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COMMENTARY

The concepts of plant pathogenicity, virulence/avirulence and effector proteins by a teacher of plant pathology

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Abstract. During the genomic and proteomic era of plant pathology in the last decade, extensive progress has been made in the understanding of the mechanisms of the interactions between plants and microbial pathogens. New models of pathogenesis have been designed, new biological phenomena have been discovered, a plethora of new molecules and functions have been determined, and new terms and senses have been added to phytopathological language. In this context, however, defects often emerge in many of the papers published on these subjects: the meanings attributed to some new terms are not always unique and do not always adhere to the basic concepts of plant pathology, including those relating to disease, disease cycles, pathogenicity, virulence, and avirulence. This paper discusses this problem, emphasizes established defintitions and proposes new ones, to assist the phytopathology community in unifying terminology for the benefit of our research discipline.

Key words: basic concepts of plant pathology, factors of virulence and avirulence, effectors, symbiont organisms, infection factors.

Introduction

It is not always easy for a teacher to give a good overview of the latest research on a subject included in his teaching programme, so as to instruct students in ways that are both accurate and stimulating. This is especially true when the subject concerns molecular aspects of plant-pathogen interactions, an area of plant pathology where remarkable progress has been made in the last few decades. The task becomes even more difficult if the teacher is not completely familiar with the research that has been and is being done, and where all the necessary information has to be extracted from the latest literature on the subject.

The generation of plant pathologists who are now investigating this complex field have made two important advances: they have succeeded in finding answers to many outstanding questions, and have thus opened new and even more complex frontiers of research. However, the overall impression that is left when one reads the hundreds of studies that have been done on pathogens infecting plants and humans, is that the subject is enormously complicated. One example of this complexity is seen in the multitude of apparently redundant effector proteins, (or in a rather small number of effector proteins, each having numerous biochemical functions), that are produced by a single plant pathogen, or by a group of pathogens. The researcher finds it difficult to assign a definite pathological trait to any given effector. Here, however, we sometimes have the impression that some of the difficulties experienced in assigning a trait to an effector protein are caused, at least in part, by a failure to correctly appreciate certain basic concepts in plant pathology.

On the other hand, plant pathology has attracted scientists from other fields of research for whom it

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Glossary

DISEASE: any deviation from normal functioning or from physiological processes, of sufficient duration to cause disturbance or cessation of vitality.

DISEASE CYCLE: the chain of events in disease development, comprising migration of the pathogen to the host, recognition and contact with host surface; penetration; establishment of infection; colonization of the host; pathogen growth, reproduction and dissemination.

DISEASE FACTOR/DETERMINANT: a pathogen component that harms the host plant (i.e. that causes disease symptoms).

EFFECTOR (effector molecule): a pathogen molecule that alters host-cell structure and function in order to cause/facilitate the formation of symptoms, to trigger defense responses and/or to avoid recognition by the host plant. (In practice, a broad term that includes direct and indirect virulence factors, non-pathogenic factors and infection factors that avoid/circumvent PAMP-triggered immunity and/or effector-triggered immunity.)

EFFECTOR GENE**:** a gene that codifies the production of disease-related molecules (effectors) delivered into the host cells by symbiotic pathogens.

VIRULENCE FACTOR: any component of a pathogen that damages a susceptible host.

INFECTION: the process by which a plant (the host plant) acquires a symbiotic micro-organism.

INOCULATION**:** penetration/introduction of a micro-organism into a host plant.

NON-PATHOGENIC FACTOR (or elicitor of incompatible responses): a factor that causes an incompatible (resistance) reaction in an otherwise susceptible host.

NON-PATHOGENIC MICRO-ORGANISM: a micro-organism that does not cause disease in a (particular) host.

NON-VIRULENT (AVIRULENT) PATHOGENIC MICRO-ORGANISM**:** a pathogenic micro-organism that does not cause measurable damage to the host; or (in an incompatible interaction), any pathogen that harbors an effector gene whose product (a non-pathogenic factor) is recognized by a host that harbors the complementary *R* gene.

PATHOGENICITY: the ability of a micro-organism to cause disease on a particular host (i.e., a quality of a micro-organism that enables it to cause disease in a particular host).

PATHOGENIC MICRO-ORGANISM: a micro-organism that causes disease in a susceptible host.

SYMBIONT: an organism that forms a close association with another organism.

SYMBIOSIS FACTOR (or INFECTION FACTOR**):** a microbial component responsible for infection.

SYMPTOM: a visible or otherwise measurable adverse change in a plant, produced in reaction to infection by a pathogenic, virulent micro-organism.

VIRULENCE: the measurable degree of damage caused by a pathogenic, virulent micro-organism to a host plant.

VIRULENCE GENE: a gene that, during the disease process, codifies the production of factors that alter host-cell structures and functions.

VIRULENT MICRO-ORGANISM: a pathogenic micro-organism which causes a measurable damage to a host plant.

may appear superfluous to have good knowledge of such basic concepts. At least, these researchers do not appear to be well disciplined in the correct use of terms or application of certain concepts, as would plant pathology specialist. But, in order not to fall into a Babel of information, where many talk, but too few understand, it is always necessary to have simple, stable and clearly defined basic concepts (such as defining what is disease, infection, pathogenicity, virulence, avirulence), and these concepts must be stable enough so that they do not require revision at every new discovery.

Perhaps now is a good time to remind ourselves of these concepts, and also, if necessary, to restate, revise and correct them in light of greater understanding of how disease develops.

This paper, which has taken the form of a lesson to be presented to students of plant pathology, deals mainly with a problem in plant pathology that needs to be solved.

The problem and scope

Plants, like human beings and animals, are exposed to infection by micro-organisms and viruses. These may reduce plant (and crop) productivity, and the quality and nutritional value of produce. The pathogens causing these infections in plants (as in

humans and animals) have a number of common features. Many have flexible genomes, or they are able to extract genes from other organisms by means of mobile genetic elements. Almost all have developed capacity to cross the barriers between kingdoms and to infect organisms belonging to different kingdoms. Still others share certain disease factors between them, such as the Type 3 secretion system, or the mechanism by which they sense the density of their population (quorum sensing). Pathogens affecting plants and animals also share some mechanisms that enable them to spread, or sometimes merely to survive, in nature. Also common to pathogens of *Plantae* and *Animalia* are the basic concepts of parasite, pathogen, pathogenicity, and virulence, and, at least in part, the mechanisms by which pathogenicity and virulence are manifested in nature.

It so happens, however, that scientists from different disciplines, and even from within the same discipline, sometimes define the terms 'pathogenicity' and 'virulence' in different ways. And what is more, in recent research reports on genomics and proteomics that deal with the relationships between plants and pathogens, there is a distinct impression that some basic concepts of plant pathology are beginning to lose their meanings, or are being interpreted in ways that differ from that in which they were interpreted in the past. Some authors view a factor of pathogenicity as a factor of virulence; others view a factor of virulence as a factor of pathogenicity, or even as a factor of both pathogenicity and virulence, without making a distinction between the two. The problem therefore is not that a misleading term is used to describe a phenomenon, or a molecular function, but that different terms are being used for what is essentially the same thing.

This is certainly not a new problem. Many researchers in the past and more recently have pointed out the difficulty with understanding of the precise meaning of some guiding concepts in plant pathology, and with the use of terms consistent with those concepts (Shaner *et al*., 1992; Andrivon, 1993; Gabriel, 1999; Casadevall and Pirofski, 1999; Wassenaar and Gaastra, 2001; Thomas and Elkinton, 2004; Bent and Mackey, 2007; Newton *et al*., 2010).

The purpose of this paper is not to review the entire process of plant disease, or how it has evolved over time, nor to exhaustively discuss each topic and each term related to plant pathology, nor even to discuss modern views about the immune system of plants, but only to examine some basic, and well-established concepts in plant pathology, and to examine if these concepts are still valid in light of the latest findings on the mechanism of disease induction. Further aspects included relate to the fact that it has become clear that a disease process as a whole is always the result of an interaction between a host plant and a micro-organism, in which both parties are significantly involved, whereas in the past our understanding of pathogenicity and virulence was pathogen-centred.

Many of the views presented here concern those infectious microbes (mainly plant pathogenic bacteria) that derive nourishment from, or have a nutritional relationship with, living plant cells (biotrophs), or those pathogens (hemi-biotrophs) that act as biotrophs but also as necrotrophs (drawing nourishment from dead or dying cells), depending on the conditions they find themselves in, or on the stage of their life cycle they are at (Lee and Rose, 2010). The hemi-biotrophs usually cause substantial host cell death at late stages in the infections they cause (Thrower, 1966). The distinction between biotrophs and necrotrophs may in some cases seem quite arbitrary. For example, the bacterial pathogen *Pseudomonas syringae* is often considered a biotroph, occasionally a necrotroph but it is probably a hemi-biotroph. Nevertheless, the distinction is important since the defences that plants call on to protect themselves against biotrophs are different from those they use against necrotrophs, and sometimes these two types of plant defence compete with each other (Glazebrook, 2005; Spoel *et al*., 2007; Mengiste, 2012). The mechanisms that biotrophs employ to harm their hosts also differ from the mechanisms used by necrotrophs.

After revisiting the concepts identified above, this paper concludes with a proposal to redefine some of the terms now in use. The comments that follow also owe much to the ideas of respected plant pathologists such as Whetzel (1929); Walker (1957); Vanderplank (1963, 1968); Horsfall and Diamond (1960).

'Plant disease', 'disease cycle' and other terms in the phytopathology tradition

Several definitions of plant disease can be found in dictionaries or in papers dealing with the subject.

One that would meet with general agreement is that given by Agrios (1997) in his textbook: 'a plant can be considered healthy, or normal, when it can carry out its physiological functions to the best of its genetic potential. Whenever the ability of the cells of a plant or plant part to carry out one of its essential functions (cambial activity, absorption of water and nutrients from the soil, photosynthesis, and so on), is interfered with by either a pathogenic micro-organism or an adverse environmental factor, the plant becomes diseased'. Disease in a plant is therefore '*any deviation from normal functioning of physiological processes of sufficient duration to cause disturbance or cessation of vitality*'.

Plants that become diseased undergo internal or external changes that may be visible or invisible. The visible changes are the *symptoms* of the disease (Agrios, 1997); although invisible, physiological changes may also contribute to economic loss. Any visible or otherwise measurable symptoms produced in a plant reacting to an infection indicate the *amount of disease* in that plant. The symptoms indicate how the plant is reacting to the pathogen, and/or how the pathogen is affecting the plant's normal functioning.

The proportion of a plant that becomes symptomatic indicates the *severity* of a disease. A disease may produce more than one symptom, and in that case all the symptoms together indicate the amount, or the severity, of the disease.

In plants, the amount of a disease is often considered synonymous with its severity.

Disease cycle. The disease cycle consists of a number of distinct steps: 1. the pathogen moves to and makes contact with the host surface; 2. it establishes itself on the host and penetrates plant tissues; 3. it initiates infection (early stages of proliferation); 4. it colonises the host; 5. it reproduces and spreads inside the host; and 6. it escapes from the host, and travels to, and infects, a new host.

Inoculation can be defined as *the penetration* (also passive penetration) *or introduction of a micro-organism into a host,* while *infection* is usually viewed as the process by which a host acquires a micro-organism (which does not necessarily have to be pathogenic to that host). (In people's minds infection is synonymous with disease but, there are examples of microbial infections that do not give rise to diseases as is the case of endophytic infections by fungi that do not cause disease in any plant. Infection is thus not synonymous of disease.)

For infection to occur, the pathogen must have the capacity to perform, with few exceptions, at least two actions: to enter the host, and to grow and spread within the host.

The entire disease cycle can therefore be simplified and reduced to three basic events:
1. the pathogen enters the host;
2. the pathogen colonises the host; and
3. the pathogen interferes with the normal physiological functioning of the host, producing disease symptoms.

In this view, the first step in a disease really occurs when a micro-organism penetrates into a host.

With respect to the relationship that a given micro-organism may establish with a given plant, micro-organisms may be divided into two main categories: those that have the capacity to penetrate a given plant, and those that do not have this capacity. A micro-organism that can penetrate a plant, and that does so, then forms with the host one of a number of possible relationships (see the section on symbiont organisms).

Pathogenicity. The earliest and still the most commonly accepted definition of pathogenicity is *the ability of a given (infectious) casual agent (*fungus, bacterium, phytoplasma, virus, viroid, nematode, or parasitic flowering plant*) to produce disease in a host organism*.

This means that when a micro-organism (the main subject of this paper) that is pathogenic to a plant enters that plant, it causes a greater or less degree of deviation from the plant's normal functioning, or its normal physiological processes, of sufficient duration to cause disturbance to the plant, leading to the onset of disease symptoms, or the cessation of vitality. In practical terms, a micro-organism is pathogenic to a plant if it damages that plant and the pathogenicity is the capacity of a micro-organism to damage a host plant.

The pathogenicity of a micro-organism is a state or quality (a "character", according to Shaner *et*

al., 1992) of the micro-organism, and as such is not measurable. Furthermore, pathogenicity usually refers not to a single micro-organism but to a group (e.g. pathovar, species or genus).

Pathogenic micro-organisms. Any micro-organism that causes a disease in a susceptible plant is a pathogenic micro-organism. To cause a disease, a micro-organism must come in contact with its host and enter it, colonise its inner tissues, draw nutrients from it; and harm it. According to several authors a pathogen is a parasite (see later) that produces a disease in its host. If so, a pathogenic micro-organism must have the traits of a parasite *and* it must also harm its host. Parasite and pathogen are not synonymous terms.

Non-pathogenic micro-organisms. Micro-organisms unable to cause a disease in a host are obviously non-pathogenic micro-organisms for that host. The key point of a plant disease is that the diseased plant suffers from a pathogen that has invaded it, and that it shows disease symptoms as a result. Since many symbiotic micro-organisms are able to perform actions propaedeutic to pathogenicity (e.g. by coming into contact, entering, and colonising a plant) and yet are not pathogens, it follows that all those micro-organisms that do not harm the host plant must be classed as non-pathogenic micro-organisms (even though they may enter the plant and colonise it, as do, for example, parasites).

Factors of pathogenicity (disease-conferring determinants). Since a pathogenic micro-organism must enter, colonise and damage its host, all the factors that enable a micro-organism function in this manner should be considered factors of pathogenicity. Pathogenicity would then be a broad term that would also include virulence, pathogen entry, and pathogen spread throughout the host. However, since it is necessary, at least for historical reasons, to distinguish pathogenicity from virulence, factors of pathogenicity are traditionally considered to be only those that enable a micro-organism to *become established in a host plant*.

Virulence. Virulence, even more than pathogenicity, has been defined in different ways. There is, however, general agreement that virulence is the quantifiable manifestation of pathogenicity, that is, it is the measure of the extent to which a micro-organism causes disease symptoms in a plant (*the amount of damage caused to a host*).The virulence of a micro-organism in a plant is therefore a property (attribute) determined by measuring, under strictly controlled conditions, the effect of a single, homogenic strain of the micro-organism whose pathogenic activity towards the host in question is already known. Virulence thus becomes measurable and can be expressed quantitatively (Ercolani, 1968).

Virulent micro-organisms and factors of virulence. If virulence, a property of pathogenicity, is the amount of damage caused to a host, then a virulent micro-organism is a pathogenetic micro-organism that causes measurable damage to the host. Consequently, virulence factors include all those pathogen factors that harm a susceptible host. In principle, any molecule (plant cell-degrading enzyme, toxin, hormone, siderophore, or extracellular polysaccharide) that occurs on the microbial cell surface or is translocated to an extracellular environment where it harms the host cells, is a virulence factor (see also Casadevall and Pirofski, 1999, 2009).

In a strict sense, virulence factors do not include any factors that indirectly influence the production of virulence factors in a pathogen, such as any factors that favour pathogen growth inside the host. This caveat is important since it is common to suggest that any factor that stimulates pathogen growth inside the host is a virulence factor.

Non-virulent (or avirulent) pathogenic micro-organisms. From the above definition of virulence it follows that a non-virulent (or avirulent) pathogenic micro-organism does *not* cause any detectable damage to its host. In other words, an avirulent pathogen does not harm the host, but only causes asymptomatic disease (the host so infected is diseased, but asymptomatic). This is because the pathogen has lost capacity to produce virulence factors (possibly amongst other things).

The gene-for-gene hypothesis and a different view of virulence/avirulence

The term avirulence was described in a new way by H. H. Flor in his research on the pathogenic fungus *Melampsora lini* and its host *Linum usitatissimum*. In presenting some of his findings, Flor stated that

Table 1. Reaction of *Linum usitatissimum* to inoculation of some *Melampsora lini* races.

Type of host reaction	Type of rust infection produced	Term used
Immune	No uredia evident	Avirulent
Resistant	Small uredia with chlorosis or necrosis	
Semi-resistant	Variable uredia with necrosis	Semi-virulent
Moderately susceptible	Small to medium uredia; little necrosis, variable chlorosis, sensitive to environmental conditions	Moderately virulent
Suceptible	Medium to large uredia without chlorosis or necrosis	Virulent

'the use of the terms avirulent, semi-virulent, moderately virulent and virulent facilitated the description of the types of symptoms produced by the host plant after artificial inoculation of some *M. lini* races' (Table 1).

On the basis of his findings, Flor then used the vigour of rust sporulation and the reaction of the host to evaluate the virulence/avirulence of the pathogen, and the susceptibility/resistance of the plant.

Flor's research convinced him that 'for each gene that conditions a reaction in the host, there is a corresponding gene (avirulence gene) that conditions pathogenicity in the parasite' (Flor, 1971). This concept has become known as the gene-for-gene hypothesis. That there was a relationship between the reaction of the host to the parasite, and the pathogenicity of the parasite to the host was pointed out elsewhere by Flor. For example, in his very first paper, (Flor, 1942) he stated that his findings 'indicated that the range of pathogenicity of a physiological race of *Melampsora lini* is determined by pathogenic factors specific for each resistance factor possessed by the host'. In a second paper (Flor, 1955) he further wrote that the '[h]ost parasite interaction in flax rust may be explained by assuming a gene-for-gene relationship between the rust reaction in the host and the pathogenicity in the parasite'. Pustule type, the criterion of both the reaction of the host and the pathogenicity of the parasite, is conditioned by specific pairs of genes, one gene of each pair being located in the host, and the other in the parasite. Though Flor always directed his attention specifically to pathogenicity, it has become common to say that 'for every gene that confers resistance in a plant, there is a corresponding gene that confers avirulence in a pathogen'. In other words, for R-gene-dependent resistance to occur in the host plant, the pathogen must express the complementary avirulence (*Avr*) gene that codifies a product recognisable by the plant. If either the *R* gene or the corresponding *Avr* gene is lacking or non-functional, resistance is not activated and disease will ensue.

The avirulence genes were so named primarily because they caused avirulence (small uredia plus a hypersensitivity reaction) in the presence of *R* genes, and perhaps also because the immunity conferred by the *R* genes overrode the pathogenic effect of any *avr* gene product on any host carrying the specific *R* genes that detect those *Avr* genes. An avirulence gene thus causes avirulence in the pathogen that harbours that gene if, and only if, it matches the corresponding *R* gene in the host. Therefore, an avirulence gene can be defined as '*any gene that codifies factors that are recognised by specific genotypes (cultivars) of a host species that contains the corresponding resistant gene.*

It follows that:

1. avirulence factors elicit avirulent responses in the host plant, e.g. a response consisting in host cell necrosis at the site of inoculation (HR);
2. a non-virulent pathogen in an incompatible (resistant) reaction is a pathogen that harbours an *Avr* gene whose product is recognised by the host, which harbours the complementary *R* gene;
3. in that case the non-virulent pathogen does not colonise the plant, the plant does not become diseased, and does not develop any symptom.

Virulence factors, on the other hand, would then be those factors that avoid the elicitation of an aviru-

lent response in the plant. Consequently a virulent pathogen in a compatible (susceptible) interaction is not recognised by the challenged host, even if the pathogen harbours an *Avr* gene. This pathogen then colonises the plant, which becomes diseased and shows symptoms.

This explanation of virulence/avirulence is completely different from that given in the previous paragraphs. In this way a split has developed between plant pathologists in how they understand avirulence. How have plant pathologists tried to resolve this apparent contradiction? They did so by positing two types of virulence/avirulence, depending mainly on the type of pathogen involved (biotrophs or necrotrophs) and on the type of interaction (compatible or incompatible). The virulence of biotrophic (and hemi-biotrophic) pathogens is viewed by them as a *qualitative* capacity, just like pathogenicity: the host fails to recognise the pathogen, and this allows the pathogen to infect the host. Biotrophic virulence is not measurable. By contrast, the virulence of necrotrophic pathogens quantifies the effect of the toxins that cause the disease symptoms. (Initially necrotrophic pathogens were not thought to comply with the gene-for-gene model. However, they have recently been reported to produce ribosomal proteins that could function as effectors causing necrosis in plants with suitable genes: Friesen *et al*., 2008a.) This type of virulence is viewed as a quantitative capacity, and as such is measurable. The limitations of these conceptual acrobatics are obvious: for one thing, there is a long list of biotrophic (and hemi-biotrophic) pathogens that rely on toxins, as well as on hormones, ESPs and other molecules to cause various measurable degrees of symptoms in host plants.

For a long time, therefore, we have effectively had to deal with two types of avirulence: one, which is intuitively obvious, was that avirulence derived from the loss or absence of the traditional factors of virulence [toxins, degrading enzymes, hormones, exopolysaccharides (EPSs)]. Opposed to that is the type of avirulence propounded by Flor, in which the R-proteins of the host somehow recognise the corresponding proteins of the pathogen, named Avr factors, and this recognition triggers a defence response often associated with HR. We now know that this recognition occurs directly by an interaction between *R*-proteins and Avr proteins, or indirectly, by detecting changes in the host targets - the guardee proteins - of the Avr proteins.

The Avr factor of the pathogen would then enable the plant to recognise the pathogen and to forestall the onset of the disease it causes, that is, it would transform the pathogen into a non-pathogen as far as that host is concerned. In that case, however, it may well be asked why these factors are called factors of avirulence, and whether it would not be better to call them *non-pathogenic* factors (or *elicitors of incompatible responses*), since any micro-organism that is non-pathogenic to a host is unable to cause a disease in that host.

All the definitions and comments given above, in particular the definitions of pathogenicity and the avirulence/virulence factors, derive from what was already known about pathogenic mechanisms in the pre-genomic era. Do these definitions and comments still have validity today? This question will be considered by viewing the process of infection in a broader light.

Symbiosis and symbionts, or the micro-organisms that enter plants

The organisms that inhabit the earth only rarely live in isolation; usually they establish more or less close relationships with other organisms. These relationships are often beneficial to both parties, but sometimes only one party benefits. Indeed, if credence is to be placed on the proponents of the hologenome theory of evolution (Rosenberg *et al*., 2007, 2009; Zilberg-Rosenberg and Rosenberg, 2008) plants (and animals) are normally associated with a multitude of micro-organisms, and the whole complex, plant or animal plus all its micro-organisms, forms a sort of superorganism (a term invented by Wilson and Sober, 1989) which is itself a unit of selection in evolution. According to hologenome theory, evolution is driven by the co-operation between microbial symbionts and their hosts [A host and all its symbiotic microbiota together are termed a holobiont in this theory (Margulis, 1993; Rohwer *et al*., 2002), and the sum of all the information codified in a host and all its microbiota is the hologenome.] The idea that groups and communities are organisms in much the same way as are individuals, and that a functional organisation rather than a gene is the ultimate unit of

selection, is borne out by the biological meaning that has been given to the many bacterial social activities (such as fractal growth in a stressful environment, biofilm formation, swarming motility and even virulence) that are regulated by cell-to-cell signals in a cell density-dependent manner known as quorum sensing (Diggle *et al*., 2007, 2010).

In this context, associations between plants and micro-organisms in nature are the rule rather than the exception, and no ecosystem consisting of only one species has yet been found. Pathologists well know that each plant in its natural or agricultural setting is a complex community, being always colonised by a diversity of microbes, both on its surface and inside. It is therefore legitimate to say that any plant is a more or less well ordered assemblage of plant cells and micro-organisms, the plant cells forming different tissues and organs, and harbouring a number of micro-organisms of certain types.

Anton de Bary (1879), the father of plant pathology, adopted the terms endophyte and mutualistic symbiosis to describe lichens, which he showed were associations between algae and fungi. He defined symbiosis as the '*Zusammenleben ungleichnamiger Organismen*' 'the living together of organisms having different names'. Oscar Hertwig later adopted the term symbiosis to denote an association between two organisms of different species.

The symbioses that form in nature can be very different from each other. Depending on whether symbionts live outside or inside the plants they colonise, symbiosis is divided, respectively, into ectosymbiosis and endosymbiosis. Endosymbiosis has, among other things, given rise to the mitochondria and the chloroplasts in plant cells, which underlines the close relationships that existed and still exist between plants and micro-organisms. When we consider not their relative position, but the trophic and logistic relationships that subsist between plants and micro-organisms, we can have *neutralism*, in which neither party derives any benefit or harm; *mutualism*, in which both benefit; *commensalism*, when the symbiosis is indifferent to the host, but advantageous to the micro-organism, which receives nutrients; *inquinilism*, when the micro-organism is only a 'tenant' that receives 'lodging', but again without detriment to the host; or, lastly, *parasitism.*

The relationships between parasites and plants are in some respects controversial. Some plant pathologists view parasites as symbionts that live at the expense of their hosts, but without damaging the hosts, whereas others see them as not only living at the expense of the host, but also as harming it. In that case, however, parasite would be synonymous with pathogen. If a micro-organism was only to live at the expense of a plant, receiving useful food and lodging and perhaps other benefits, but not harming the plant, it would be merely a commensalist, or a tenant, and in that case there would be no need for the word parasite. If the term parasite is to be retained, therefore, we must distinguish it from commensal-tenant by defining it as a symbiont that not only benefits from its relation with the plant, but also harms the plant. The harm caused to the plant may be slight, as when it diverts a certain (but usually negligible) amount of nutrients, in which it resembles the commensal symbiont. The harm may range, however, to the point where serious damage is caused, and which turns into disease when the normal physiological functioning of the plant is impaired. In that case the parasite is raised to the rank of a true pathogen. A pathogen is a parasite that harms its host to an extent where the plant is evidently diseased (see also Hentschel *et al*., 2000). On the other hand, a pathogen that has lost all its factors of virulence and is no longer able to harm its host can also be down-classed to the rank of parasite.

The dividing line between parasite and pathogen is thus rather hazy, and the two terms are often used interchangeably. To eliminate this overlap, the terms could be distinguished by defining a parasite as *a micro-organism that lives at the expense of its host but does not harm it*, or at least does not cause disease, whereas a pathogen, as has already been mentioned, is a symbiotic micro-organism that causes a disease in its host. When a parasite in a host-parasite relationship is non-pathogenic to its host, there is no virulence, and no disease, and none of the host's typical reactions to a disease (symptoms). The parasite has no factors of virulence, or if it has them is unable under normal circumstances to deploy the processes that bring these factors to bear. Nevertheless, the interaction between plants and all these symbionts is not static but is a dynamic process, in that it is subject to change over time. A commensal or tenant symbiont, for example, can become a parasite when it moves

to a host it does not normally infect. A parasite may cause significant visible harm in a weakened host (*opportunism*) or following changes in host ecology. If this is true, then the disease process may be divided into at least two parts: one that extends from the first contact made by the symbiont with the host to the establishment of the symbiont in the host; and the second that extend from the establishment of the symbiont in the host to the appearance of symptoms. Between these two there could be a time period (hours or longer to months or even years).

We can therefore suppose that a close relationship has existed between plants (or what would eventually become plants) and micro-organisms from the very beginning of evolution, and that this relationship thereafter became an important component of evolution. Plants and micro-organisms have learned to live together, for better or for worse. But how can there be such a continual coming and going of micro-organisms, as there is for instance in cyclical symbiosis, where a new generation of the same partners has to renew the association at every turn of the cycle? Surely there is a need here for an exchange of chemical signals to enable micro-organisms to recognise or deceive their hosts. Control mechanisms are also requisite, whether genomic or metabolic, to enable the symbiotic relationship to function. The idea is steadily gaining ground that '*all known species of animals and plants are infected with symbiont micro-organisms, whose effect on the host ranges all the way from beneficial to harmful*' (Sachs *et al*., 2011), and that the mechanisms which these symbionts employ to invade and colonise their hosts are similar between symbionts. This idea is not new: Flor (1971) wrote that 'parasitism is an antagonistic rather than a mutualistic form of symbiosis'. Though the data are still few, we can therefore say that before a micro-organism can initiate establishment in its host, and irrespective of the type of relationship that it will eventually establish within the host, it is first necessary for the host to become infected. The act of infection, among other things, requires overcoming the active and passive defensive barriers deployed by the host plant. It is only after infection has occurred (though it may be immediately afterwards) that all those factors that cause damage to plants, be they few or many, can become active. It is these harmful factors that distinguish the parasite, which does not have them, from the pathogen, which does.

The immune system of plants

Micro-organisms have thus contributed to the evolution of plants and are continuing to do so. Plants, for their part, have evolved to provide at least 'room and board' to many micro-organisms, enabling them to fulfil their functions and to perpetuate their DNA. Inevitably or by pure chance, however, some of these micro-organisms then began to impose a burden on their host plants by interfering with their normal physiological functions, since the micro-organisms were also evolving. And the plants obviously had to respond to this interference if they did not want to see their own vigour, or even survival, compromised.

In an environment containing many micro-organisms, some of which were at least potentially harmful, plants first tried to safeguard their survival by relying on some preformed and strong physical and chemical barriers, their so-called passive defence. This significantly reduced the number of micro-organisms that managed to invade and harm the plant. To protect themselves against the relatively few micro-organisms that still succeeded in overcoming these outer barriers set up against them, plants further evolved an effective system of micro-organism recognition, consisting of a fast and efficient response to deal with them, the so-called active defence. All these means of active defence constitute the immune system of plants, which is effective against all manner of potential invaders, including bacteria, fungi, oomycetes, viruses, nematodes and insects.

Plant immune systems essentially have two levels of active defence (the zigzag model introduced by Jones and Dangle, 2006). In the first level, the plant recognises conserved pathogen-associated molecular patterns (PAMPs, once known in a general sense as elicitors), which are evolutionarily stable. Recognition at this level elicits a 'general defence response', which is weaker than the second level of active defence, discussed below. This first level of recognition provides a type of basal immunity, PAMP-triggered immunity (PTI), or, since these immunity-triggering molecules also occur in non-pathogenic micro-organisms, microbe-triggered immunity (MTI) (Boller and Felix, 2009). This level of recognition was probably the first to evolve, since it responded to all micro-organisms, harmful or not, that confronted the plant. PAMPs include molecules such as flagellin, pepti-

doglycan, lipopolysaccharide, the elongation factor TU, fungal chitin, and oomycete glucans (Ayers *et al*., 1976; Felix *et al*., 1993, 1999; Dow *et al*., 2000; Gust *et al*., 2007; Erbs *et al*., 2008). PAMPS are perceived by transmembrane pattern recognition receptors (PRRs) located on the surfaces of the plant cells.

It has been suggested that in non-pathogenic (mutualistic or commensal) interactions, the determinants of basal immunity should be termed 'symbiont-associated molecular pattern molecules' (SAMPs) (Hirsch, 2004).The first or basal level of immunity is thus directed against all those micro-organisms that overcome the passive defences and succeed in entering the plant.

The second level of immunity is termed effector-triggered immunity (ETI), and evolved in plants to enable them to block those micro-organisms that had 'learned' to overcome the first level of immunity. With this second level of defence, the plant recognises microbial effector proteins, e.g. Type III effectors (T3Es) (formerly generically known as avirulence factors), either directly (as in the case of T3E, PopP2 and the R protein RRS1-R of *Ralstonia solanacearum*: Deslandes *et al*., 2003) or more often indirectly (the 'guard hypothesis': Van der Biezen and Jones,1998; Dangl and Jones, 2001), when these Type III effector proteins are injected into host cells by a syringe-like apparatus known as the type III secretion system (T3SS), which is codified in bacteria by their hypersensitive responses and pathogenicity/conserved (*hrp/hrc*) genes.

The *hrp* genes are so-called because any mutations in them abolish the bacterial elicitation of hypersensitive responses (HR) in resistant host and non-host plants, and generally abolish pathogenesis in susceptible host plants (Lindgren *et al*., 1986). The *hrc* genes are so-called because some of them are highly conserved.

Effector-triggered immunity is thus a recognition process at a more evolved and effective level, achieved by resistance (*R)* proteins in host plants. This immunity occurs when plant recognise and protect themselves against specific microbial species or populations. T3Es are perceived by nucleotide-binding, leucine-rich repeat (NB-LRR) proteins in the cytoplasm of host cells. ETI initiates rapid and localised programmed cell death, known as hypersensitive response (HR).

Let us now look at the immunity response from the point of view of the micro-organism. Why did pathogenic micro-organisms develop capacity to produce effector proteins? The most likely answer is that, at a certain stage in evolution, pathogens attempting to invade plants had to overcome the first level of immunity in the prospective host, and did so by developing effector proteins. The first purpose of effector proteins was to overcome this innate basic resistance of plants. And how did the hosts respond to this newly evolved capacity of micro-organisms? They evolved resistance genes (*R* genes), whose products were able, directly or indirectly, to recognise the effector proteins of bacteria and render them innocuous. Plant breeders have exploited ETI to transform susceptible hosts into resistant ones. Genes that codify resistance proteins have been inserted into genome of susceptible hosts, that recognise pathogen T3Es and neutralise them. What will be (or has already been) the next step in this evolutionary process? Obviously micro-organisms will have to avoid or circumvent the plant's ETI by shedding or diversifying the effector gene that is now being recognised by the ETI, or by acquiring additional effector genes that suppress the ETI. For the host, the next step will be to evolve new R genes able to recognise these new defence response elicitors in the micro-organism and to cope with them (Jones and Dangle, 2006).

What has been outlined here is obviously only a general model, which can apply to many, but not all, situations. For instance, there are groups of effectors, e.g. the LysM effectors, that are so widespread that they can be qualified as PAMPs (Thomma *et al*., 2011). On the other hand, some PAMPs are only very narrowly conserved (Brunner *et al*., 2002; Lee *et al*., 2010).

Susceptibility

Plants can potentially be infected with very many pathogenic micro-organisms, but normally each plant species is only infected by a small cohort of pathogens. This is because, firstly, the passive plant defence, and its PTI and ETI, protect against most of the potential pathogens of the host species or varie-

ties. If such safeguards exist to protect plants, why are they nevertheless infected by pathogenic micro-organisms? This is because some pathogens manage to overcome the passive plant defences, and, at the same time or immediately afterwards, overcome active defences by neutralising or circumventing both the first and the second level of the host immune system. It is only when this happens that a plant becomes infected.

This tactic of micro-organisms has been documented for many T3Es, and is referred to as effector-triggered susceptibility (ETS) in host plants (Espinosa and Alfano, 2004; Grant *et al.*, 2006; Jones and Dangle, 2006), as opposed to ETI. T3E proteins thus have two opposite effects, which produce two opposite responses in plants: firstly they activate immunity, i.e. prevent disease, and secondly they create susceptibility, i.e. permit disease.

How can a plant be made susceptible by a pathogen when it has a functioning immune system? In simple terms, plants can be made susceptible in two ways:

1. if they do not possess the cognate R genes, the micro-organism uses its effectors to overcome the first level of immunity, enter the plant, multiply, and cause disease;
2. if plants possess the cognate R-genes, micro-organisms use their effectors to overcome the first level of immunity, and then use the same or different effectors to overcome the second level as well, and in this way cause disease.

If we consider a pathogen X, which is attempting to invade a non-host plant, there are three main outcomes:

1. the pathogen does not overcome the passive defence of the plant;
2. it overcomes the passive defence, but is then blocked by the first level of immunity;
3. the pathogen overcomes the passive defence and the first level of immunity, but is then blocked by the second level of immunity. (In this case it is presumed that all the populations of the host species have R genes.)

If we consider a pathogen X attempting to invade a host plant, there is only one outcome:

1. the pathogen overcomes the passive defence, overcomes or circumvents the first level of immunity, and, if it exists, the second level of immunity; it then invades the plant, eventually causing disease symptoms.

In this scenario it is assumed that the plant possesses not only passive defence, but also at least the first level of immunity.

It is further assumed that:

1. some effectors are particularly effective at overcoming the first level of immunity;
2. some effectors are particularly effective at overcoming the second level of immunity in plants with R genes; and
3. some effectors are particularly effective at overcoming both the first and second levels of immunity (Guo *et al.*, 2009).

When effectors act to overcome the immunity system they are considered as virulence factors, also because they may harm the host cells directly, and/or favour multiplication of the pathogen. And yet as has been said these effectors may also be recognised by the PRRs and/or by the NB-LRR proteins in the plant, eliciting PTI and/or ETI. When the effectors act in this way they act as avirulence factors, since they then render the micro-organism avirulent (or in other words, **non-pathogenic**).

Type III effector proteins

At least 57 families of effectors, with each bacterial strain expressing about 15–30 effectors, have been identified in the bacterial pathogen *Pseudomonas syringae* alone (Lindeberg *et al.*, 2012). Effectors are produced by all the major species of pathogenic bacteria infecting plants and animals, and also by fungi, oomycetes and nematodes. Effectors of *P. syringae* and other phytopathogenic bacteria are generally designated as Hop proteins (Hrp outer proteins, i.e. proteins that have the capacity to travel through the T3S system), though several still have their original designation 'Avr' protein, which was attributed to them by their avirulence phenotype on resistant cultivars of various crops (Lindeberg *et al.*, 2012). Avr proteins are the products of *Avr* genes that match the corresponding products of the R genes in the host, making that host resistant to the pathogens that possess those *Avr* genes (gene-for-gene hypothesis). In that case pathogens possessing those *Avr* genes were by definition pathogens blocked when they tried to enter the plant, even though they retained their diversity of virulence factors. In other words, they

were avirulent not because they had lost those factors that were, and still are, considered virulence factors (toxins, hormones, or enzymes and the like), but because their phenotypes had been avirulent on resistant cultivars.

Which of those dozens of molecules identified as PAMPs or effectors are factors of pathogenicity, and which are factors of virulence/avirulence in accordance with the definitions of pathogenicity, virulence and avirulence given above? This is not a trivial question. As mentioned at the beginning of this paper, some authors writing on the immune systems of plants, effector proteins and PAMPs, do not always use the terms pathogenicity, virulence and avirulence appropriately, and at the same time they do not trouble to give a clear new definitions of these phenomena. Currently, the substances (effectors) that the T3SS secretes into the host plant cells are taken to be either virulence or avirulence factors, or sometimes also pathogenicity factors.

Some examples illustrate this point. Bocsanczy *et al*. (2012) state that in *Erwinia amylovora* the two most important types of *pathogenicity factors* that have been described are the T3SS and its associated secreted proteins (Type III effectors) and the exopolysaccharides (EPSs) amylovoran and levan. T3SS is a pathogenicity factor because 'if any gene comprising the secretion apparatus or its regulation is disrupted, the disease-causing ability of the bacterium is reduced or eliminated'. The EPSs are pathogenicity factors because these molecules are 'related to the ability of the pathogen to colonize and grow competitively in the environment, where the EPSs presumably protect bacteria from oxidizing agents' (the cited reference is to Geider, 2000). The T3SS is also essential for the pathogenicity of *Xanthomonas*, but here the type III effectors are called 'important virulence factors' (Büttner *et al*., 2003; Tampakaki *et al*., 2004; Gürlebeck *et al*., 2005; Kay and Bonas, 2009). In Jones and Dangle (2006), effector molecules are likewise primarily termed avirulence factors, but they are also factors that contribute to pathogen virulence. In Lindeberg *et al*. (2006) the term effector denotes a virulence-related protein delivered into the host cell by a pathogen, but these authors (Lindeberg *et al*., 2012), in their review "*Pseudomonas syringae* type III effector repertoires: last words in endless arguments", also state that the 'cytoplasmic effector repertoires of the bacteria, fungi and oomycetes that attack plants … is composed of proteins that are collectively essential but individually dispensable for pathogenesis'. Baltrus *et al*. (2012), in a paper entitled 'Dynamic evolution of pathogenicity revealed by sequencing and comparative genomics of 19 *Pseudomonas syringae* isolates', state that the Type III secretion system is a key virulence determinant, but also that Type III effector proteins are essential for pathogenicity because once inside the plant they promote pathogenesis by disrupting and suppressing the host defence response at multiple levels. They also state that the 'pathogenesis of *P. syringae* on any given plant species results from both the absence of avirulence factors and the presence of multiple virulence factors acting coordinately to promote disease and to suppress [the] host immune response'. A final example is from a review by O'Brien *et al.* (2011) entitled 'Evolution of plant pathogenesis in *Pseudomonas syringae*: a genomics perspective', where it is stated that 'the type III secretion system has been identified as one of the key virulence determinants in a number of gram-negative pathogens of both plants and animals, including *P. syringae'*.

It is clear even from the examples given here that there are differences in points of view between the researchers working in the field. This is possibly because these researchers do not uniformly consider the basic plant pathology concepts such as pathogenicity, virulence and avirulence, and because they do not all attach the same values to these concepts.

The T3SS is a structural component of symbiotic pathogens, which provides 'a continuous channel for effector proteins to travel from the bacterial cytoplasm directly into the cytoplasm of eukaryotic cells' (Büttner and He, 2009). Irrespective of whether the effector proteins are thought of as pathogenicity factors or as virulence/avirulence factors, when the T3SS ceases to function in a given phytopathogenic micro-organism, this is recognised by specific receptors in the plant, and disease does not occur. A functioning T3SS is clearly necessary to cause disease, even when all possible exceptions are taken into account. The T3SS is thus primarily a factor/structure necessary for pathogenicity, and hence also inevitably for virulence, since virulence is a property of pathogenicity. But the T3SS and its associated effectors are also found in non-pathogenic symbiotic

micro-organisms. Therefore if we wish to give a broader definition of the T3SS, we cannot restrict the definition to a pathogenic context.

The first bacterial *Avr* gene was cloned in 1984 (Staskawicz *et al*., 1984), the first fungal *Avr* gene in 1991 (van Kan *et al.,* 1991), and the first oomycete *Avr* gene in 2004 (Shan *et al*., 2004). Over the last two decades, numerous novel *Avr* genes have been identified, and this has considerably increased understanding of plant-microbe interactions.

Products of bacterial *avr* genes from pathovars of *P. syringae* and *Xanthomonas campestris* (and also from other microbial pathogens) were initially found to elicit a HR in those plants that carried the corresponding *R* genes (for reviews see Dangl, 1994; Leach and White, 1996; Vivian and Gibbon, 1997). Later, it was discovered that the inactivation of these bacterial *avr* genes could lead to the bacterium becoming virulent to previously resistant host cultivars (Leach and White, 1996). This made it certain that the *avr* genes conferred selective advantage on bacteria, and it was found that the *avr* genes of *Erwinia amylovora* (Bogdanove *et al*., 1998), and a number of *avr* genes of *P. syringae* pathovars and *Xanthomonas* spp., had pathogenicity/virulence functions in compatible interactions, or had roles in bacterial fitness (Leach and White, 1996).

It has been reported that the "parasitic" success of *P. syringae* may depend on the ability of T3Es to suppress PTI, while suppressing or evading detection by ETI (Cunnac *et al*., 2009; Lindeberg *et al*., 2012). For example, the gene *AvrPto* interfers with PAMP perception and *HopM1* interfers cell wall defence-associated vehicle trafficking (Nomura *et al*., 2006; Shan *et al*., 2008; Xiang *et al*., 2008). As an effector, on the other hand, AvrPtoB has various domains: it suppresses PTI, and both elicits and suppresses ETI (Abramovitch *et al*., 2003; Rosebrock *et al*., 2007; Xiao *et al*., 2007). Moreover, $HopXI_{Ea}$ of *Erwinia amylovora* is an avirulence gene in apple shoots and in the non-host *Nicotiana tabacum*, but is a HR suppressor in *N. benthamiana,* also a non-host (Bocsanczy *et al*., 2012). Moreover, overexpression of $HopXI_{Ea}$ in *E. amylovora* strain Ea273, which is highly virulent on apple and pear, reduces disease development in apple shoots. AvrXa7, PthXo1, and other AvrBs3-like proteins from *X. oryzae* pv. *oryzae* support strong bacterial growth and lesion development in rice (Kay and Bonas, 2009). The effectors of the AvrBs3 family so far identified in *Xanthomonas* spp. and *R. solanacearum* act as plant transcriptional activators and directly modify the transcriptome of their hosts by binding to cognate promoter boxes (Kay and Bonas, 2009). In infected pepper plants, the central repeat domain of AvrBs3 binds to a conserved element in the *upa20* promoter and the AvrBs3 activation domain induces *upa20* expression. Upa20 is a bHLH transcription factor and master regulation of cell expansion. Consequently, AvrBs3-mediated induction results in hypertrophy of the mesophyll tissue (Marois *et al*., 2002; Kay *et al*., 2007; Lewis et al., 2009). AvrB2, HopAB1, HopI1, HopR1, HopAS1 and HopR1 from *P. syringae* pv. *phaseolicola* 1448a are individually required for wildtype-like growth during the entire course of infection or at its later stages (Macho *et al*., 2012). Numerous T3SEs manipulate plant hormone signalling pathways. AvrRpt2 upregulates auxin levels in *Arabidopsis* and enhances susceptibility to disease (Chen *et al*., 2007). Hop $I1_{Pma}$ES4326 causes remodelling of chloroplast thylakoids and suppresses the accumulation of salicylic acid (Jelenska *et al*., 2010). The hormone abscissic acid, which causes greater drought tolerance and suppresses growth, is upregulated by AvrPtoB; HopAM1 probably manipulates abscissic acid signalling (Torres-Zabala *et al*., 2007). *AvrPphF* from *P. syringae* pv. *phaseolicola* confers either pathogenicity, virulence, or avirulence, depending on the host plant (Rivas *et al*., 2005). A final example is the *awr* gene family from *R. solanacearum* which codifies effectors that display both virulence and avirulence activities (Solé *et al*., 2012).

For pathogenic fungi and oomycetes (for a review see De Wit *et al*., 2009), it has been shown that two effectors from *Hyaloperonospora parasitica,* namely ATR1 and ATR13, suppress the host's PTI when the host interaction is compatible, whereas in resistant *Arabidopsis* accessions they trigger ETI. Moreover, three alleles of ATR13 suppress PAMP-triggered callose deposition on the leaf cell walls of *Arabidopsis*, while $ATR13^{Maks9}$ suppresses PAMP-triggered ROS burst in susceptible host plants. In *Fusarium oxysporum* f.sp. *lycopersici*, which is a pathogen of tomato, the effector Sixl is required for full virulence to be displayed on that host (Rep *et al*., 2004; 2005), but this effector also triggers ETI in the presence of the cognate resistance gene *I-3* (Huang and Lindhout,

1997; Rep *et al.*, 2004). Six3 contributes to virulence, but it also triggers ETI in the presence of the cognate resistance gene *I-2* (Houterman *et al.*, 2009). Six4 suppresses *I-2* and *I-3*-mediated resistance (Houterman *et al.*, 2008). Pep1 is an effector protein from the corn smut fungus *Ustilago maydis* (for this fungus no gene-for-gene interaction has been established), and this protein is essential for the fungus to penetrate host epidermal cells, but it also elicits a strong defence response in the maize host (Doehlemann *et al.*, 2009). Avr2 and Avr4 of *Cladosporium fulvum* inhibit plant cysteine proteases, which are important for host defence (Kruger *et al*., 2002; Rooney *et al.*, 2005; Shabab *et al*., 2008), but they also protect chitin in the fungal cell walls against plant chitinases (Van Den Burgh *et al*., 2003, 2006).

The picture of T3E activity is therefore complicated and diverse. Many pathogen virulence factors are likely to be components of complex systems (Schneider and Collmer, 2010); they possess multiple functional domains and have several additional activities. Nevertheless, the most common definition of effectors is still that they are 'virulence-related proteins delivered into the host cells by pathogens'. However, as Lindeberg *et al*. (2012) rightly pointed out, the term effectors is often used more broadly, to denote other pathogen molecules such as phytotoxins as well. There is thus a tendency to broaden the original meaning of 'effector' and to make it cover all, or almost all the substances involved in pathogenicity/virulence. Another definition of 'effector' was provided by Hogenhout *et al.* (2009), and was accepted by Schneider and Collmer (2012) in one of their latest reviews on the subject. According to Hogenhout *et al.* (2009), effectors are 'all pathogen proteins and small molecules that alter host cell structure and function'. Schneider and Collmer (2012) added to this that 'these alterations either facilitate infection (virulence factors and toxins) or trigger defence responses (avirulence factors and toxins), or both, and that 'This broader definition of effectors includes many molecules, such as pathogen-associated molecular patterns (PAMPs), toxins, and degradative enzymes'.

It is therefore proposed to change the definition of effector. This term should have broad meaning, denoting all those substances that in any way, directly or indirectly, individually or in competition with each other, have roles in ensuring the normal functioning of the host cells or in safeguarding their structural integrity.

The definition of effector given by Hogenhout *et al.* (2009) was a laudable and welcome attempt to put some order into a terminology that currently is only creating confusion. This has occurred as the activity of molecules released by the T3S gradually becomes better known, as more effector molecules and effector-functions are discovered, and as advances are made in understanding of the mechanisms of disease, and of how host plants defend themselves against disease. Knowledge of the role of effectors is certainly not yet complete, and much work still remains to be done. Of this Schneider and Collmer (2012) were well aware when they pointed out that 'in the absence of more information, it would be suitable to call these molecules effectors until the exact activities of the pathogen molecules are revealed, after which they may be renamed to reflect their specific activities'.

But the problem is not merely one of delimiting the concept of effector in general, but also to assign to each effector its own specific activity, in accordance with shared and unique concepts of pathogenicity, virulence and avirulence. It is then necessary, and it must surely be possible, to bring greater clarity into the terminology of plant pathology.

Proposals

Every plant in a natural or agricultural setting is colonised by a multitude of microbes both on the plant surface and within. Microbes living inside plants include mutualistic, commensal, parasitic and pathogenic symbionts, latent pathogens, latent saprotrophs, and saprotrophs.

Regardless of the relationships that the micro-organisms eventually establish with their hosts, all of these micro-organisms, with inevitably a few exceptions or special situations, must go through all the preliminary steps to become established in their hosts: they must make contact with the host, enter it, colonise its inner tissues and derive nutrients from it. Since micro-organisms perform all these tasks in different ways, one suggestion is to classify all those factors that enable these tasks to be performed

(enabling micro-organism contact, enabling micro-organism entry into the host, and enabling them to derive nutrients from it) ***symbiosis factors***, or ***infection factors***. Alternatively, they could be named after the particular type of relation they establish with their hosts: mutualistic factors, commensal factors, parasitic factors, pathogenic factors, always bearing in mind that, while all these micro-organisms vary greatly in the relationships they have with their hosts, the target of their activity is always the same.

The main difference between pathogenic micro-organisms and all other micro-organisms that live in plants is that pathogens harm their hosts. Since the damage they cause is indicated by the symptoms that appear, and since these symptoms indicate the extent of the disease, those microbial factors that cause the symptoms should be called ***disease factors/disease determinants***.

Infection and disease factors will be synthesised by three groups of genes in pathogens:

1. symbiosis genes, which codify factors that promote the process of infection from the first contact of the micro-organism with the plant to the full colonisation of the host, passing through the intermediate stages of invasion and of finding a source of nutrients;
2. true virulence genes, which codify factors (toxins, hormones, EPSs, degradative enzymes, and other compound) that interact with the host, and that damage the host directly in the process of infection;
3. virulence-associated genes, which codify those factors that are involved in the deployment (regulation, secretion, processing) of the products of the true virulence genes.

The effectors can likewise be divided into at least three groups:

1 factors whose primary function is to translocate true effectors through host barriers (helper proteins or translocators);
2. factors which elicit resistance responses in incompatible interactions, or that suppress host immunity (PTI and/or ETI) in compatible interactions (these factors are now known as avirulence factors); and
3. factors which enable the micro-organism to complete the disease cycle once it overcomes the immune system of the host, and which directly cause, or assist in causing, the onset of symptoms. To this last group belong the effector proteins, and also molecules that are not transported through the plant by the T3SS.

Of these effectors, those in the first group can continue to be defined as helper proteins or translocators; those in the second group, are non-pathogenic factors if the interaction is incompatible, and symbiont/infection factors if it is compatible; and those in the third group are the true disease factors.

With this new terminology, AvrPto, for example, would be an *effector protein* acting as a non-pathogenic (formerly, avirulence) factor when it elicits a resistance (non-disease) response (Pedley and Martin, 2003). It would be a symbiosis/infection factor whenever it occurs in a situation in which it suppresses innate immunity and enables the micro-organism to enter the plant and spread through it (Hauck *et al.*, 2003; Kang *et al.*, 2004). AvrPto would be a disease/virulence determinant when it contributes to symptom appearance (Chang *et al.*, 2000; Shan *et al.*, 2000; Alfano and Collmer, 2004).

The original view of avirulence genes as 'genes that codify factors that can be recognised by specific genotypes of the host species that contain the corresponding resistant genes' has now been superseded. It is therefore suggested that avirulence and similar genes should be called *effector genes*, since they codify the production of disease-related proteins (effectors) that are then delivered to the host cells by the pathogens. This is particularly pertinent because of the discovery that the products of these genes can have more than one function (an effector protein is not merely a virulence-related protein delivered into a host cell by a pathogen, nor is it merely a suppressor or modulator of PTI or ETI).

In conclusion, it is important to remember that a disease is a complex phenomenon that is relatively rare in nature. Furthermore, the pathogenic micro-organisms that we know today are the result of spontaneous or induced mutations, of the acquisition of genetic material from other micro-organisms (and even from higher organisms) and of genetic rearrangements and mingling, which continually create new functions. These have to be explained, and ex-

planatory schemes of the biological phenomena to which they are related have to be developed. These schemes are not static but dynamic: as research opens up new insights, they must be revised and completed accordingly. It is also true that the facts that have been mentioned in this paper are a considerable simplification of reality, since micro-organisms represent a vast field of research with innumerable variations in all the plant-pathogen combinations that are found. This paper, with its limits and its peculiarities, is not presented with the ambition to rewrite Plant Pathology dogma, or to discuss the latest details of the mechanisms of the interactions between plants and their microbial enemies. The aim here has been to call the attention of plant pathologists to the opportunities provided by a standard nomenclature of key plant pathology terms.

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I have aimed in this paper to address current issues relevant to knowledge transfer in modern plant pathology, and to promote thought and discussion on how these may be addressed. My ultimate goal has been to assist the communication of concepts that are relevant in our research discipline. I sincerely thank the numerous colleagues who have reviewed drafts of the paper, and provided valuable (sometimes sceptical) feedback, which has assisted presentation of the ideas I have outlined.

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