolton et al., 2008; De Jonge et al., 2010; Sanchez-Vallet et al., 2013). Now a weak form of effector-triggered susceptibility (ETS) is induced, leading to weak disease symptoms. *C. fulvum* can now colonize tomato to some extent (**Figure 25B** *page 24*).

However, it needs to produce more effectors to become a stronger pathogen. Effectors like Avr2 and Avr4 can fulfill this function. This leads to more significant disease symptoms and *C. fulvum* has become a real pathogen (Van Esse et al., 2008) (Van den Burg et al., 2006) (**Figure 25C** *page 24*).

However, in the arms race, tomato fights back by sequentially developing the Cf-Ecp6, Cf-2 and Cf-4 immune receptors linked to co-receptor SOBIR1(**Figure 25D** *page 24*). Development of the Cf-Ecp6 immune receptor is supposed to be the first

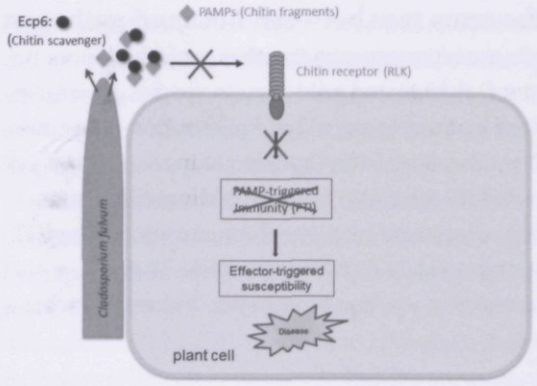


Figure 25B. C. fulvum *secretes Ecp6 effector that scavenges chitin fragments to prevent PAMP-triggered immunity*

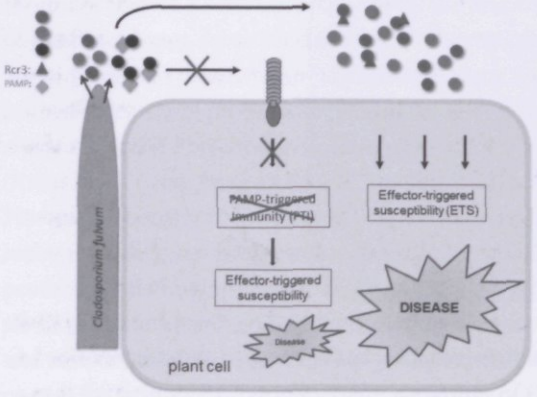


Figure 25C. C. fulvum *secretes many effectors targeting apoplastic host targets to increase virulence*

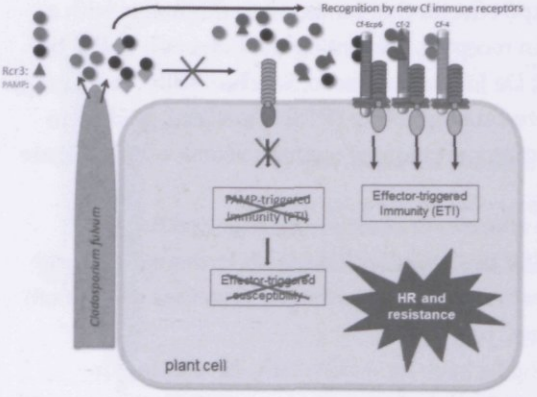
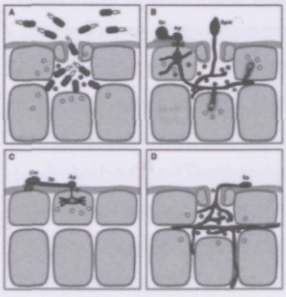


Figure 25D. C. fulvum *develops new Cf immune receptors to recognize new effectors to trigger ETI*

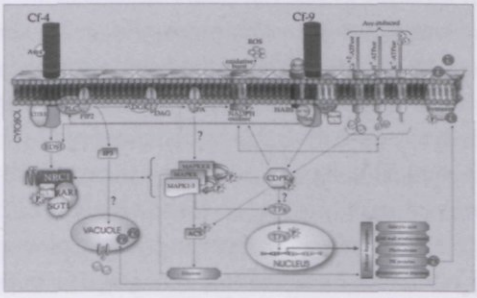
defence weapon developed by tomato, but many more followed, and maybe up to 100 effectors might be employed by *C. fulvum* for the attack, and a similar number of immune receptors might have been developed by tomato.
From the genome of *C. fulvum* we know that it secretes more than 100 potential effectors. Imagine the arms race between *C. fulvum* and tomato making use of 100 effectors as weapons and tomato fighting back with 100 corresponding Cf immune receptors. The fight becomes a real trench warfare! The strongest effectors we might have discovered already, but there are many more to be discovered with smaller effects. You will now understand why my work of a (molecular) phytopathologist is not finished yet.

Many more plant pathogens with different modes of infection

I have now discussed extracellular fungal pathogens, with *C. fulvum* as an example. However, there are other fungi, oomycetes and bacteria that show different modes of infection (Dou and Zhou, 2012) (**Figure 26**). They exploit the cytoplasm of plant cells by injecting effectors that interact with cytoplasmic targets to suppress PTI (Whisson et al., 2007; Stergiopoulos and De Wit, 2009). Several of these host targets have been identified, but I have no time to discuss them. The cytoplasmic effectors are usually recognized by cytoplasmic NBS-LRR immune receptors, also known as NLRs (Maekawa et al., 2011). If one assumes that tomato is infected by 10 different fungal pathogens of which some infect the apoplast, and some the cytoplasm or cell organelles, and every pathogen produces 100 effectors, then by simultaneous infection, tomato is attacked by 1000 effectors. Assume that in response tomato needs to develop 1000 corresponding immune receptors. A lot of work needs to be done to figure out the strategy of attack assuming that not all enemies of tomato act as allied forces.



A. Bacteria
B. Rusts and downy mildews
C. Powdery mildews
D. Extracellular pathogens



➢ Defence signaling pathways mediated by Cf-4 and Cf-9 immune receptors

Figure 26. Different pathogens, different infection modes, different effectors but similar arms race/defence

How can plants survive in the presence of so many different pathogens?

After recognition of effectors by RLPs, ETI is activated and many research groups are identifying and dissecting downstream defence signaling pathways. In **Figure 26** (right panel), a few defence signaling pathways activated and mediated by RLP receptors Cf-4 and Cf-9 are shown (De Wit et al., 2009). I will not go into further detail. It is now the subject of Matthieu Joosten's research group at the Laboratory of Phytopathology. In the past, former PhD students Sandor Snoeijers, Camiel de Jong, Suzan Gabriëls, Iris Stulemeijer and Ahmed Abdel-Haliem, have worked on this research topic (Perez-Garcia et al., 2001; De Jong et al., 2004; Gabriels et al., 2007; Stulemeijer et al., 2007; Vossen et al., 2010).

Consider the huge difference in generation time between a pathogen and a plant. The generation time of a bacterium is around 20 minutes, the generation time of a fungus a few days, that of a plant 3-6 months, and that of man is 10 years. Consider the speed at which pathogens develop mutations in effectors and develop new effectors. One wonders, why we and plants still exist. Some plant immune receptors can work together by making receptor complexes active against more than one pathogen (Lozano-Torres et al., 2012). Also downstream defence responses in plants activated during PTI and ETI partly overlap and are effective against a broad spectrum of pathogens. These responses include the generation of toxic reactive oxygen species, antimicrobial phytoalexins and the antimicrobial enzymes like chitinases, glucanases, proteases, and often the HR (Stotz et al., 2014). Former PhD student Jos Wubben has worked on the *in planta* localization of chitinases and glucanases (Wubben et al., 1992). Work on PR proteins has remained a recurring theme in our research, especially the work on chitinases that play a crucial role in the host-pathogen interaction. These, in particular, have been extensively studied, and the cloning of their genes was initiated by Jan van Kan (Van Kan et al., 1992; Danhash et al., 1993).

There are also examples of one immune receptor being active against two different pathogens. Recently our colleagues at the Laboratory of Nematology showed that the Cf-2 immune receptor also works against the cyst nematode *Globodera rostochiensis* (Lozano-Torres et al., 2012).Both the Avr2 effector of *C. fulvum* and the nematode Gr-VAP1 effector inhibit the cysteine protease Rcr3, which triggers Cf-2-mediated immunity. How can plants develop so many highly specific immune receptors? Most of them occur in clusters allowing them to generate new specificities by inter- and intra-locus recombination between their homologues. In this way, immune receptors with new specificities can be generated (Van der Hoorn et al., 2001; Kruijt et al., 2004). This is somewhat reminiscent of the mechanisms by which specific antibodies are produced in our own adaptive immune system. New antibodies are generated by rearranging germ-line DNA segments to form new antibody genes. Joining different segments of DNA encoding the variable light and heavy chains allows the

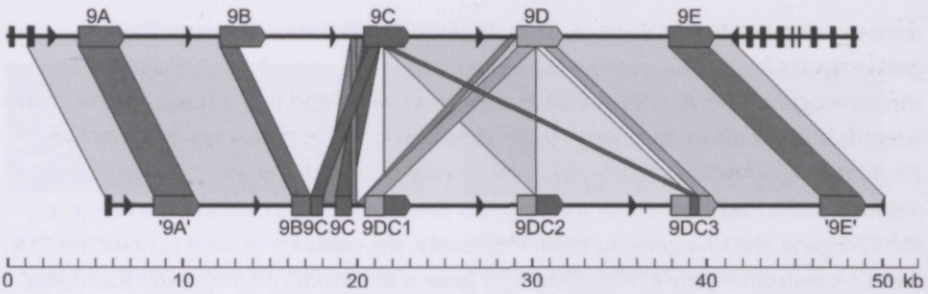


Figure 27. Development of new Cf-9 immune receptors by intra- and inter-locus recombination

production of millions of different antibodies (Angelin-Duclos and Calame, 1998). In **Figure 27** you see an example of recombination between homologues of the *Cf-9* immune receptor genes. You notice the mosaic structure of the homologues in the cluster in two different genotypes of tomato. Former PhD students Marco Kruijt and Renier van der Hoorn studied this together with members of the Jonathan Jones lab (Kruijt et al., 2004; Wulff et al., 2004). Thus, the wild tomato population is very diverse and contains numerous different homologous immune receptor genes, of which few are characterized in their defensive role. It is expected that in the future, many will be shown to be involved in recognizing microbe-derived PAMPs and effectors which are currently unknown.

Valorisation of research

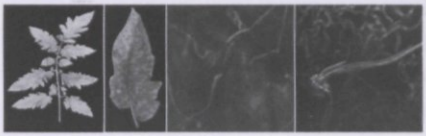
Modern crop plants should be equipped with more than one immune receptor against a particular pathogen. With a mutation rate of one in one million nucleotides per *R* gene per season, the chance of overcoming five *R* genes is estimated to be 1000.000.000.000.000.000.000 times smaller than overcoming one *R* gene. Pyramiding (also known as stacking) five different *R* genes in one cultivar or using multi-lines or mixed lines, each carrying one of the five different *R* genes in time and space in many different crops, is expected to be durable (Wolfe, 1985); (Brunner et al., 2012). However, even when we have developed tools and methods to prevent diseases, this does not always lead to implementation in practice. Application depends on the attitude of breeders, growers and consumers. I can illustrate this with two examples The first example comes again from *C. fulvum*. The five resistance genes *Cf-2*, *Cf-4*, *Cf-4E*, *Cf-5* and *Cf-9* are very effective against this fungus. In the first half of the last century *C. fulvum* was an economically important disease of tomato, when no resistance genes were available yet. In a textbook written by Butler and Jones published in 1949, seven pages were devoted to *C. fulvum* (Butler and Jones, 1949). In the 1980s, only one or two *Cf* genes were present in tomato cultivars grown in greenhouses. This led to frequent outbreaks of new strains. Since the 1980s most

tomato cultivars contain three or more *Cf* genes which prevented new disease outbreaks for more than 25 years. However, after 2010, new *C. fulvum* outbreaks were reported in greenhouses of organic tomato growers in The Netherlands and neighboring countries. Diseased leaves were sent to the Laboratory of Phytopathology and were diagnosed. Only race 0, race 2, race 9 or race 2.9 strains were identified indicating that the diseased tomato cultivars contained no *Cf* gene, the *Cf-2* gene, the *Cf-9* gene, or both. Obviously, the cultivars did not contain the *Cf-4* and *Cf-5* resistance genes. Why? The *Cf-9* gene was introduced in the 1980s and was so powerful that during the last decades some breeders only introduced the *Cf-9* gene in new cultivars and did not care for the others explaining why these cultivars became heavily infected by old races that are still out there in the field. This example shows that breeders and growers need always to be alert, as outbreaks occur when tomato cultivars are not equipped with sufficient number of *Cf* genes.
The second example refers to the introduction of genetically modified disease-resistant plants. Nobody wants to eat food that is contaminated with pesticides, but we are in favor of growing disease-resistant cultivars. However, in some cases this might require using GM plants when traditional resistance breeding is not possible. However, GM plants meet much opposition in our society, despite objective information about their safety. With all the knowledge generated we can now produce new resistant genotypes with multiple *R* genes that do not need chemicals to be protected against pathogens. Hopefully GM disease-resistance plants will eventually become accepted by the public and we can grow them to secure our crops (Brunner et al., 2012; Zhu et al., 2012).

La dernière étappe

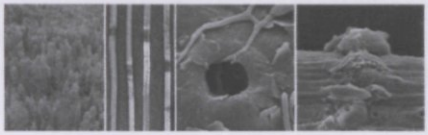
Now I come to the last episode of my career. I was very happy with the appointment as KNAW professor five years ago. It felt like doing my second PhD thesis. I cannot defend it today with the Rector Magnificus Martin Kropff chairing the defence committee and the professors left and right of me on the podium being members of the defence committee firing questions at me. I am sorry, I need another year of research before my second PhD has been completed and can be defended.
I was happy that I could form an excellent international research team around me consisting of motivated young scientists: Dutchmen/women Harrold van den Burg, Ate van der Burgt, Evy Battaglia and Henriek Beenen, Frenchman Jérôme Collemare, Greek Ioannis Stergiopoulos, Welshman Scott Griffiths, Iranians Rahim Mehrabi and Mansoor Karimi, Turkishman Bilal Ökmen, Japanese Yuichiro Iida and New Zealander Carl Mesarich. Many of them have affinity with bioinformatics, especially Ate van der Burgt, former Dutch Champion of 800m and 1500m speed running. Together we managed to sequence and annotate the *C. fulvum* genome and compare it with its closest relative, the pine needle pathogen *Dothistroma septosporum*, and

Cladosporium fulvum



- Biotroph
- Genome invaded by many retrotransposons
- Shares effectors with *D. septosporum*
- Produces tomatinase
- Does not produce dothistromin toxin
- Shares introner-like element with *D.septosporum*
- Contains more secondary metabolite genes than *D. Septosporum*
- Originates from a (pine) tree pathogen?

Dothistroma septosporum



- Hemibiotroph
- Genome with few retrotransposons
- Shares effectors with *C. fulvum*
- Lacks tomatinase gene
- Does produce dothistromin toxin
- Shares introner-like elements with *C. fulvum*
- Contains less secondary metabolite genes than *C. fulvum*
- Originates from a solanaceous plant pathogen?

Figure 28. Dothistroma septosporum *the pine needle pathogen is the closest relative of* C. fulvum

discover many new phenomena in their genomes. Thank you guys! I cannot discuss all your achievements due to time limitation. Some highlights are provided in **Figure 28**. We could address research questions like:

- Where does *C. fulvum* come from?
- Was *C. fulvum* a pine tree pathogen before it became a tomato pathogen?
- How did *C. fulvum* adapt to its host plant tomato?
- Where did the transposons that invaded *C. fulvum* come from?
- Are the discovered introner-like elements ancestral to regular spliceosomal introns?
- Is *C. fulvum* a real biotroph?
- If a real biotroph, why does *C. fulvum* contain so many secondary metabolite genes and do they produce functional products?
- Can pseudogenization explain adaptation to new host plants?

Many of these research questions are answered by you already, but many are still under investigation. For those of you who are interested, you can read the papers that were published as a result of this research in recent years: (Bradshaw et al., 2012; Ohm et al., 2012; Van der Burgt et al., 2012; Bradshaw et al., 2013; Chettri et al., 2013; Okmen et al., 2013; Stergiopoulos et al., 2013; Collemare et al., 2014; Mesarich et al., 2014; Okmen et al., 2014; Stergiopoulos et al., 2014; Van der Burgt et al., 2014a; Van der Burgt et al., 2014b).

The future of next generation sequencing

The genomes of numerous fungi can now be compared based on data obtained from genome sequences of " the one thousand fungal genome project". We can now identify and isolate useful genes from many of these fungal genomes. The KNAW Institute CBS in Utrecht headed by Pedro Crous hosts one of the world's largest collections of fungi. It is important to know what these fungi can produce. Some might be producers of new antibiotics that are badly needed to cure human and animal diseases.

This afternoon was a journey through forty years of my own research, as a PhD student, a postdoc, assistant, associate and full professor. The research was mainly performed by talented MSc students, PhD students and postdocs and I thank them all. I have good memories of exciting discoveries that we made during that journey. I am happy that many of you could be present today.

The Laboratory of Phytopathology

For 23 years I was head of the Laboratory of Phytopathology, consisting of six independent research groups and I had the privilege to lead this motivated group of scientists as the "primus inter pares". The group leaders could all have presented a similar talk about their fascination and passion for research.

- Former group leader Maarten de Waard would have talked about ABC transporters in fungi,
- Pedro Crous about fungal biodiversity and evolutionary phytopathology,
- Francine Govers about the genome and effectors of *Phytophthora infestans* and potato immune receptors,
- Matthieu Joosten about effector-triggered Cf-mediated defence signaling in solanaceous plants, as he did this morning during the farewell symposium,
- Jan van Kan about virulence factors of the necrotrophic pathogen *Botrytis cinerea* and comparative genomics of its relatives,
- Jos Raaijmakers about molecular microbial ecology and soil-borne, antimicrobial and growth stimulating bacteria; Jos, much success as future head of the Terrestrial Microbial Ecology department of the KNAW Institute NIOO,
- and finally Bart Thomma about effectors of vascular pathogen *Verticillium* species and host immune receptors.

Your research groups have all made great contributions to the reputation of The Laboratory of Phytopathology by your excellent research. This is also why not only sponsors like the EU, NWO, STW and the KNAW, but also breeding companies have always supported the Laboratory very well. Our work was also appreciated by the board of Wageningen University by extra financial support for education and research. Also, ALW and STW have granted many Veni, Vidi and Vici fellowships to

our young talents. They are too many to mention them all. I would also like to thank secretary Ali Ormel and the technicians Grardy van den Berg, Rob Weide, Ester Dekkers and Henriek Beenen for their contributions to research and teaching, and managing the office and laboratory. Your work was, and still is of crucial importance. MSc students, PhD students and postdocs come and go, but they are the ones that carry out the innovative research. Many of you who passed through the Laboratory got prestigious positions in The Netherlands and abroad.

I am also privileged that my successor was appointed already before I stepped down as head of the Laboratory of Phytopathology. Bart Thomma, you came like Julius Cesar: "Veni-Vidi-Vici", but what Cesar could not manage, you did. You came from the south after having conquered the Belgicae, you passed the river Rhine and decided to stay permanently among the Batavians. I wish you much success as the new head of the Laboratory. You have new challenges ahead, but I am sure you will master them with your team of excellent scientists.

I would also like to thank my former teachers and colleagues, the late professor Johan Dekker through his wife Hanny Dekker, Jan Carel Zadoks, Mike Jeger, Leen Davidse, Adriaan Fuchs, Tijmen Hijwegen, Gerrit Bollen, Herman Frinking, Theo Ruissen and Aad Termorshuizen, as well as our former secretary Elly Depryck. I keep good memories of all of you.

I would also like to thank my colleague professors in the former crop protection section for stimulating discussions and collaborations, Just Vlak who replaced the late Rob Goldbach, Jaap Bakker, Joop van Lenteren and Marcel Dicke. Also our collaborators on the *C. fulvum* project outside the Laboratory I would like to thank: Jacques Vervoort, Pim Lindhout, Gert Kema, Geert Smant and Henk van den Broek. Internationally we have collaborated with many colleagues. I am happy that some of them are here today and some even presented their last research in the farewell symposium this morning. They are Verna Higgins, Jonathan Jones, David Jones, Richard Oliver, Rosie Bradshaw, Nick Talbot and recently Brian Staskawicz, our Wageningen University honorary doctor. These collaborations were sometimes competitive, but they always worked synergistically and accelerated progress in research. I am thankful to all of you.

As former director of the graduate school EPS, I interacted with many colleagues in Plant Sciences from Wageningen University and other Dutch universities. It was great to hear when I later became member of the ECOS committee of the KNAW that the graduate school EPS was always taken as the example of an excellent national graduate school. It was the pioneering work of Cees Karssen, Ab van Kammen and

founding director Evert Jacobsen who made this all possible. I wish the present director Ton Bisseling and his team much success in the future.
I would also like to thank the department of Plant Sciences through its director Ernst van den Ende and Wageningen University through its Rector Magnificus Martin Kropff for providing conditions that stimulated students and scientists to perform at their best.

I also like to thank my tennis team mates with whom I played for forty years and some even longer; it gave me a lot of pleasure and diversion after long hours at the laboratory. However, fitness decreases with age. I still enjoy tennis, but then the team decided to play golf, which requires less physical abilities. First I did not like golf, then I started to like it and finally I decided to play competition. I don't think that was a good Idea. Our team lost this Spring, but like fungi in the arms race, one has to lose before one can win. Thank you, team mates.

Finally I would like to thank Els for so many years of support during this journey. I was often away, physically or with my thoughts, but we always found time for joint activities at the tennis court, golf course, jogging, cycling or travelling. I am sure we will get more time for all these activities in the future. We will also have more time to visit Matthieu and his wife Renee, and Christiaan, Katie and our grandson Elye who all live abroad. Matthieu and Christiaan, you have gone different ways, but it was good you followed your heart, Matthieu as a scientist studying the functioning of our brains, and Christiaan in the music industry. I wish you much success.
With the last two slides I would like to thank all past and present members of Laboratory of Phytopathology (**Figure 29**) for a wonderful time, as well as Wageningen University and all outside sponsors (**Figure 30**) who made this all possible by their support.

Thank you for listening. It is now time for drinks.

Figure 29. Thank you Laboratory of Phytopathology!

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Figure 30. Thank you sponsors of the Laboratory of Phytopathology!

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