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2. A multitude of attackers lies in wait

In the vast majority of natural ecosystems, green plants are the basis of the food chain, because they are able to produce complex organic matter from simple inorganic matter. Other organisms directly or indirectly depend on plants as a source of nutrition. This is the main reason why green plants, including our crops, are the main nutrition source for a wide diversity of organisms. In agricultural production systems, such plant-consuming organisms affect the quantity and quality of the produce. The diversity of plant-consuming or -exploiting organisms is shown in Table 1, where we list the various groups, ranging from the most simple pieces of infectious RNA (viroids) to very highly developed and complex organisms such as vertebrates. In this book we call these pathogens and pest species 'attackers' of plants.

The present chapter presents a general classification of these attackers. A more detailed discussion of the categories of these attackers is presented in Chapter 6.

Classification of attackers is performed mainly according to size and method of feeding. Attackers belonging to the micro-organisms (or even lower in level of complexity, e.g. viroids and viruses), are called **pathogens**. They usually live inside the plant, and, as individuals, can only be observed by light- or electron microscope. With the naked eye it is possible to see fruiting bodies (like urediosori of rust fungi), or larger colonies of mycelium (powdery mildew). Often, the presence of the pathogens is not immediately obvious, but their effects on the plant (**symptoms**) stand out: wilting, leaf yellowing, etc.

Small animals (nematodes, scale insects, aphids, mites) are usually called **parasites**. They suck fluids from the plants, and may have little mobility. They can be observed and counted by the naked eye or loupe. Entomologists prefer the term '**phytophagous** insects'. Parasitic higher plants (*Striga, Orobanche*) are also considered parasites.

Herbivore is a term used for larger, mobile animals that consume plant tissue and cause **biting damage** (caterpillars, locusts, rodents). Herbivorous insects are also called phytophagous insects.

In the relevant literature there is no consensus about the terminology. Many people call the powdery mildew fungus a pathogen (because it is a microbial organism). However, powdery mildew colonies are as clearly visible as scale insects (a parasite, see above), and therefore it may also be regarded as a parasitic fungus. Therefore in Table 1 fungi are classified as parasites as well as pathogens.

Table 1. Classification of various attackers and the nature of their effect on plants.

Taxonomic classification	Classification of the natural enemy	Effect on the plant
Viroid, Virus, Phytoplasm, Bacterium, Oomycete, Fungus	pathogens	disease, infection
Higher plant, Nematode, Mite, Insect	parasites	infestation[1]
Snail and slug, Vertebrate	herbivores	biting damage

[1] In many cases of infection by nematodes and some cases of infection by insects the physiology of the whole plant is disturbed: in such a case the term 'disease' is also correct.

A plant on which a parasite or pathogen feeds successfully, is called a **host plant.** The plant provides nutrients and shelter: a place to live. For example, cucumber is a host plant to the spider mite and to the cucumber powdery mildew. Plants are called **food plants** in relation with herbivores (grass is a food plant for the cow). However, the borders between these are not clear-cut.

Many principles that hold true for pathogens also apply to parasites. In order to prevent the clumsy phrase 'parasite and/or pathogen' in this book, we often opt for pathogen, attacker or pestilent organism instead.

2.1. What is disease?

The word 'pathogen' literally means 'generator of disease'. It is questionable, however, whether many pathogens really cause disease. In the narrow sense, the word **disease** indicates a physiological disturbance in the whole plant, leading to **symptoms** such as yellowing, wilting, stunting and malformation. Such symptoms are the most prominent indicators of **infections** by viruses, phytoplasmas, bacteria and vascular wilt fungi. Local reactions of plant tissue to an attacker, like necrotic flecks caused by a resistance reaction or necrotic lesions caused by a local infection, may also be called symptoms.

The intensity of symptoms is not always a reliable indicator for the amount of pathogen present in the plant (see § 3.3 on **tolerance**).

Many pathogens and parasites are (partially) directly visible, and can be counted and measured directly. Colonies of powdery mildew on a leaf are actually not symptoms, any more than scale insects on a plant. However, even in many scientific publications such colonies are referred to as symptoms. Actually such visible parts of pathogens are better described as **signs**, by which they can be recognised. The consequences of infection to the plant (in terms of yield reduction, **damage**), are not directly visible, nor predictable from the amount of infection. Plant genotypes may vary in level of tolerance in a similar way to humans in the degree to which a flu infection is tolerated.

> Leaf blotch fungi cause local lesions. These lesions are the direct consequence of local presence of the pathogen in the tissue. One cannot see the pathogen itself, but the amount of pathogen can be estimated indirectly by the size and number of the lesions. Such pathogens take an intermediate position in between pathogens, causing symptoms and directly visible pathogens.

We prefer to avoid the term disease, since infection by e.g. a scale insect or powdery mildew does not bring about a physiological disturbance in the whole plant. Instead, we use the term **infection** if the plant is being exploited by pathogens or parasites ('a plant severely infected by a rust fungus'). The terms 'disease' and 'diseased plant' are used when the pathogen more or less upsets the physiology of the whole plant (like mosaic virus, wilting fungi, etc.).

The term '**pest**' is used to indicate animal parasites and herbivores. The word indicates the pest species ('White fly is a pest on tomato'). A population of a pest species that reaches such a size that **damage** occurs, is called a **plague** ('In 1993 there was a severe plague of white fly on tomato'). Plants harbouring many individuals of the pest species, are heavily **infested** by that pest.

2.2. Classification of pathogens

Pathogens are subdivided according to the characteristics of the infection process. **Biotroph**s (e.g. viruses, powdery mildew, rust fungi, loose smuts) depend on living plant tissue. Many biotrophic fungi form **haustoria** in the plant cells, and withdraw nutrients from them. Such haustoria invaginate the plasma membrane, and do not invade the cytoplasm. The host plant cells remain alive, despite the presence of such a haustorium. The haustoria probably also release molecules to reprogramme the plant cell to suppress

possible defence reactions. Most biotrophic pathogens cannot be cultured on artificial media, or only with sophisticated protocols.

Necrotrophs (e.g. *Septoria*, *Helminthosporium*, and other leaf spot fungi, some moulds like white (S*clerotinia sclerotiorum*) and grey mould (*Botrytis cinerea*) fungi) extract the nutrients from plant tissue that they have killed. To this end they usually produce toxins. They are similar to **saprophytes** (see glossary), but provide themselves actively with dead plant tissue. They are easily cultivated on artificial media.

Hemibiotrophs (e.g. *Phytophthora infestans*, downy mildews, rice blast) are intermediate between the biotrophs and necrotrophs. Quite soon after the beginning of the infection process, plant tissue will die. Some species can be grown on media (e.g. *P. infestans*), others cannot (*Bremia lactucae*).

Further groups of pathogens seem to have an **opportunistic** lifestyle, as they may infect many plant species, and especially infect plants or plant parts that have an impaired defence capacity such as maturing fruits, seedlings and plants growing under sub-optimal conditions. Examples are the oomycete *Pythium* and the fungus *Rhizoctonia* which cause damping-off, i.e. the killing of young seedlings. Other groups are the **vascular wilts,** root rot and foot rot fungi, such as *Fusarium oxysporum*, *Verticillium dahliae* and *Ophiostoma ulmi*. The former two fungi cause blocking of the xylem, and hence wilting of plants. The latter species is the causal agent of the infamous Dutch elm disease.

Q1 What are the differences and similarities between the terms pathogen, parasite and herbivore?

4. Plant-pathogen interaction: a fundamental concept

In order to understand the concept of plant-pathogen interactions, it is helpful to consider the **co-evolution** between plants and their attackers as follows.

Green plants are the basis for (almost) all life on earth. Plants are organisms that turn inorganic matter (like carbon dioxide, water and minerals) into organic matter (carbohydrates, fats, proteins) with light as the primary energy source. This renders them an attractive source of nutrients for organisms that are not able to photosynthesise. In order to discourage their consumption by microbes and animals, plants have developed defence strategies. These vary in nature, and may differ per plant species. Plants quickly and effectively repair sites where the epidermis or bark is disrupted, hampering the entry of microbes. Plants may contain toxic compounds (e.g. alkaloids), or produce such compounds as a response to wounding or infection attempts. These properties are called '**general defence**', and many of them have probably arisen very early in **evolution** due to selection pressure by a broad range of potential pests and pathogens. This general defence is effective against almost all potential attackers. Part of this general defence is preformed, another part is induced (Figure 3, left). The latter is generally prompted by so-called

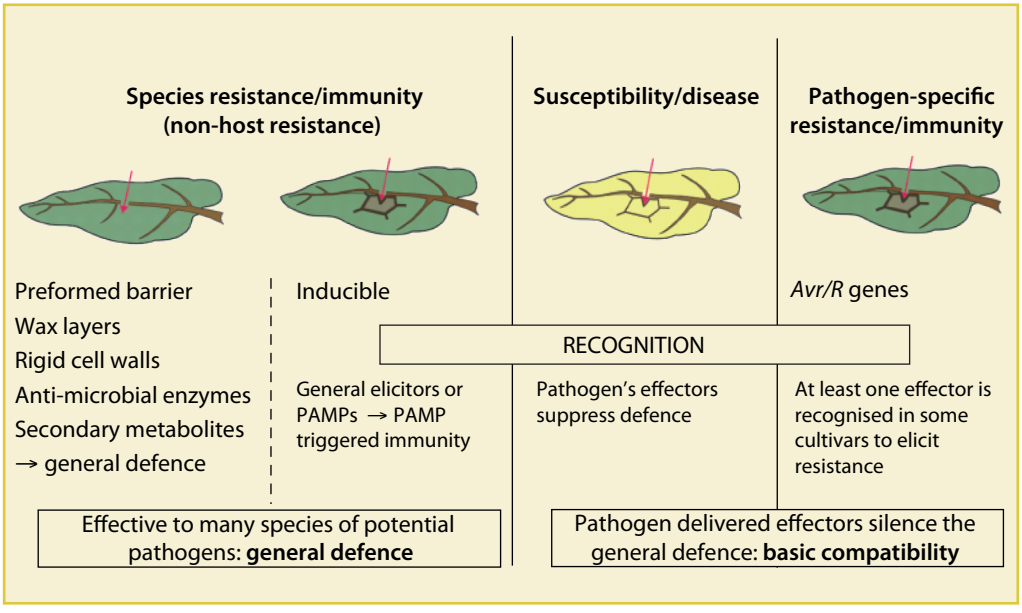


Figure 3. Schematic representation of general and cultivar-specific resistance, and their dependence on recognition between plant and pathogen factors. After Nürnberger et al. *(2004).*

PAMPs – pathogen-associated molecular patterns. These are biochemical compounds or peptides that are often indispensable for the microbial lifestyle and are alien to plant tissues. Examples of PAMPs are a motif in the flagella of plant-pathogenic bacteria, and the chitin cell wall compound of fungi. These PAMPs are perceived by plant receptors. Such a perception initiates a defence response leading to what is called **PAMP-triggered immunity** (Figure 4, left).

PAMPS are by definition conserved across a wide range of microbes, and because these molecules are essential for viability or lifestyle of the microbe, microbes are not likely to evade host immunity through mutation or deletion of PAMPs. PAMP-triggered immunity (PTI) contributes to the plant innate immunity. It is activated even during a susceptible (compatible) interaction, but adapted pathogens are able to suppress this PTI in their host species. PTI is believed to constitute an important aspect of non-host resistance, which explains why most plants are resistant to the majority of pathogens.

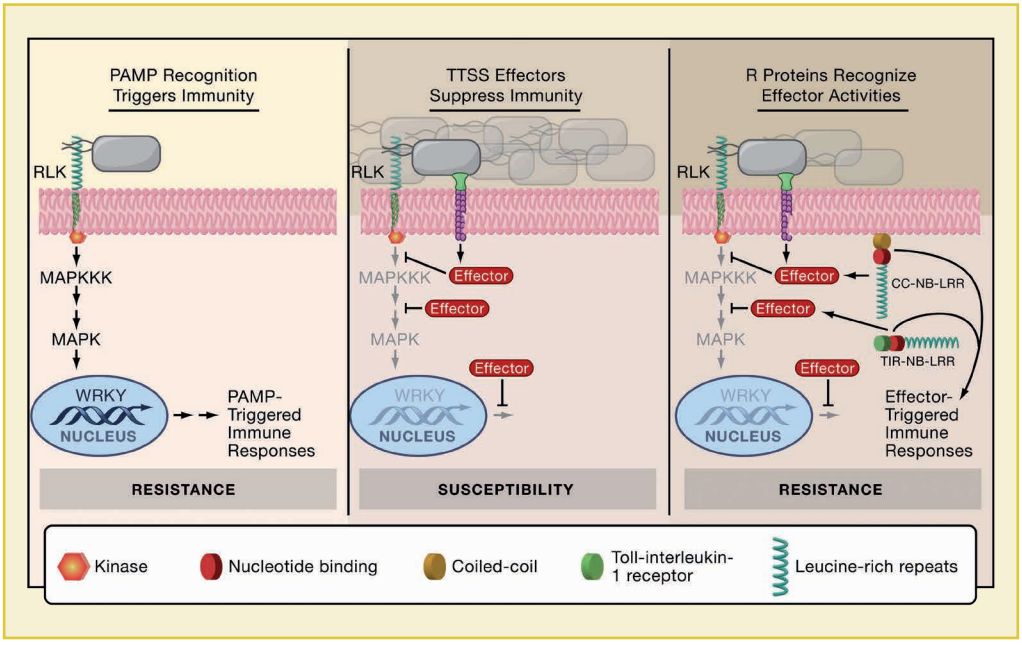
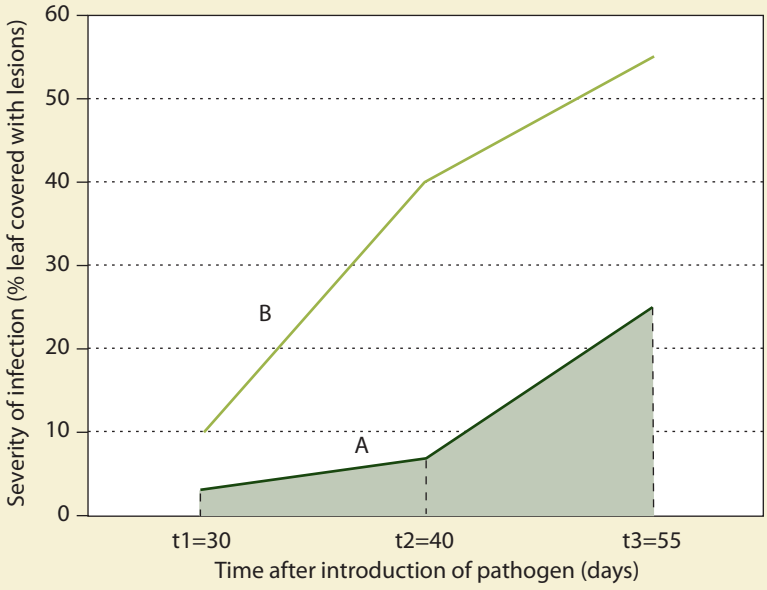


Figure 4. Model for the interaction between a bacterial pathogen and plants. (Left) Perception of pathogen-associated molecular patterns (PAMP, such as bacterial flagellin) by extracellular receptor-like kinases (RLKs) promptly triggers immunity. This involves signalling through MAP kinase cascades and transcriptional reprogramming mediated by plant WRKY transcription factors. (Centre) Pathogenic bacteria secrete effector proteins that target host proteins to suppress the PAMP triggered immunity responses. (Right) Plant resistance proteins (represented by CC-NB-LRR and TIR-NB-LRR) recognize effector activity and confer resistance through effector-triggered immune responses. After Chisholm et al. *(2006).*

In field tests, the amount of infection by **polycyclic** leaf pathogens is usually assessed by estimating the percentage of leaf tissue covered with lesions, mycelium or pustules: the percentage infected tissue. When such observations are repeated several times in a season on the same plants or field plots, an impression is obtained of the increase in the infection over time. From a graph in which the amount of infection is plotted against time, the 'Area Under the Disease Progress Curve' (AUDPC) can be calculated, a figure that is a good indicator of the amount of infection during the season (Figure 25).

Q29 Calculate the AUDPC for cv A, if the infection severity at t1 is 3%, at t2 7% and at t3 25%. Calculate also the AUDPC for cv B.

Measuring the percentage of infected tissue with the eye is a rapid method, but not really objective or sufficiently reproducible. When the same rust-infected leaf is shown to several persons, estimations of the percentage of leaf covered by the pustules will vary greatly. To improve the objectivity and reproducibility, several researchers have designed assessment



AUDPC: $(t2 - t1) \times (y_{t1} + y_{t2})/2 + (t3 - t2) \times (y_{t2} + y_{t3})/2 + ...$
in which y_t is the severity of infection at time t

Figure 25. Infection severity measured at three time points for cv A and cv B. The surface of the shaded area represents the Area under the Disease Progress Curve (AUDPC) for cv A.

keys. These are pictures (drawings or photographs) of plants or plant parts, with several degrees of infection (Figure 26). Under each picture the percentage of infection is given. The assessment keys serve as a reference in field and greenhouse evaluations. There are also computer programmes that are helpful for training the eye of the observer to estimate the amount of infection as precisely as possible.

In 1892 Cobb developed an assessment key, the so-called Cobb scale, for rusts in cereals. Using pictorial presentations of five infection levels of the stem rust fungus he could obtain quick and quite reliable assessments. This principle is still used. James (1971) presented similar assessment keys for several more pathogens. Most keys are pictorial, some are descriptive (see Table 17). The percentages of infection that are estimated this way are percentages of really infected tissue. The observer should decide for himself what he considers to be infected tissue. With many rust species uredosori are usually surrounded by some pale or chlorotic tissue. This may or may not be regarded as infected tissue. Furthermore, not all leaves and leaf parts are equally severely infected. The observer should try to estimate the weighted average of infection. Experience is an important asset in this matter.

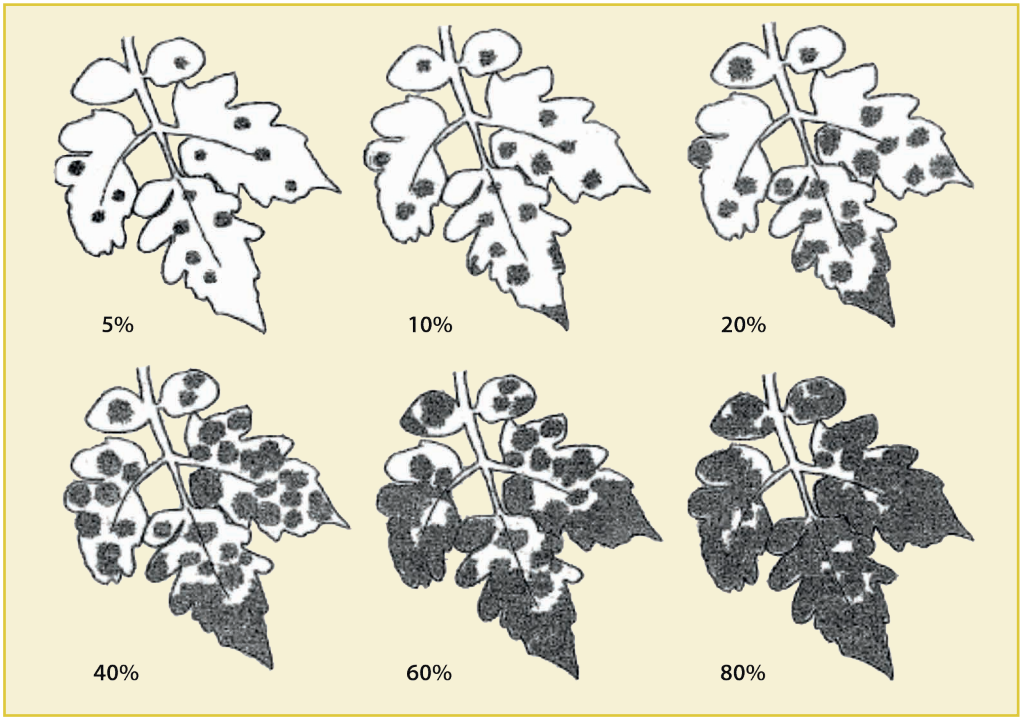


Figure 26. Home-made pictorial key to standardise assessment of infection severity of tomato powdery mildew.

About the authors

Rients Niks (1953) is Assistant Professor in the Plant Breeding Laboratory at Wageningen University. He studied plant breeding in Wageningen, where he worked under the supervision of Jan Parlevliet on aspects of partial resistance of barley to barley leaf rust, to obtain his MSc degree (1978) and PhD degree (1983). He obtained a post-doc position at ICARDA (Syria), where he ran the durum wheat improvement programme for two years. When Jan Parlevliet was appointed Full Professor at the plant breeding laboratory in Wageningen in 1983, Rients had the honour of succeeding Jan, and continued the latter's successful research on partial resistance of barley and extended the scope of his research to include the genetics of non-host resistance. Rients also further developed the courses on breeding for disease resistance, and taught many courses on plant breeding at home, in Spain, South America , South Africa, Kenya, Iran, Thailand and China. His research on mechanisms of durable resistance and related subjects has resulted in over 100 (co-) authored publications and book chapters. He will retire in 2020.

Jan Parlevliet (1932) is retired Professor in Plant Breeding at Wageningen University, the Netherlands. He studied plant breeding in Wageningen, from where he graduated in 1960. After that he worked at Unilever to obtain his PhD degree (1967) on the physiology of spinach. From 1967 to 1971 Jan was a plant breeder at the Pyrethrum Experiment Station, Molo, Kenya. In that period several good clones and cultivars were released as new cultivars for the production of natural insecticides.
In 1971 Jan joined the department of Plant Breeding at Wageningen University to investigate aspects of durable resistance, and trained numerous students in breeding for resistance. His research subject was barley-barley leaf rust, in which both the presumed polygenic durable resistance and the non-durable major-genic resistance occur. During his time there Jan initiated research on a wide range of host/pathogen combinations, including wheat, rice, maize, beans, potato, ground nut and pathogens belonging to the fungi, oomycetes, bacteria, viruses and nematodes. Durability of resistance remained his main focus.
In 1995 Jan retired, but remained involved in a plant breeding support project in the Andean Region, to enhance local breeding programmes with emphasis on durable disease resistance in various crops. He (co-)authored more than 130 scientific publications and book chapters.

Pim Lindhout (1953) studied chemistry and biology at Leiden University, the Netherlands. There he also defended his PhD thesis on 'the translation of alfalfa mosaic virus RNA1' (1985). He was appointed to run the tomato breeding research programme at Institute for Horticultural Plant Breeding (Dutch: IVT), Wageningen, where he evolved into a real 'tomatologist'. He initiated and coordinated several multidisciplinary research programmes to unravel the genetics of important traits in various horticultural crops, mainly tomato.
In 1994, Pim was appointed Associate Professor in Plant Breeding at Wageningen University. His main focus was the mapping and understanding of quantitative traits and implementing new technologies in plant breeding. He coordinated tomato genomics research in a national plant genomics programme (CBSG), and has (co-)authored more than 100 scientific papers.
In 2006 he moved to the private sector to become head of R&D at DeRuiterSeeds, a successful vegetable breeding company. This attracted the attention of Monsanto, which, seeking to strengthen its vegetable division, acquired DeRuiterSeeds Company (2008). Soon Pim became head of vegetable breeding in Monsanto, but left the company in 2010. Nowadays he is director of the technology company Solynta aimed at implementing innovative technologies in hybrid potato breeding from true seeds.

Yuling Bai (1964) is Professor at Plant Breeding, Wageningen University. She obtained her first MSc (1988) in the field of Plant Genetics and Breeding at Henan Agricultural University in China. There, she was appointed Associate Professor (1995). In 1997, she came to Wageningen University in the Netherlands, where she studied 'Biotechnology' for her second MSc (2000) and did her PhD (2004) and post-doc research on resistance of tomato to tomato powdery mildew. In 2007 Yuling was appointed Assistant Professor at Wageningen University and set up her research group 'Breeding for resistance in Solanaceae'. In 2013 she was promoted to Associate Professor and in 2018 to Professor. Her research focuses on the understanding of genetic and molecular mechanisms underlying disease resistances in vegetable crops to different pathogens. Her research ambition is to translate scientific findings into breeding programmes by developing novel breeding strategies and exploiting new techniques to make breeding more efficient. Besides doing research, Yuling is involved in teaching and developing courses related to plant genetics and breeding for Wageningen University as well as other universities, for example in China and Spain.