Chapter from the book *Pesticides in the Modern World - Pesticides Use and Management*

The Millardetian Conjunction in the Modern World

Marie-Pierre Rivière\textsuperscript{1, 2, 3}, Michel Ponchet\textsuperscript{1, 2, 3} and Eric Galiana\textsuperscript{1, 2, 3}

\textsuperscript{1}INRA (Institut National de la Recherche Agronomique), Unité Mixte de Recherche 1301 Interactions Biotiques et Santé Végétale, F-06903 Sophia Antipolis, France

\textsuperscript{2}CNRS (Centre National de la Recherche Scientifique), Unité Mixte de Recherche 6243 Interactions Biotiques et Santé Végétale, F-06903 Sophia Antipolis, France

\textsuperscript{3}Université de Nice-Sophia Antipolis, Unité Mixte de Recherche Interactions Biotiques et Santé Végétale, F-06903 Sophia Antipolis, France

1. Introduction

This chapter deals with the review of literature related to the impact of study of biotic interactions on the development of modern methods to control plant diseases. Only diseases caused by fungi and oomycetes, the two major phylogenetic groups of microbial eukaryotic plant pathogens, were considered. To fight these pathogens, the chemical treatment with fungicides is a long-established method and the most usually used still today. The first report of effective chemical control was related to the use of a fungicide. Its discovery results from the conjunction between a double need, that to protect the vineyards from robbers and downy mildew disease, and a gift for observation which led Millardet to a fertile conclusion for vine protection, but also, for the rise of the chemical treatments of crops. Millardet initially observed that the rows of vineyard, in border of road, treated with an aqueous mixture of copper (II) sulphate to protect against the disease and of lime to dissuade the grape thieves, were preserved from mildew. After experimentations he elaborated a treatment based on a combination of these chemicals now known as the Bordeaux mixture which became the first fungicide (Millardet, 1885; Rapilly, 2001). The mixture is nowadays still used, but it was widely supplanted by synthetic fungicides from various chemical natures (carbamates, triazol, amines, amides, quinines, phenol and benzene derivatives, etc....). Today large quantities of fungicides are applied each year to crops and seeds in the agriculture sector. For example, a mean of 40 000 tons of industrial fungicides are now used each year in France (Aubertot et al., 2005).

Until the 1980s, the productivist and intensive injunction allowed to nourish the vast majority of the human populations in the developed countries. Because of their low cost and their efficiency, fungicides were used in most countries without restrictions to maximize yield profitably and protected crops. From a phytopathological point of view, plants were mainly looked like simple receptacles, both for the pathogens and the fungicidic molecules. Regarded as a nutritive soup for the first ones and as a simple excipient for the second ones, the protected crop plants laid their fruits with abundance. These last decades, the ecological
imperativeness succeeded the productivist one. This has contributed to impose a radically different view of plants in Science and in Agriculture. Host plants are now self-defending organisms, endowed of an innate immune system, and able to develop various strategies against infections, from the burned ground to the targeted striking. In the same way, substantial knowledge has been gained on the biology of plant pathogens, the epidemiology of diseases and the co-evolution between a host plant and a pathogen. This knowledge constitutes a remarkable sink for genetic and ecological innovations in plant protection. Such alternatives to chemical control have become imperative.

The use of fungicides as well as of the other pesticides (insecticides, herbicides, rodenticides) is now questioned. Their efficiency to control plant pests is counterbalanced by their undesirable and various effects on human health, on sustainability of ecosystems and on biodiversity. There is also the problem of the rapid adaptation of plant pathogenic populations in response to systematic use of pesticide molecules. Within the sustainable development framework, countries and international organizations have a stated political aim of reducing use of pesticides. In France, the Ecophyto 2018 plan constitutes the engagement of the recipients to reduce by 50% the use of the pesticides at the national level within a deadline of ten years, if possible (http://agriculture.gouv.fr/IMG/pdf/PLAN_ECOPHYTO_2018.pdf, 2008). Several fungicides have already been judged like harmful substances which can cause acute or chronic toxicity. In some cases the marketing authorizations of the preparations containing alarming active substances are withdrawn; their distribution and their use are prohibited. In the European Union the directive 2009/128/EC establishes a framework for community action to achieve the sustainable use of pesticides (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri =CONSLEG:2009L0128:20091125:EN:PDF, 2009). The pesticide program helps government of the Organisation for Economic Co-operation and Development to reduce the risks associated with pesticide use, through a variety of actions to supplement pesticide registration and further reduce the risks that may result even when registered pesticides are properly used (http://www.oecd.org/department/0,3355,en_2649_34383_1_1_1_1_1,00.html). In the same time, the main challenge in agriculture is to increase crop yields for feeding seven billion individuals today and about nine billion on the horizon 2050 (http://km.fao.org/fileadmin/user_upload/fsn/docs/ SUMMARY_2050.pdf, 2009). The impact of the absence of fungicidal protection in plant diseases may reduce crop quality and quantity. The limitation of the fungicide use beyond the optimisation may be harmful for some crops (Butault et al., 2010) (http://www.inra.fr/l_institut/etudes/ecophyto_r_d/ecophyto_r_d_resultats). The development of integrated pest management, linking all appropriate options including, but not limited to, the judicious use of pesticides, as well as the development of organic food production, limiting the use of pesticides to those that are produced from natural sources, require to prospect new biological resources for plant protection.

The necessity to reduce pesticide use while maintaining high crop yields is today the double need of what we name in this review the millardetian conjunction. What is today the substrate(s), the scientific fields from which could emerge a seminal(s) observation(s) that would supplement the conjunction? Of course it is advisable to say at once how much is hard to anticipate that today. This chapter is focused on the control of plant diseases caused by fungi and oomycetes (Figure 1). Fungi and oomycetes are today mainly and effectively controlled by fungicide applications. We review our knowledge in three domains that we
consider as potentially fruitful for such emergence and for rupture in phytoprotection. In the context of studies on plants-pathogens interactions, we underline in the section 2 how this knowledge may help to reduce fungicide use. We also highlight in the section 3 how rapidly expanding investigations on interactions between cells of a pathogen, and between a pathogen and microbial species living in the same biotope may promote environmental friendly innovations in plant protection.

![Fig. 1. Examples of eukaryotic pathogens and plant symptoms](image)

(A) Sporulation of the ascomycete *Botrytis cinerea* on a kalanchoe stem. (B) Oat crown rust pustules (basidiomycete) on an oat leaf. (C) Sporangium and mycelium of a polyphagous oomycete, *Phytophthora parasitica*.

### 2. The plant-pathogen interaction

Beside constitutive physical and chemical barriers preventing infection, plants use their innate responses to ward off pathogens. Plants have evolved the ability to detect microbes through the recognition of conserved microbial leitmotivs which are referred to as Pathogen- or Microbe-Associated Molecular Patterns (PAMPs or MAMPs). The molecular responses are mediated by Pattern-Recognition Receptors (PRRs), a class of innate immune response-expressed proteins that respond to PAMPs. This recognition level initiates MAP kinase signaling and PAMP-triggered immunity (PTI), a key aspect of plant innate immunity which contributes to prevent microbial growth (Nurnberger et al., 2004). Pathogens may suppress PTI responses by secreting effectors in the apoplast or directly into the cytoplasm of host cells, leading to effector-triggered susceptibility (Gohre and Robatzek, 2008). Through evolution and by the driving force of natural selection, plant R gene function has emerged resulting in direct or indirect recognition of specific effectors by R proteins. This second level of microbial recognition, specific to certain races or strains of a pathogen, leads to effector-triggered immunity (ETI). ETI is associated in the host with a local programmed cell death, a response which is referred to as the hypersensitive response (HR), and with the establishment in the whole plant of systemic acquired resistance (SAR) which is long lasting and effective against a broad spectrum of pathogens (Chisholm et al., 2006; Dodds and Rathjen, 2010; Jones and Dangl, 2006; Zipfel, 2009).

#### 2.1 Recognition by plants of molecular signatures from pathogens

One way to prevent crop diseases, and in the same time to reduce frequency of chemical treatments, is to enhance the ability of plants to stimulate their own innate immune system. Understanding how plant receptors recognize molecular signatures from pathogens is important to approach such a goal. Over the past 20 years many R genes
have been discovered and evaluated to engineer disease resistance in crop (Hammond-Kosack and Parker, 2003). On the other hand, only a few plant PRRs have been identified up to now, and our knowledge of the molecular mechanisms underlying PTI is limited. Nevertheless, new agricultural applications could ensue from recent studies on pattern-recognition receptors. A PRR gene from the cruciferous plant *Arabidopsis thaliana*, occurring only in the Brassicaceae family, was transferred into two plants, *Nicotiana benthamiana* and *Solanum lycopersicum*, in order to determine if adding new recognition receptors to the host arsenal would lead to better resistance (Lacombe et al., 2010). This EFR gene encodes a surface-exposed leucine-rich repeat receptor kinase EFR, and mediates recognition of the bacterial pathogen-associated molecular patterns EF-Tu (elongation factor Tu). It was chosen by the authors because the high level of conservation of EF-Tu protein sequences across bacteria offered the possibility that EFR could confer resistance against a wide range of bacterial pathogens. Based on triggering of an oxidative burst and on induction of defense-marker genes, expression of EFR in *N. benthamiana* and *S. lycopersicum* transgenic plants was found to confer responsiveness to bacterial elongation factor Tu. The heterologous expression of EFR makes also transgenic lines more resistant to a range of phytopathogenic bacteria from different genera (*Pseudomonas*, *Agrobacterium*, *Ralstonia*, *Xanthomonas*). These results were obtained with host plants and pathogens growing in controlled laboratory conditions. Nevertheless, they constitute a first step for the evaluation of the deployment of new PAMP-recognition specificities in crop species. This strategy could be used to engineer pathogen broad-spectrum resistance in crop plants, potentially enabling more durable and sustainable resistance in the field (Dodds and Rathjen, 2010; Gust et al., 2010; McDowell and Stacey, 2008).

### 2.2 Exogenous application of natural compounds stimulating plant defense responses

As mentioned above, one of the main change in the philosophy of plant disease management has been these last twenty years to abandon the systematic use of biocide treatments against pathogens for alternate solutions among which the bio-activation of plant innate immune system. In some cases it has become reasonable to prevent crop diseases by exogenous application of natural compounds used as elicitors of immune defence responses or of systemic acquired resistance (Vallad and Goodman, 2004). This constitutes a potential alternative or a complement to the intensive use of chemical fungicides with the view to reduce their negative effects on environment and human health. Conventional fungicides are metabolic inhibitors (of electron transport chain, of enzymes, of sterol synthesis of nucleic acid metabolism or protein synthesis) while in contrast elicitors have no direct effect on pathogens. Most of elicitors are natural compounds extracted from microorganisms, algae, and crustacean. Due to their biodegradability and to the low doses applied, the risk of environmental contamination by residues appears weak. Also, they don’t show, a priori, a profile to present dangers to human health (Lyon et al., 1995). They appear particularly attractive in the case of integrated production and are evaluated in the frame of the organic farming which lacks anti-fungus substances.

The screening for such natural compounds has led to the characterization of some active molecules now used in the field as a supplement to classic fungicidal treatments. Laminarin, a beta-1→3 glucan, derived from the blue green algae, *Laminaria digitata*, elicits defense
responses and resistance to disease in different plants (Aziz et al., 2003; Joubert et al., 1998). Several countries have approved its use particularly on diseases of wheat and barley. Chitosan, another polysaccharide (a deacetylated derivative of chitin, beta-1,4-linked glucosamine) has also been approved by the food and drug administration of the USA first as a wheat seed treatment (El Ghaoouth et al., 1994; Hadwiger, 1995). Because of its properties to activate various plant defense responses (phenyl ammonia lyase and peroxidase activities, phytoalexins synthesis, cell wall lignifications) and to trigger resistance, it is considered as an interesting alternative for enhancing natural resistance against Botrytis cinerea and other pathogens (Aziz et al., 2006; Povero et al., 2011). Harpin is a proteinaceous stimulator of plant defenses, produced by the plant pathogenic bacterium, Erwinia amylovora. When applied to plant surfaces by conventional means, harpin may elicit resistance to pathogens and insects and also enhances plant growth (Wei and Beer, 1996; Wei et al., 1992). Its use is approved in United States on a series of diseases for a wide range of plants: cotton, citrus, wheat, tomatoes, cucumbers, rice, strawberries, peppers, tobacco.

While these elicitors interfere or are suspected to interfere with the early step of recognition by plants of microbial molecular signatures, downstream events of defense signaling pathways have also been subjected to molecular dissection as well as technological evaluation for improving plant resistance to diseases. Two molecular entities have been particularly studied: the NPR (for Nonexpressor of PR genes) gene family and the salicylic acid (SA), two key positive regulators of systemic acquired resistance (Cao et al., 1994; Vernooij et al., 1994). Salicylic acid has been identified by several lines of evidence as a positive component playing an essential role in the SAR transduction pathway. SA levels are elevated at the onset of SAR in cucumber (Metraux et al., 1990; Rasmussen et al., 1991), tobacco (Malamy et al., 1990), and Arabidopsis (Uknes et al., 1993). The exogenous application of SA to leaves of tobacco or Arabidopsis induces resistance against the same spectrum of pathogens and activates the same set of SAR genes, as with pathogen-induced SAR (Ward et al., 1991). Transgenic plants expressing a bacterially derived gene that encodes salicylate hydroxylase (nahG), an enzyme that converts SA to catechol, are unable to induce SAR (Delaney et al., 1994; Gaffney et al., 1993). The observation that treatment of plants by exogenous SA induces resistance to viral, bacterial and fungal, particularly biotrophic, pathogens has led to application of SA-induced defense responses in plant protection. A SA derivative, the BTH, benzo(1,2,3)thiadiazole-7-carbothioic acid Smethyl ester, is mainly used. BTH activates the same set of defense genes and induce similar wide spectrum resistance with lower phytotoxic effect than SA (Gorlach et al., 1996; Lawton et al., 1996). BTH treatment protects against a broad spectrum of pathogens in several fruit, vegetable crops and ornamental plants (Abo-Elyousr et al., 2009; Brisset et al., 2000; Godard et al., 1999; Hukkanen et al., 2007; Iriti et al., 2005; Malolepsza, 2006; Narusaka et al., 1999).

Members of the NPR gene family are also key positive regulators of systemic acquired resistance (Cao et al., 1994; Tada et al., 2008). Genetic studies in Arabidopsis have demonstrated that AtNPR1 encodes an ankyrin repeat protein which is involved in SA perception and downstream SAR responses (Cao et al., 1994; Cao et al., 1997; Ryals et al., 1997). Nuclear localization of NPR1 is essential for SA-induced gene expression (Kinkema et al., 2000). Upon pathogen infection accumulation of SA triggers a change in cellular reduction potential, resulting in partial reduction of NPR1 oligomer to monomers, and then in their translocation in the nucleus where they interact with members of the TGA family of basic Leucine zipper transcription factors (Després et al., 2000; Kinkema et al., 2000) that bind to PRI promoter elements. NPR1-mediated DNA binding of TGA factors appears to be
critical for activation of defense genes (Fan and Dong, 2002; Jupin and Chua, 1996; Lebel et al., 1998; Qin et al., 1994) among which PR genes, which encode antimicrobial effectors (Van Loon and Van Strien, 1999). The potential of over-expression of AtNPR1 from Arabidopsis thaliana or of its orthologues in crop species is a current approach for the development of more resistant cultivars. Over-expression of the AtNPR1 gene in citrus and of the MpNPR1 gene in apple increases resistance to citrus canker (Zhang et al., 2010) and to fire blight (Malnoy et al., 2007), respectively. In some cases negative impacts of the NPR1 expression have been observed in transgenic plants. In apple, the overexpression of Malus NPR1 does not create detrimental morphological changes, but side effects of overexpression of NH1 (rice homolog of AtNPR1) have been noted in rice. The NH1 overexpression leads both to constitutive activation of defense genes and developmentally controlled lesion-mimic phenotype (Chern et al., 2005; Fitzgerald et al., 2004). On the other hand, overexpression of AtNPR1 in Arabidopsis not only potentiates resistance to different pathogens, but also enhances plant response to BTH and effectiveness of three Oomycete fungicides: metalaxyl, fosetyl, and Cu(OH)$_2$ (Friedrich et al., 2001). The authors suggest that a combination of transgenic and chemical approaches may lead to effective and durable disease-control strategies.

Despite their great potential for control of diseases, treatments of crops with elicitors are not however considered as the panacea for replacing fungicide application. It can be rather considered as a fungicide supplement when fungicide application may be reduced. Indeed treatments with elicitor provide between 20 and 85% disease control and in several cases their application provides no significant level of resistance. To improve their efficiency in the field, information of the influence of the environment, plant genotype, and crop nutrition on plant responses leading to effective resistance remains required (Walters et al., 2005).

2.3 Disease management and plant developmental resistance

In this section we have paid particular attention on knowledge on plant developmental resistance. An increasing number of studies show that induction of resistance to disease during plant development is widespread in the plant kingdom (see for review Develey-Riviere and Galiana, 2007; Panter and Jones, 2002; Whalen, 2005). The scientific community that has investigated this question has used enough diversified approaches, from genetics to epidemiology, to delineate possible and robust contributions of this field for reducing fungicide uses in crop protection.

2.3.1 A parameter for modeling epidemics and to minimize chemical use

One important exciting and difficult challenge in plant protection is to define epidemiologic state both to ensure high crop yields and to manage chemical treatments. A precise definition of the defense and resistance potential of each host plant throughout its life cycle is a key element for the control of pathogen infection. In the context of the ecological awareness, developmental resistance may be considered as a very important factor in the rationalization of cultural practices, the main statement being to reduce fungicide application to shorter periods of high host susceptibility. To achieve this, at least two time parameters have to be properly defined: the precise time point at which establishment of developmental resistance occurs and the length of time during which resistance is effective against the disease. Thus the time required for a plant or for new leaves to acquire developmental resistance is now often integrated as one of variables used in modeling plant diseases (Ficke et al., 2002; Gadouy et al., 2003; Kennelly et al., 2005). For example modeling
of the dynamics of infection caused by sexual and asexual spores during *Plasmopara viticola* epidemics considers that only young grape leaves are receptive to infection because of developmental resistance (Burie et al., 2010; Rossi et al., 2009). Such considerations are also explored for powdery mildew of strawberries. Young leaves, flowers and immature green fruits are much more susceptible to the powdery mildew, caused by the biotrophic fungus *Podosphaera aphanis*, than mature tissues. The high susceptibility to powdery mildew at the early developmental stages seems coincident with the succulent nature of the fruits at this stage, making it easy for penetration and establishment of mildew (Asalf et al., 2009; Carisse and Bouchard, 2010). Control measures targeting at these critical windows of fruit susceptibility are likely to reduce yield loss. The authors of these studies concluded that timing fungicide sprays based on periods of high leaf and berry susceptibility should greatly improve management of strawberry powdery mildew. These few examples illustrate how studies on developmental resistance may help for the development of decision-making tools to minimize environmental and public health risk of fungicide application while maintaining high crop yields.

### 2.3.2 Genetic tools for breeders

The excavation of various and new genetic resources constitutes an additional window opened by studies on plant developmental resistance. This form of resistance has been now reported for a large number of crop plants. An increasing number of studies have shown that disease resistance governed by major genes (*R* genes) or minor genes (quantitative trait loci, QTLs) may be plant stage-specific. When it occurs the persistence of the phenomenon throughout the rest of the plant life cycle once it has been induced is of clear agronomic interest. The influence of development on race-specific resistance genes has been first studied in detail in rice and wheat, to assist breeders in their decision-making processes (for review Develey-Riviere and Galiana, 2007). A recent finding indicates that QTLs controlling constitutive expression of defense-related genes co-localizes with QTLs for partial resistance of rice to *Magnaporthe oryzae* (Vergne et al., 2010). Such studies also concern other crop plants. Fruits from several cucurbit crops were tested for the effect of fruit development on susceptibility to the oomycete *Phytophthora capsici*. The seven crops tested represent four species: melon (*Cucumis melo*), butternut squash (*Cucurbita moschata*), watermelon (*Citrullus lanatus*), and zucchini, yellow summer squash, acorn squash, and pumpkin (*Cucurbita pepo*). For all of these fruits, a pronounced reduction in susceptibility accompanied the transition from the waxy green to green stage (Ando et al., 2009). The importance to consider developmental resistance for breeding has been underlined in a review on genetic approaches to the management of blister rust (*Cronartium ribicola*) in white pines. The authors have defined developmental resistance, *R*-gene resistance and partial resistance as the three broad categories of resistance that breeders have to take into account for resistance in North American white pines (King et al., 2010).

### 2.3.3 A putative source for bio-fungicides

Researches on developmental resistance also provided opportunities for characterizing new host molecules influencing pathogen growth in planta. Metabolite compounds accumulating in late phases of host plant development may enable the plant to inhibit the infectious cycles of pathogens (Hugot et al., 1999; Kus et al., 2002). However the nature of these compounds remains unknown and it is difficult to define their interest as adaptive resources for plant
protection and for their application to crop fields. It has been merely observed that in *Arabidopsis* the intercellular accumulation of SA is critical for antibacterial activity associated with developmental resistance to *Pseudomonas syringae* (Cameron and Zaton, 2004).

### 2.4 The pathogen in interaction with its host

During the current decade the main research effort on eukaryotic plant pathogens has been and still is the release of genome sequences for pathogens causing the most devastating crop diseases (Dodds, 2010). As a result, an increasing number of gene collections involved in regulation of the interaction with host plants as putative PAMP or effectors have been identified. The identification within these collections of effectors that are crucial for virulence offers the opportunity to select plant targets for more durable resistance (Houterman et al., 2008). In a functional genomics studies Vleeshouvers and coll. (2008) developed an effector-based method for identification of late blight resistance gene in potato. They used a repertoire of secreted and translocated effectors. The putative effectors were predicted computationally from the oomycete *Phytophthora infestans* genome for the presence of a signal peptide and of a RXLR translocation motif into plant cell (Birch et al., 2006; Kamoun, 2006). In an initial set of 54 candidates, two variants of the effector *ipiO*, *ipiO1* and *ipiO2*, were found to trigger HR-associated responses in *Solanum bulbocastanum*, a species carrying the late blight resistance gene *Rpi-blb1*. Both effectors were also found to induce HR responses in *Solanum stoloniferum*, and *ipiO* was found to be the avirulence gene of the *Rpi-blb1* resistance gene. Based on these results and on the hypothesis that the resistance genes were orthologous or at least members of the same family, the authors cloned *Rpi-sto1* from *S. stoloniferum* and *Rpi-pta1* from *S. papita* by gene-capture PCR (Polymerase chain reaction). Both genes were found to be functionally equivalent to *Rpi-blb1* and are now used for selective breeding (Pankin et al., 2010).

Comparative genomics of phylogenetically proximal species helps to delineate genome evolution and should also be useful in designing rational strategies for plant disease management. The analytical potential of this approach was illustrated on these two aspects by several articles published in the Science review in 2010 (Dodds, 2010). One of these studies was based on the resequencing of six genomes of four sister species *Phytophthora infestans*, *P. ipomoeae P. mirabilis* and *P. phaseoli* (Raffaele et al., 2010). These species infect diverse plants and form a tight clade of pathogens sharing 99.9% identity in their ribosomal DNA internal transcribed spacer region. The aim of the study was to determine how host jumps affect pathogen genome evolution. Genome sequencing allowed the identification of gene-sparse regions and gene-dense regions. Most pathogen genes and genome regions were found highly conserved. But more than 44% of the genes located in the gene-sparse regions showed high diversity suggesting signature of a rapid evolution, when only 14.7% of remaining genes show such signatures. Gene-dense regions were enriched in genes induced in sporangia. Gene-sparse regions were highly enriched in genes induced during plant infection, especially those encoding the predicted RXLR-containing effectors. This is in accordance with the hypothesis that genes induced *in planta* are supposed to evolve faster in a context of a co-evolution with the host. A similar strategy was developed to reveal pathogenicity determinants in two maize smut fungi, *Ustilago maydis* and *Sporisorium reilianum* (Schirawski et al., 2010). These two closely related Basidiomycetes species present an example of differentiation of two closely related pathogens parasitizing the same host. Both genomes were compared and variable genomics regions were identified. These regions
were supposed to contain genes encoding virulence proteins since one could expect that pathogen secreted effectors should rapidly evolve. On the other hand, both genomes comprise conserved effector genes as expected for pathogens infecting the same host. Eighty nine percent of the *U. maydis* putative effectors are conserved in *S. reilianum*. This statement could enable to target genomics regions involved in virulence on the same host plant and common to the Basidiomycetes. These studies illustrate how comparative genomics allow identifying the biological functions that are evolutionarily the most stable and that could be targeted to create more durable resistance.

Comparative genomics of more distal pathogenic species within a clade, that of the oomycetes, was also fruitful to define signatures associated with adaptation to a particular trait of life, the obligate biotrophy (Baxter et al., 2010; Spanu et al., 2010). The genome of the obligate biotrophic pathogen *Hyaloperonospora arabidopsidis* was sequenced. The identified gene functions were compared to those of three hemibiotrophic *Phytophthora*, *P. infestans*, *P. sojae* and *P. ramorum* (Baxter et al., 2010). Among a total of 14,543 predicted genes in *H. arabidopsidis*, 6882 had no identifiable orthologs in sequenced *Phytophthora* species. Those genes are potentially involved in biotrophic functions. On the other hand the genome of *H. arabidopsidis* showed a drastic reduction in the number of genes encoding enzymes for assimilation of inorganic nitrogen and sulfur, and proteins associated with zoospore formation and motility. Unsurprisingly, the drastic reduction also concerns genes involved in pathogenicity encoding for degradative enzymes (such as secreted proteinases or cell-wall degrading enzymes), for necrosis and ethylene-inducing (Nep1)-like proteins (NLPs) and for PAMPs. The *H. arabidopsidis* genome also exhibited no more than 134 potential effector proteins with RXLR cell translocation motifs that likely function to suppress host defenses while they have been found to be hundred in the *Phytophthora* genomes (Jiang et al., 2008; Tyler et al., 2006; Whisson et al., 2007). Only 36% of them showed significant similarity percentages with *Phytophthora* effectors. With the aim of obtaining specific targets of biotrophic oomycetes these genes could represent good candidates.

### 3. Ex planta biotic interactions and plant health

In Phytopathology the plant-pathogen interaction has caught for a long period the attention of most studies at the molecular level. The aim for controlling disease was to develop the scientific bases for genetic engineering of crops (breeding, genetically modified plants). However at least two other kinds of interactions occur at the host plant surface and are crucial for the disease outcome and also for the development of alternative crop protection strategies. Still today, too few studies deal with these two biotic interactions: (i) the cell-cell interaction governing, within the pathogenic species, the biology of the microorganism; (ii) the diverse interactions between the pathogen and the microbial community in their shared habitat. In support to this observation we investigated the features of literature on biotic interactions and plant disease outcome based on bibliometric means. The MEDLINE database was searched via the PubMed access for articles indexed under the publication type “Plant Fungus”. Growth of the literature and thematic distribution were addressed. From 1980 to 2010, a total of 35,767 citations were retrieved dealing with a plant-fungus interaction. The literature growth rate is gradually and exponentially growing (Figure 2A). Throughout this period, studies on microbial community and on cell signaling in pathogenic fungi are scarce. These two topics represent respectively 1 % and 1.9% of the whole analyzed literature (Figure 2A and 2B), and for the topic “cell signaling”, most of
Fig. 2. Bibliometric of the literature on plants-fungi interactions (19. 02. 2011).

PubMed was used to access to the MEDLINE database for searching articles under the publication category “Fungus Plant” (blue), “Fungus Plant Microbial Community” (red), “Fungus Plant Cell Signaling” (red). The retrieved articles were counted and analyzed using Microsoft Excel. (A) growth of the Plant Fungus literature, 1999–2010. (B) Overall number of articles per category. The volume of the literature related to the “Fungus Plant Microbial Community” category is surely underestimated (a search for “Plant Fungus biological control” led to retrieve 1,729 articles but most of them however did not question microbial community as an entity). Similar results were obtained when the data was screened for the publication category “Plant Oomycete” (data not shown).
studies concern the host or fungus responses during the interaction with 46% of them strictly dealing with the plant responses. Thus investigations on interactions between cells of a pathogen and between pathogenic species and the other microorganisms sharing the same biotope are insufficient. Nevertheless, biology and microbial ecology of pathogens must offer opportunities to extend our knowledge on causal relationships between biotic interactions and the epidemiology of a disease. They also open new ways for development of new sustainable agro-ecosystems that should have both agricultural value, by preventing disease, and ecological value, by reducing environmental risks.

3.1 The cell-cell interaction within a pathogenic species

3.1.1 Target cell-to-cell signaling to slow microbial adaptation to treatment

Before infection, cells, spores, zoospores, or mycelia from an eukaryotic pathogen live mainly in groups attached to surfaces, each biological entity interacting with its neighborhood. The influence of these interactions within the species have been until recently neglected, the pathogen being considered at the unicellular level for investigating the interaction with the host. However, prokaryotes and microbial eukaryotes naturally form multicellular aggregates in particular on the surface of putative hosts. The study of these aggregates has shown that microorganisms are capable of complex differentiation and behaviors. The cells communicate and cooperate to perform a wide range of multicellular behaviors, such as dispersal, foraging, biofilm formation, quorum sensing (Atkinson and Williams, 2009; West et al., 2007), these behaviors contributing to the virulence as well as to the dynamics of interactions with the host.

For phytopathogenic bacteria, it has been shown that aggregation promotes virulence in *Ralstonia solanacearum* (Kang et al., 2002), that quorum sensing regulates a variety of virulence factors in *Pectobacterium atrosepticum* (Liu et al., 2008) and that the transition from an aggregated lifestyle to a planktonic lifestyle promotes dissemination in *Xanthomonas campestris* (Dow et al., 2003). As a consequence, the molecular machinery for cell-to-cell signaling constitutes a novel target for the design of antagonists able of attenuating virulence through the blockade of bacterial cell-cell communication (Williams et al., 2000). As mentioned above, cell-to-cell signaling is not limited to the bacterial kingdom. Oomycetes produce and use molecules to monitor population density of biflagellate motile cells, the zoospores. These cells coordinated their communal behaviors by releasing, detecting, and responding to signal molecules (Kong and Hong, 2010). In the species *Phytophthora parasitica*, zoospores may form biofilms on the host surface, using a quorum sensing-like phenomenon to synchronize behavior (Galiana et al., 2008; Theodorakopoulos et al., 2011). Whether such cell-to-cell interactions contribute to the virulence of oomycetes or fungi is not known. However, the fact that, as bacteria, cells of eukaryotic pathogens cooperate to perform multicellular behaviors, indicate that from the dissection of related transduction pathways could emerge new tools for the management of cellular populations on the surface of host plants. Treatments against disease targeted to cell-cell signaling machinery could have an additional benefit and not the least, that to circumscribe the problem of pathogen resistance to fungicides for a larger period, to some extent at least. By performing modeling of multicellular organization in bacteria as a target for drug therapy to predict the speed of resistance evolution, André and Godelle (2005) concluded that this adaptation may be several orders of magnitude slower than in the case of resistance to usual antibiotics. The hypothesis of the authors makes sense in the context of the hierarchical selection theory (Gould, 2002). By targeting treatments
against adaptive properties of groups instead of individuals, the relevant unit of organization generating resistance and submitted to selection shifts one level up. Instead of facing billions of cells with a very rapid evolutionary rate, these alternate treatments face a reduced number of larger organisms with lower evolutionary potential (André and Godelle, 2005). Nevertheless, to our knowledge the molecules for such treatments are not yet available for eukaryotic pathogens, and anyway it would be advisable to be sure that they have not pleiotropic or toxic effects.

3.1.2 Biomimetism to trap pathogens
The formation of biofilms is a widely spread property of microbial life governed by cell-cell signaling (Costerton et al., 1999; Danhorn and Fuqua, 2007; Hall-Stoodley et al., 2004; Harding et al., 2009). Biofilm generation is a high spot of research because these structures represent for pathogens an important influence on the virulence as well as on the dynamics of interactions with hosts (Costerton et al., 1999; Hall-Stoodley and Stoodley, 2005). They constitute microbial communities living in co-operative groups attached to surfaces and embedded in a self-producing polymeric matrix. Their formation involves first that planktonic (free-swimming or free-floating) cells become attached to a solid surface, leading to the formation of microcolonies, which then differentiate into exopolysaccharide-encased and fluidfilled channel-separated mature sessile biofilms. Biofilms confer several advantages to pathogens promoting attachment, dissemination or virulence and protecting cells against host defenses and biocide treatments. For human pathologies the failure to eradicate them by standard antimicrobial treatments results in several cases in development of chronic and nosocomial diseases (Costerton et al., 1999; Davies, 2003). The impact of biofilm persistence is not really appreciated for the epidemiology and management of plant diseases (Ramey et al., 2004). To our knowledge nothing is known about potential antimicrobial resistance mechanisms to thwart the efficiency of treatments with fungicides or bactericides. But researches on biofilm may offer an attractive option to diversify biologically-based alternatives to systematic treatments with synthetic fungicides. During the biogenesis of biofilms by an eukaryotic plant pathogen concomitant cellular processes are mobilized to synchronize cell behaviour: chemotaxis, adhesion and aggregation (Galiana et al., 2008; Theodorakopoulos et al., 2011). The elucidation of molecular aspects of these processes should help to elaborate biomimetic materials for the development of trapping systems for pathogens, exactly on the same principles than for the design of insect traps used for many years to monitor or reduce insect populations and based on behavioural confusion techniques (Silverstein, 1981).

3.2 The interactions of the pathogen within a microbial community
In an ecosystem, a plant pathogen evolves within a microbial organized community which has a great influence on the local environment and disease. Before infection various species interact with the pathogen on the host surface shaping the distribution, density and genetic diversity of the inoculum. Such a community is considered and studied as a driving force for natural selection and pathogenicity (Kuramitsu et al., 2007; Siqueira and Rocos, 2009). Concomitantly present metagenomics studies of soils provide pictures of a community structure. The abundance distribution and total diversity can be deciphered. The analyses of the released datasets open a great opportunity to explore into the enormous taxonomic and functional diversity of environmental microbial communities (Simon and Daniel, 2011).
combining studies on function and structure of soil communities, it becomes possible to increase our ability to modify disease states and to question practices of fungicides. We considered here two levels.

The first one is to re-evaluate the analyses of suppressive soils. Pathogen-suppressive soils have been defined as soils in which the pathogen does not establish or persist, establishes but causes little or no damage, although the pathogen may persist in the soil (Cook and Baker, 1983). Examination of the microbial community compositions in soils possessing various levels of suppressiveness has been referred as a population-based approach (Borneman and Becker, 2007). The strategy leads to establish positive correlation between the population densities of some species and suppressiveness levels, suggesting that they may be involved in the disease suppressive process. The exploration of available metagenomic data will change the dimension of such analyses. As transcriptome analysis reveals gene networks for particular cellular functions, Metagenomics may help now to characterize microbial species networks for ecosystemic functions such as pathogen-suppressive properties of soils. This should help to reveal the huge potential of suppressive soils for managing soilborne pathogens. Characterization of the potential may be “easy” when biological nature of the suppression is known as illustrated by studies of soils with known chitinase and antifungal activities (Hjort et al., 2010). Metagenomics may also lead to screen uncultured microorganisms from soil which represent a potentially rich source of useful natural products. During the screening of seven different soil metagenomic libraries for antibacterially active clones, long-chain N-acyltyrosine-producing clones were found in each library. Of the 11 long-chain N-acyl amino acid synthases that were characterized, 10 were unique sequences. The heterologous expression of environmental DNA in easily cultured hosts as *Escherichia coli* has then been used by the authors to illustrate the access to previously inaccessible natural products (Brady et al., 2004).

The second level is more prospective. It consists in screening the functional diversity of microorganisms within communities in which pathogenic species evolve in respect to the disease outcome. In soil as in the other biotopes there is a myriad of microorganisms interacting with each other or with the environment, and performing a wide range of functions (organic decomposition, reduction/oxidation of different forms of elements, nitrogen-fixation...). The set of biotic interactions involving a pathogen constitutes a key factor for the natural population dynamics and emergence of pathogenic clones. In most cases this set remains uncharacterized and one great challenge for improving disease control is to identify in it the biotic interactions which contribute to the negative and also positive control of a pathogenic population. For this aim methods for screening microbial communities to select species associated with a pathogen and impacting the related host disease are missing and must be developed. As a contribution to resolve this problem we have developed a selection method and applied it to a soilborne plant pathogen, *Phytophthora parasitica*, for screening the microbial community from the rhizosphere of the host plant *Nicotiana tabacum*. Two of the selected microorganisms interfered with the oomycete cycle. An ascomycete strongly suppressed the tobacco black shank disease and a ciliate promoted the disease (Galiana et al., unpublished results). In this case the efficiency of the method must be further tested by characterizing other species that affect the tobacco disease. It must also be evaluated for other eukaryotic pathogens before giving food for thought on disease control in two directions. Firstly, the identification of the key suppressive microorganisms will help to diversify material for biological control, a method which have been recommended to replace chemical control methods since it is more
economical and environmentally sustainable (Fravel, 2005; Herrera-Estrella and Chet, 1999; Shennan, 2008; Weller et al., 2002). The molecules supporting the suppressive activity of microbial species should be analyzed for their bio-fungicide properties and for their impact on human health and on the rest of the microbial environment. Secondly, the produced information will gradually allow revealing the set of species interacting with a pathogen. In the same time, their abundance in each soil, in each biotope could be easily determined through metagenomic approaches. Thus the combination of both parameters, richness and identity of microbial species affecting a disease cycle, should be an important consideration to define the status of the biotic environment with respect to the occurrence of an epidemic. It could be fruitful to define new decision-making tools that will have to be considered by farmers to decide serenely to restrict fungicide applications or not if required.

4. Conclusion

How protect crops against diseases caused by fungi and oomycetes with both agricultural and ecological value? The treatment of this complex question combines a lot of parameters (crop rotation diversification, crop diversity, rationalization of N-fertiliser application, environment, climate, farmers practices…) mainly treated in the frame of integrated plant disease management. This chapter focuses only on what could emerge from studies on biotic interactions in plant pathology for contributing to the reduction of fungicide use, the development of alternative methods and the selection of crops more tolerant to diseases (Figure 3).

The concern about reduction of fungicides came forward very early from the advent of their use. Based on experimentations, in the lab first and then in the field, Millardet and Gayon (1888) recommended to winegrowers to use a Bordeaux mixture less rich in copper (II) sulphate and lime than in the first formulation. The new mixture was at once more adhesive on leaves than the former, without danger for the vineyard (which did not present any more foliage injuries), and more effective against the mildew. Today a trend to achieve significant fungicide reduction is to diminish frequencies rather than doses. The use of forecasting epidemics systems to assist in the timing of fungicide applications may be one of the appropriate tools. Fungicide treatments would be performed only when necessary. But this may be acceptable by farmers if the risk of an epidemic development of the disease is very low. With the increasing resources on several biotic parameters (timing for establishment of plant developmental resistance, dynamics of clones within a pathogenic population, presence and richness of microbial species affecting a disease cycle) there is an urgent need to associate and integrate the related number of variables to develop more refined and integrated models. They could serve as a starting point to carefully decide in which timing and in which biotic environment the gain from a reduced number of fungicide applications will not alter the potential risk of loss resulting from an incorrect control strategy. Another way of decreasing the frequencies is to combine fungicide treatments with exogenous applications of natural compounds stimulating plant defense responses. Studies on this subject appear scarce and few pieces of information are available on the efficiency of such approach.

Different aspects may also be considered for the development of alternative methods. In the field of genetics, greater possibilities now result from determination of crop plants and pathogens genomes for selecting new varieties of plants capable of resisting to eukaryotic pathogens. Functional and comparative genomics programs have expanded the resource of genes that can be used into crop species, as it has been recently illustrated through the development of new PAMP-recognition specificities in agricultural species or through
The representation by a pyramid symbolizes the integration of different knowledge to develop and properly articulate plant disease management strategies with a low impact on environment and human health. At the bottom the quadrilateral frustum represents the different knowledge of biotic interactions on which may be built crop protection innovations. Three of four base edges mention the biological entities involved in these interactions and which were discussed in this chapter: plant, pathogen, microbial community. The fourth one represents the other biological entities which are important in the biotic environment of a plant (plant community, insects, nematodes, …) and that we did not consider here in the context of the control of disease caused by fungi or oomycetes. At the top of the pyramid are mentioned the topics of emergence of innovations in crop protection. There is no particular consideration for the location of each topic except for modeling at the apex of the pyramid. To our mind this means that robust mathematical models must integrate several biotic variables, often not still parameterized, for building exploitable forecast in terms of rationalization of crop protection.

screening of wild relatives of crop plants to identify new sources of resistance. In the field of biology of organisms, the possibility to elaborate biomimetic materials for the development of behavioural confusion techniques against pathogens must emerge from the molecular elucidation of chemotaxis and aggregation processes. This could lead to design local traps for pathogens associating molecules with specific attractive, aggregative and biocide properties. What is effective to control the populations of insects (pheromone-based trap, sticky fly traps, …) and what was made possible by studies of molecular bases of the behavior of pest insects, must also be effective and possible for the control of pathogens. The validity of disease management by this way should be easily evaluated at the crop scale in hydroponic systems. Hydroponics as an agricultural production system is one of the fastest growing sector, which is more and more used to produce flowers, fruits or vegetable. “Sticky” pathogen traps could contribute to the sanitary quality of the nutrient circulating
solutions that is crucial in hydroponic systems. In the field of ecology, new ways could also emerge from exploitation of genomics and metagenomics data to manage pathogenic population in the greenhouse or in the field. Based on appropriate screening of microbial communities they will help to develop and to vary biological control strategies. Beyond the challenge to develop new strategies for crop protection, the biggest defy remains to associate, to articulate them in an adequate way in order to conciliate environmental concerns, safety for human health and agricultural imperativeness.

5. Acknowledgement

This work was supported by a research-aid fund of the CNRS-Cemagref Ecological Engineering program, Programme de recherche interdisciplinaire : « Ingénierie Ecologique».

6. References


Cameron, R. K., and Zaton, K. (2004). Intercellular salicylic acid accumulation is important for age-related resistance in Arabidopsis to Pseudomonas syringae. Physiological and Molecular plant Pathology 65, 197-209.


www.intechopen.com


This book brings together issues on pesticides and biopesticides use with the related subjects of pesticides management and sustainable development. It contains 24 chapters organized in three sections. The first book section supplies an overview on the current use of pesticides, on the regulatory status, on the levels of contamination, on the pesticides management options, and on some techniques of pesticides application, reporting data collected from all over the world. Second section is devoted to the advances in the evolving field of biopesticides, providing actual information on the regulation of the plant protection products from natural origin in the European Union. It reports data associated with the application of neem pesticides, wood pyrolysis liquids and bacillus-based products. The third book section covers various aspects of pesticides management practices in concert with pesticides degradation and contaminated sites remediation technologies, supporting the environmental sustainability.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following: