Molecular Plant Pathology

MOLECULAR PLANT PATHOLOGY (2016) 17(9), 1506–1518 DOI: 10.1111/mpp.12436

Review

Bacterial disease management: challenges, experience, innovation and future prospects

Challenges in Bacterial Molecular Plant Pathology

GEORGE W. SUNDIN[1,*], LUISA F. CASTIBLANCO[1], XIAOCHEN YUAN[2], QUAN ZENG[3] AND CHING-HONG YANG[2]

[1]*Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, MI 48824, USA*
[2]*Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI 53211, USA*
[3]*Department of Plant Pathology and Ecology, Connecticut Agricultural Experiment Station, New Haven, CT 06504, USA*

SUMMARY

Plant diseases caused by bacterial pathogens place major constraints on crop production and cause significant annual losses on a global scale. The attainment of consistent effective management of these diseases can be extremely difficult, and management potential is often affected by grower reliance on highly disease-susceptible cultivars because of consumer preferences, and by environmental conditions favouring pathogen development. New and emerging bacterial disease problems (e.g. zebra chip of potato) and established problems in new geographical regions (e.g. bacterial canker of kiwifruit in New Zealand) grab the headlines, but the list of bacterial disease problems with few effective management options is long.

The ever-increasing global human population requires the continued stable production of a safe food supply with greater yields because of the shrinking areas of arable land. One major facet in the maintenance of the sustainability of crop production systems with predictable yields involves the identification and deployment of sustainable disease management solutions for bacterial diseases. In addition, the identification of novel management tactics has also come to the fore because of the increasing evolution of resistance to existing bactericides. A number of central research foci, involving basic research to identify critical pathogen targets for control, novel methodologies and methods of delivery, are emerging that will provide a strong basis for bacterial disease management into the future.

- Near-term solutions are desperately needed. Are there replacement materials for existing bactericides that can provide effective disease management under field conditions?
- Experience should inform the future. With prior knowledge of bactericide resistance issues evolving in pathogens, how will this affect the deployment of newer compounds and biological controls?
- Knowledge is critical. A comprehensive understanding of bacterial pathosystems is required to not only identify optimal targets in the pathogens, but also optimal seasonal timings for deployment.
- Host resistance to effectors must be exploited, carefully and correctly. Are there other candidate genes that could be targeted in transgenic approaches? How can new technologies (CRISPR, TALEN, etc.) be most effectively used to add sustainable disease resistance to existing commercially desirable plant cultivars?
- We need an insider's perspective on the management of systemic pathogens. In addition to host resistance or reduced sensitivity, are there other methods that can be used to target these pathogen groups?
- Biological systems are variable. Can biological control strategies be improved for bacterial disease management and be made more predictable in function?

The answers to the research foci outlined above are not all available, as will become apparent in this article, but we are heading in the right direction. In this article, we summarize the contributions from past experiences in bacterial disease management, and also describe how advances in bacterial genetics, genomics and host–pathogen interactions are informing novel strategies in virulence inhibition and in host resistance. We also outline potential innovations that could be exploited as the pressures to maximize a safe and productive food supply continue to become more numerous and more complex.

Keywords: bactericide resistance, biological control, CRISPR/Cas9 genome editing, nanoparticles, TAL effectors, virulence inhibitors.

* *Correspondence*: Email: sundin@msu.edu

INTRODUCTION

Many general characteristics of plant bacterial diseases (examples shown in Fig. 1) favour disease incidence, pathogen dissemination and pathogen survival, and confound management strategies in

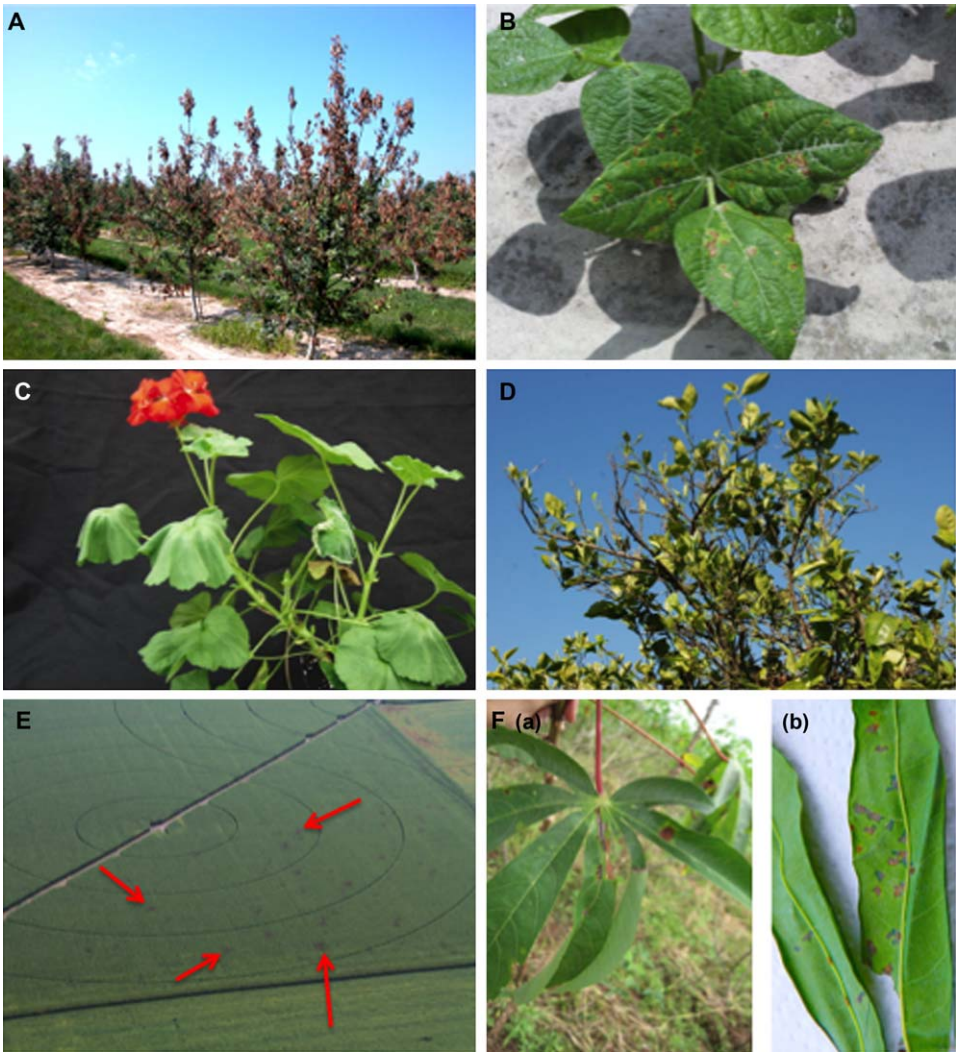

Fig. 1 Symptoms of selected plant bacterial diseases illustrating a range of problems affecting the potential for successful disease management. (A) Fire blight of apple caused by *Erwinia amylovora*. (B) Bacterial brown spot of bean caused by *Pseudomonas syringae* pv. *syringae*. (C) Bacterial wilt of geranium caused by *Ralstonia solanacearum*. (D) Huanglongbing of citrus caused by '*Candidatus* Liberibacter spp.'. (E) Goss's wilt (aerial view) of sweet corn caused by *Clavibacter michiganensis* ssp. *nebraskensis* (arrows point to representative areas of diseased plants within the field). (F) (a, b) Bacterial blight of cassava caused by *Xanthomonas axonopodis* pv. *manihotis*. Photograph credits: (A, B) George Sundin; (C) Ana Maria Bocsanczy (University of Florida); (D) Nian Wang (University of Florida); (E) Megan Botti-Marino and Martin Chilvers (Michigan State University); (F) Cesar Medina and Adriana Bernal (Universidad de Los Andes).

agricultural ecosystems. Examples of these include: (i) the growth rate of bacterial pathogens under optimal environmental conditions can quickly result in population sizes that favour both increased levels of infection and the potential for rapid spread to new infection sites, driving disease epidemics (Fig. 1A – fire blight, *Erwinia amylovora*); (ii) epiphytic growth on symptomless leaf surfaces can result in the attainment of sufficient population size for disease occurrence (Fig. 1B – bacterial brown spot, *Pseudomonas syringae* pv. *syringae*); (iii) some bacterial pathogens are soil-borne and, with the absence of effective fumigants, reductions in bacterial populations in soil are extremely difficult to achieve (Fig. 1C – bacterial wilt, *Ralstonia solanacearum*); (iv) many bacterial pathogens predominantly colonize internal locations within plants that are inaccessible to most spray-applied chemical and biological controls that target plant surfaces (Fig. 1D – Huanglongbing (HLB), '*Candidatus* Liberibacter spp.'); (v) populations of insect vectors, especially of destructive xylem- and phloem-dwelling pathogens, are as difficult to manage as the diseases (Fig. 1D); (vi) pathogen survival and growth on crop debris and on alternative hosts defeat strategies of inoculum reduction (Fig. 1E – Goss's wilt, *Clavibacter michiganensis* ssp. *nebraskensis*); and (vii) asymptomatic survival of pathogen cells on planting material for extended periods of time can result in disease dissemination to new geographical locations (Fig. 1F – bacterial blight of cassava, *Xanthomonas axonopodis* pv. *manihotis*).

Management strategies for plant bacterial diseases require a thorough knowledge of the pathosystem in order to identify the most appropriate timing for the targeting of pathogen populations and to determine when the critical host tissues are vulnerable to infection. An integrated management approach, including the use of plant host resistance or the growth of less susceptible cultivars, intervention with chemical and/or biological controls, and cultural practices aimed at inoculum reduction, typically represents the best overall strategy for effective and sustainable disease management. However, integrated approaches depend on the availability of suitable host plant cultivars, efficacious chemical and

biological controls, and cultural practices that are physically achievable and economically suitable, such that growers will deploy them.

In recent years, genomic advances have facilitated a rapid increase in our understanding of bacterial pathogen–host interactions and have led to the invention of novel techniques to incorporate resistance into horticulturally desirable plant cultivars. However, the timeline from promise to practice of genomics to effective drugs and disease control outcomes has proven to be long and tortuous in clinical microbiology (Brown and Wright, 2016; Silver, 2011). Moreover with much more limited resources available to plant agricultural research, we can expect similar results. It is important to realize, however, that these pathways differ, because of our ability to manipulate host plant populations, which could significantly tilt the scale in favour of plant disease management.

Thus, the management of bacterial plant diseases remains a formidable 'grand challenge' for plant pathologists. In this article, we first assess some of the history of bacterial plant disease management, asking the question: 'what can we learn from past experience and how can this fuel the development of future innovative and integrated approaches for disease management?' From there, we continue into the current plant bacteriology arena to discuss novel anti-virulence approaches and the potential for the targeted genetic manipulation of host plants.

MANAGEMENT OF BACTERIAL DISEASES WITH COPPER AND ANTIBIOTICS AND THE EVOLUTION OF RESISTANCE

The potential for the chemical management of individual bacterial diseases has been largely driven by factors such as the availability of effective modes of action, the opportunity to access the pathogen on plant surfaces, the susceptibility of the pathogen to the specific chemical, the economic value of the crop threatened and the market potential of the use of the chemical from an industrial perspective. Compared with fungicides, for example, relatively few chemicals targeting plant bacterial diseases have been marketed. Probably the most important reason for this is that the best antibacterial compounds available are antibiotics, and almost all antibiotics historically have been developed for use in clinical medicine and not for plant agriculture.

Initial forays into the chemical management of bacterial diseases focused on a 'kitchen sink' approach, involving the testing of a wide range of available compounds against a wide range of diseases (e.g. Zaumeyer, 1958). From these types of study, copper compounds (introduced in the 1880s) and the antibiotic streptomycin (1950s) proved to be the most efficacious, and have been the most commonly used bactericide spray treatments for bacterial disease management on plants, mainly targeting *Pseudomonas* spp., *Xanthomonas* spp. and *E. amylovora* (McManus *et al.*, 2002). Although, in general, these bactericides have been relatively successful disease management tools, the use of both copper and streptomycin has been impacted by the evolution of resistance in populations of plant pathogens (Cooksey, 1990; McManus *et al.*, 2002).

The extensive use of copper and antibiotic sprays over multiple years and/or the use of high numbers of applications within individual seasons is correlated with the selection of resistance in pathogen populations. In addition, in the majority of cases, resistance has evolved as a result of the acquisition of genes encoding resistance determinants, thus also implicating non-target microbiota in the horizontal transmission of resistance determinants within agricultural ecosystems. We now know that horizontal gene transfer, a mechanism for the acquisition of resistance determinants by bacteria even from phylogenetically distinct species, has driven the antibiotic resistance crisis currently affecting the human population (Perry and Wright, 2013; Smillie *et al.*, 2011). The linkage of disparate ecosystems on a global scale is also apparent when we consider the breadth of geographical locations and bacterial species harbouring identical antibiotic resistance determinants (Blair *et al.*, 2015; Forsberg *et al.*, 2012; Sundin and Bender, 1996). The inclusion of plant pathogens and other populations of plant-associated bacteria in this globally connected ecosystem also became apparent when the genetic determinants of resistance to copper and streptomycin were revealed (discussed below).

Copper resistance is encoded by a copper-inducible operon (*copABCD*) in *P. syringae* pv. *tomato* (Mellano and Cooksey, 1988) and by related variants in other *P. syringae* pathovars (Gutierrez-Barranquero *et al.*, 2013), *Xanthomonas campestris* pv. *juglandis* (Lee *et al.*, 1994) and in the citrus pathogens *Xanthomonas citri* ssp. *citri* and *Xanthomonas alfalfae* ssp. *citrumelonis* (Behlau *et al.*, 2011). Horizontal gene transfer has been implicated in the transfer of copper resistance genes, usually via the conjugation of copper resistance plasmids; evidence of genetic exchange at a global level exists in *Xanthomonas* spp. (Behlau *et al.*, 2013). In *E. amylovora*, the first instances of streptomycin resistance were conferred by a chromosomal mutation altering the ribosomal protein target of the antibiotic (Moller *et al.*, 1981). More recent analyses of streptomycin resistance in *E. amylovora*, *P. syringae* and *X. campestris* pv. *vesicatoria* have shown that resistance is conferred by the *strAB* genes, which encode aminoglycoside phosphotransferase enzymes that modify streptomycin to a non-toxic form. Of significance, in all reported cases among these different pathogen genera isolated on several continents, the *strAB* genes are located on the transposon Tn*5393* and related variants that differ only in the presence of the insertion sequences IS*1133* or IS*6100*, which provide promoters for the expression of *strAB* (Chiou and Jones, 1993; Förster *et al.*, 2015; Sundin and Bender, 1995). Streptomycin, discovered in 1944, represents one of the longest and most widely utilized antibiotics in human history (administered to control diseases of humans,

animals and plants, and also used in animals for growth promotion). The world-wide distribution of *strAB* genes in an ever-growing number of bacterial genera from disparate habitats illustrates the tremendous capacity and breadth of horizontal gene transfer in bacterial populations as a mechanism to disseminate ecologically useful traits (Sundin and Bender, 1996).

A few additional antibiotics have been used as alternatives to streptomycin either because of resistance or in some pathosystems in which streptomycin is not effective. Oxytetracycline has been used on pome fruit trees to control fire blight (*E. amylovora*) in the USA and Mexico, on peach and nectarine targeting bacterial spot (*Xanthomonas arboricola* pv. *pruni*) in the USA, and on vegetable crops targeting *Pseudomonas* spp. and *Xanthomonas* spp. in Latin American countries (McManus *et al.*, 2002). Gentamicin has been used in Latin American countries to control fire blight and various vegetable diseases, and oxilinic acid has been used in Israel to control fire blight (Shtienberg *et al.*, 2001). In these cases, gentamicin-resistant bacteria were recovered from lettuce in Costa Rica, and oxolinic acid resistance in *E. amylovora* was detected in Israel 1–3 years after its introduction (Kleitman *et al.*, 2005; Rodriguez *et al.*, 2006). More recently, kasugamycin has been registered for use in the USA to target fire blight, especially in orchards containing streptomycin-resistant *E. amylovora* (McGhee and Sundin, 2011), was used in European Union countries for more than three decades until antibiotic use was banned in 2007, and has been used in Japan as a seed treatment for bacterial diseases. Kasugamycin does not have any applications outside of these plant agricultural uses and does not appear to be as broad spectrum as streptomycin. However, the use of antibiotics has generally been discouraged or is not allowed in some regions of the world because of the potential impact of antibiotic use on the transfer of antibiotic resistance into clinical pathogens. In addition, experience with horizontal gene transfer and the acquisition of streptomycin resistance genes by plant pathogens in many ecosystems suggests that antibiotic resistance evolution is an eventuality with any new antibiotic deployed. Thus, the main reality of using antibiotics for crop diseases is that any strategy that selects for resistance in the target pathogen is not sustainable.

ALTERNATIVES TO COPPER BACTERICIDES AND ANTIBIOTICS FOR BACTERIAL DISEASE MANAGEMENT: COMMERCIALLY AVAILABLE AND NEAR-TERM OPTIONS

The realization that the use of copper and/or antibiotics for bacterial disease management is less than ideal or is not allowed for regulatory reasons has driven discovery and research efforts into alternative strategies. In this section, we briefly focus on biological control, the use of antibacterial peptides and the use of inducers of systemic acquired resistance (SAR).

Biological control

Biological control can be broadly defined as the use of beneficial microbes or their byproducts or byproducts/extracts from plants or animals in the suppression of plant disease. Strategies to manipulate populations of biological control agents (BCAs) in field situations are typically either inundative, in which the BCA is introduced in a sufficient quantity to suppress disease, even without reproduction, or augmentative, in which the BCA is introduced in sufficient quantity to generate a stable, replicating population suitable for disease suppression (Johnson, 2010). In some situations, molecules secreted from antagonistic microbes, typically antimicrobials, are used instead of the microbes themselves. The topic of biological control and the scientific bases of biological control have been reviewed extensively, and it is not our intention to re-summarize this field. Rather, we wish to highlight ongoing efforts in biocontrol and shed light on innovations that could influence future management efforts.

The best chances for success with biological control at a commercial level appear to be in systems in which there is a short window of time in which management is needed. For example, the most notable example of success of biological control for bacterial diseases has been the use of *Agrobacterium radiobacter* strain K84 in the protection of exposed surfaces of woody plant cuttings from crown gall caused by *Agrobacterium tumefaciens* (Kerr, 2016). Another system in which biological control has achieved some success is in the management of the blossom blight phase of fire blight during the relatively short apple or pear bloom window (Johnson and Stockwell, 1998), although management outcomes have shown major differences on a regional basis, probably caused by differences in environmental conditions (Sundin *et al.*, 2009).

Unfortunately, for many bacterial diseases and for leaf- and fruit-spotting pathogens and soil-borne pathogens in particular, the window of infection is much longer, significantly reducing the chances for consistent success with biological controls. In these situations, difficulties arise because of the need to sustain sufficient populations of antagonists to protect host tissues at the specific sites at which pathogens colonize. In these situations, some success has been achieved with the use of *hrp* (*hypersensitive response and pathogenicity*) mutants as competing antagonists (Hert *et al.*, 2009; Moss *et al.*, 2007), although the lack of commercial development of such strains is a conspicuous sign of likely 'deal-breaking' limitations inherent in the utilization of this strategy. Indeed, biosafety concerns, in particular with the classification of some biocontrol bacteria (e.g. *Burkholderia* spp., *Pantoea agglomerans*) as opportunistic human pathogens, has also more recently limited deployment, and signifies a need for the discovery of novel biocontrol strains categorized as GRAS/QPS (generally regarded as safe/qualified presumption of safety).

The use of bacteriophage for bacterial disease management is another biological control method that has been the subject of

research activity at varying times over the past four decades (Jones *et al.*, 2007). Bacteriophages are abundant in most if not all ecosystems on Earth, and phage capable of lysing plant-pathogenic bacterial species can be readily isolated from host plant tissue or soil. Inundative application strategies are typically used for disease management, and the two main limiting factors affecting bacteriophage efficacy are stability in the environment and spontaneous resistance mutations in target bacterial pathogens. Interest in bacteriophage for disease control is undergoing a resurgence, particularly in clinical medicine, as antibiotic resistance becomes more problematic. Likewise for plant bacterial diseases, there is interest in using phage, but significantly more field research and the optimization of formulations and spray timings are necessary to facilitate the development of successful applications.

Ultimately, a thorough understanding of the ecology of potential antagonists, interactions with plants, positive and negative interactions with the plant microbiome and interactions with pathogens is needed for improved and sustainable deployment in commercial systems. Functional genomic analyses appear to represent a solid route to obtaining knowledge on current gold-standard biocontrol agents (Massart *et al.*, 2015), but this work needs to be coupled with significant, dedicated field efforts, such that the behaviour of these organisms can be assessed under a wide range of environmental and plant host combinations.

Antimicrobial peptides

Small antimicrobial peptides (AMPs; typically 50 amino acid residues or smaller) are synthesized by bacteria, fungi and oomycetes, functioning in inter-microbial competition, and by animals and plants, as part of the innate immunity system in response to microbial challenge. Most of these peptides are cationic and can either insert into and disrupt cell membranes or can be taken up by cells and inhibit nucleic acid or protein synthesis (Brogden, 2005). Investigations of the potential for peptides in the management of bacterial plant diseases have been ongoing for more than a decade with strategies mainly involving the inundative application of AMPs to plant surfaces, the use of native fungal or bacterial antagonists that express and secrete AMPs as a BCA, the expression of an animal defensin, such as cecropin, in a transgenic plant or the use of a modified synthetic analogue of an animal or plant defensin in a transgenic situation (Montesinos, 2007).

The deployment of an AMP via spray application onto crop plants may be a more desirable method than the use of transgenic plants, especially in specialty food crops, because the source of some of the more efficacious AMPs includes insects and amphibians. However, one critical limiting step to this process is the cost of AMP production. The use of plants as 'biofactories' for AMP synthesis (Nadal *et al.*, 2012; Parachin *et al.*, 2012) may represent an economically suitable mode of production, ultimately facilitating the use of AMPs for bacterial disease management. Nevertheless, although the AMP field is productive and mature, unfortunately, for plant disease management, the promise of AMPs remains a work in progress, and is still dominated by screening efforts searching for new candidates (Breen *et al.*, 2015).

SAR, induced systemic resistance (ISR) and related strategies

The induction of SAR (Durrant and Dong, 2004) in plants through the application of a chemical inducer, such as the commercially available product acibenzolar-*S*-methyl (ASM), represents one of the greater successes in the transfer of basic research findings to disease management applications. This strategy of pre-conditioning of plants to pathogen challenge through the foliar application of ASM has been successfully applied in a number of pathosystems, including bacterial canker of tomato, bacterial wilt of tomato and fire blight (Maxson-Stein *et al.*, 2002; Pradhanang *et al.*, 2005; Sen *et al.*, 2015). Soil drenches with ASM and other SAR inducers have also been found to be effective in the control of citrus canker on young grapefruit trees (Graham and Myers, 2011). In addition, SAR inducers continue to be evaluated as candidates for the management of emerging significant disease issues, such as Huanglongbing in Florida (Li *et al.*, 2016). Although ASM is not typically sufficiently effective as a stand-alone material, the incorporation of ASM into an integrated programme can result in the achievement of the desired control levels, whilst reducing the applications of other materials, such as copper or antibiotics (Roberts *et al.*, 2008).

The use of ISR involves the priming of plant defence through the actions of certain plant growth-promoting rhizosphere bacteria and fungi, including *Bacillus* spp., *Pseudomonas* spp., *Trichoderma* spp., etc., and is conferred through plant hormone-mediated signalling (Pieterse *et al.*, 2014). ISR has proven to be an effective strategy in the management of a variety of bacterial pathogens, including *P. syringae*, *Xanthomonas oryzae* pv. *oryzae* and *R. solanacearum* (Kloepper *et al.*, 2004). Other strategies used to increase plant defences against bacterial pathogens, such as *R. solanacearum*, include the application of inorganic elements, such as silicon. Silicon has been hypothesized to induce enhanced structural barriers against pathogens and has also been shown to induce resistance through hormone-controlled signalling pathways, similar to ISR (Ghareeb *et al.*, 2011).

PLANT HOST RESISTANCE AND DURABLE MANAGEMENT OF BACTERIAL DISEASE

Breeding for disease resistance has been a desirable method for bacterial disease management for many years, even up to 100 years ago. During the past two decades, much effort has been made to obtain an understanding of the key determinants of plant-pathogenic bacteria–host interactions. The increasing

amount of genomic and transcriptomic data from both plants and pathogens, together with the continuous improvements in high-throughput sequencing technologies, have resulted in a more complete understanding of the defence mechanisms deployed by plants and how bacterial pathogens subvert such immune responses in order to cause disease. In general, the plant immune response to biotic stresses depends on the deployment of defence mechanisms based on pathogen recognition within a two-layered response (Jones and Dangl, 2006). The first line of defence is initiated by contact with highly conserved pathogen elicitor molecules, named pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs). Such molecules, including bacterial flagellin, EF-Tu elongation factor and lipopolysaccharides, are recognized with high affinity by membrane-bound receptor proteins, named pattern recognition receptors (PRRs; Dodds and Rathjen, 2010). This recognition triggers a general defence response, termed pattern-triggered immunity (PTI; Dodds and Rathjen, 2010), which is generally sufficiently strong to restrict non-adapted pathogens. Pathogens evolutionarily adapted to their host are able to overcome PTI by translocating effector proteins that suppress PTI signalling into the plant cytoplasm via the type III secretion system (T3SS). In some cases, a more robust line of defence is then triggered after the recognition of these pathogen effectors by more specialized receptors encoded by resistance (R) proteins, which recognize either the effector protein *per se* or the molecular modification of a decoy target in the host, inducing effector-triggered immunity (ETI; Dodds and Rathjen, 2010; van der Hoorn and Kamoun, 2008; Macho and Zipfel, 2015).

The deployment of specific *R* genes into agronomically important crop plants has generated positive momentum for disease management in a wide variety of bacterial disease pathosystems. However, in many cases, the resistance is not durable, as mutational modification of effector targets results in the evolution of new pathogen races that can overcome the resistance. Although most known examples of durable resistance are quantitative (polygenic), this type of resistance is typically too difficult to transfer by breeding. Thus, the pyramiding of groups of *R* genes into new cultivars has been a very common breeding strategy, and success in durability is predominantly dependent on combinations containing *R* genes that have been known to be highly effective over long periods of years, such as the *Xa3*, *Xa4* and *Xa21* genes in the rice–bacterial blight (*X. oryzae* pv. *oryzae*) system (Leach *et al.*, 2001).

Improvement of these breeding strategies will require more intensive studies of the mechanisms of action of *R* genes and of the importance of effector targets to bacterial pathogenesis and the ability of these targets to tolerate mutations. One promising area for genetic manipulation has come through the analysis of transcription activator-like effector (TALE) proteins, secreted by *Xanthomonas* spp. and *R. solanacearum*. These TALEs are imported to the plant nucleus, bind to specific promoter DNA fragments, named effector-binding elements (EBEs), in a nucleotide-specific manner (Boch *et al.*, 2009) and, with the assistance of transcription helper proteins, activate the transcription of the downstream genes required for disease development, termed susceptibility (*S*) genes (Boch *et al.*, 2014). Some plants have evolved mechanisms to avoid the activation of *S* genes by modifying the EBE promoter sequences, synthesizing variants of the transcription helper proteins or by encoding *R* (executor) genes in the place of *S* genes, which restrict disease progression by ETI induction (Boch *et al.*, 2014; Doyle *et al.*, 2013). The development of a comprehensive understanding of the expression and function of executor *R* genes has definitive promise in the construction of new crop varieties with durable resistance (Zhang *et al.*, 2015).

Potential examples of the modification of TALE–host interactions resulting in resistance include the editing of EBEs to create 'blind' promoters upstream of susceptibility genes and the introduction of synthetic EBEs into the promoter regions of executor genes, creating 'promoter traps' that confer resistance to effectors from multiple pathogens (Schornack *et al.*, 2013). As reported by Hummel *et al.* (2012), six additional EBEs have been introduced in tandem in the promoter region of *Xa27*, an executor gene in rice that confers resistance to *X. oryzae* pv. *oryzae* secreting the effector protein *AvrXa27*. This genetically engineered promoter confers broad-spectrum resistance against bacterial blight and bacterial leaf streak in rice as it contains three EBEs that correspond to three additional effectors from *X. oryzae* pv. *oryzae* and three effectors from *X. oryzae* pv. *oryzicola* (Hummel *et al.*, 2012). More recently, Zeng *et al.* (2015) have reported the genetic engineering of the promoter region of *Xa10*, a TALE-dependent *R* gene that provides narrow-spectrum resistance to only a few strains of *X. oryzae* pv. *oryzae*. The introduction of five EBEs to the *Xa10* promoter, corresponding to the TAL effectors PthXo1, PthXo6, PthXo7, AvrXa10 and AvrXa27, has been reported to confer broad-spectrum resistance to 29 *X. oryzae* pv. *oryzae* strains from different geographical origins (Zeng *et al.*, 2015). Although considerations of transformation stability, gene regulation mechanisms and potential pleiotropic effects need to be taken into account, TALE-assisted engineering of resistance is a promising strategy for the development of durable broad-spectrum resistance to disease caused by *Xanthomonas* spp. and *R. solanacearum*.

INNOVATION AND FUTURE PROSPECTS IN BACTERIAL DISEASE MANAGEMENT

In the preceding sections, we have described the management of bacterial plant diseases from a historical perspective. From this discussion, it is clear that management has relied on two main intervention strategies, namely the application of chemical or biological materials to plant surfaces targeting pathogen populations or the utilization of some form of host resistance targeting

pathogens at the host interface. As we search for innovative future methods of disease management, the attempts may become more specific and strategic, for example through the targeting of pathogen virulence or the fine-tuning of the genetic manipulation of host plants. Thus, as discussed below, the genetics/genomics age has the potential to fuel novel management strategies that are hypothesized to be environmentally benign and potentially less prone to resistance evolution.

Targeting the T3SS

The T3SS is a needle-like apparatus that enables pathogens to translocate effector proteins directly from the bacterial cytoplasm into the host cell cytoplasm. These secreted effectors inhibit the host immune response and play critical roles during bacterial infection (Stavrinides *et al.*, 2008). As the T3SS is required for pathogenesis in many Gram-negative bacterial pathogens, this system represents an obvious target for the inhibition of pathogenesis in the design of novel disease management strategies. Two plant phenolic compounds, *o*-coumaric acid (OCA) and *t*-cinnamic acid (TCA), have been identified as inducers of the T3SS genes in *Dickeya dadantii* (Yang *et al.*, 2008). OCA and TCA are the biosynthetic precursors of the plant defence hormone salicylic acid (SA) and have also been reported to play a role in plant defence (Montesano *et al.*, 2005; Vidal *et al.*, 1997). This finding suggests that plant phenolic compounds could be good resources for the identification of potential T3SS inhibitors. A collection of phenolic compounds was screened for the ability to inhibit the expression of *hrpA*, which encodes the structural component of the T3SS pilus in *D. dadantii*. This approach enabled the identification of a new phenolic compound, *p*-coumaric acid (PCA), as a T3SS inhibitor (Li *et al.*, 2009). PCA is an intermediate in the phenylpropanoid biosynthesis pathway. Phenylpropanoids belong to a group of secondary metabolites produced by plants and function as defence molecules in response to microbial attack (Dixon and Paiva, 1995; Feys and Parker, 2000).

The identification of PCA as a potential inhibitor of T3SS was further employed to improve the inhibition efficiency through compound modification; subsequent screening of a library of PCA derivatives resulted in the identification of one compound (*trans*−4-hydroxycinnamohydroxamic acid), exhibiting an eightfold higher inhibitory potency towards T3SS structural and regulatory genes relative to PCA (Li *et al.*, 2015). Other studies have also indicated that several plant phenolic compounds are able to modulate T3SS gene expression in *E. amylovora* and in the multihost pathogen *Pseudomonas aeruginosa* (Khokhani *et al.*, 2013; Yamazaki *et al.*, 2012), similar to salicylidene acylhydrazide family compounds, which not only exhibit an inhibitory role over T3SS-related genes, but also suppress amylovoran exopolysaccharide production in *E. amylovora* (Yang *et al.*, 2014).

In addition, many of the above-mentioned PCA derivatives showed little effect on bacterial growth *in vitro*, suggesting that these compounds do not have as high a selective pressure for resistance development as traditional bactericides. We conducted field assays with the phenolic T3SS inhibitors *trans*−4-phenylcinnamic acid and *trans*−3-fluorocinnamohydroxamic acid in 2014 and 2015, investigating their efficacy against fire blight caused by *E. amylovora* on apple trees. Our results indicated that these two compounds, applied individually to trees at a concentration of 5 mM, reduced blossom blight in the orchard with an efficacy similar to kasugamycin (C.-H. Yang and G. W. Sundin, unpublished data).

Targeting bacterial biofilms

Biofilm formation provides bacteria with effective protection from environmental stresses, antimicrobial drugs and host defence mechanisms, and is a critical virulence factor of many plant-pathogenic bacteria (Danhorn and Fuqua, 2007; Hall-Stoodley *et al.*, 2004; Koczan *et al.*, 2009; Rodrigues *et al.*, 2008). Thus, the development of inhibitors of biofilm formation to control bacterial infection has become an attractive research area. Biofilm development typically contains three main stages, including surface attachment, biofilm maturation and biofilm dispersal (Kostakioti *et al.*, 2013). Currently, most of the developed anti-biofilm inhibitors, such as D-amino acids and indole derivatives, have been applied to prevent early biofilm formation, resulting in reduced disease symptoms caused by animal and plant pathogens, and increased bacterial susceptibility to conventional antibiotics and copper (examples include Brackman *et al.*, 2011; Kolodkin-Gal *et al.*, 2010; Li and Wang, 2014; Sarojini *et al.*, 2010; Worthington *et al.*, 2012). Bis-(3′,5′)-cyclic dimeric guanosine monophosphate (c-di-GMP) is a bacterial secondary messenger that participates in the regulation of multiple cellular behaviours and virulence in many bacterial species (Romling *et al.*, 2013; Yuan *et al.*, 2015). Recent studies have demonstrated that a decrease in intracellular c-di-GMP levels can trigger biofilm dispersal, which has been proposed as a promising strategy for biofilm eradication (Chua *et al.*, 2015).

One compound showing excellent promise is 2-aminoimidazole (2AI), which inhibits biofilm formation by the bacterial spot pathogen *Xanthomonas euvesicatoria in vitro*. When applied in mixtures with copper hydroxide, 2AI suppressed bacterial spot infection in field experiments on tomato more effectively than either material alone (Worthington *et al.*, 2012). Two other compounds, 3-indoylacetonitrile and D-leucine, inhibit biofilm formation by the citrus canker pathogen *X. citri* ssp. *citri*. When applied alone or in combination with copper sulfate, these compounds reduced bacterial populations and disease severity on citrus leaves in glasshouse assays (Li and Wang, 2014). In addition, the compound *N*-acetylcysteine reduces adhesion and inhibits

biofilm formation in the xylem-dwelling pathogen *Xylella fastidiosa*. Although *N*-acetylcysteine uptake into the xylem of citrus plants via fertigation or other methods did not reach the minimal inhibitory concentration of 6 mg/mL, this compound reduced symptom expression and bacterial populations in glasshouse tests (Muranaka *et al.*, 2013). The development of effective biofilm inhibitors could represent a critical breakthrough, especially in the management of foliar pathogens, as biofilm formation can confound the efficacy of bactericides (Redondo *et al.*, 2015). Thus, inhibitors need not be developed as stand-alone products, and potentially could be better used as mixing partners to enhance the efficacy of existing bactericides.

Targeting quorum sensing

Quorum sensing (QS) is a cell-to-cell communication process involved in the regulation of various traits in response to cell density via extracellular signalling molecules, such as acyl homoserine lactones (AHLs) or diffusible signal factors (DSFs) (von Bodman *et al.*, 2003; Rutherford and Bassler, 2012). QS regulates critical traits involved in host–pathogen and pathogen–vector interactions in at least nine plant pathogen genera (Helman and Chernin, 2015). Because QS relies on bacterial perception of an external signalling molecule, this process represents an excellent potential target for manipulation, either through signal degradation or overproduction of the signalling molecule. For example, AHL-degrading enzymes have been reported in many bacteria, and this strategy has been used in disease management efforts, either as a biological control strategy via inundative application of AHL-degrading organisms or through the expression of AHL-degrading enzymes in transgenic plants (e.g. Dong *et al.*, 2001; Molina *et al.*, 2003). In other situations, the synthesis of AHL molecules in transgenic plants has been used to thwart pathogen attack, as the stimulation of QS-controlled traits at inappropriate timings can cause malfunctions in the orchestration of pathogenesis through a process that has been termed 'pathogen confusion' (Lindow *et al.*, 2014). The observation that some plants also respond to QS molecules and that others produce quorum-quenching enzymes indicates that the host can also influence this bacterial population process (Bauer and Mathesius, 2004; Dudler and Eberl, 2006), and suggests that there is still much to learn prior to the sustainable use of anti-QS strategies in plant disease management.

Nanoparticles

Nanoparticles (NPs) are particles with at least one dimension within the 1–100-nm range. Particles within this size range have unique physical and chemical properties, including large surface to mass ratio, high reactivity and unique interactions with biological systems (Zhang *et al.*, 2008). Some of the above properties make them excellent antimicrobials, and some properties make them ideal carriers/delivery systems for other antimicrobials. The evaluation of NPs for plant disease management to date has mainly focused on oomycete and fungal diseases (reviewed in Servin *et al.*, 2015), but promising results have also been obtained for bacterial diseases (discussed below).

The antimicrobial activities of most NPs result from three major aspects: photocatalysis, physical damage to the bacterial cell envelope and the release of toxic metal ions. NPs with photocatalysis activity are mostly metal oxides, such as CuO, TiO_2, ZnO and Fe_3O_4 (Li *et al.*, 2012), and metals, such as Ag (Choi and Hu, 2008). During photocatalysis, reactive oxygen species (ROS) are generated in the form of hydrogen peroxide, hydroxyl radicals and peroxide (Manke *et al.*, 2013). These ROS are toxic to bacteria, as they can damage cellular constituents, such as DNA, lipids and proteins, resulting in bactericidal and bacteriostatic effects (Storz and Imlay, 1999). In plant disease management, an NP formulation of titanium dioxide (TiO_2) induced photocatalysis, resulting in antimicrobial effects against the bacterial spot pathogen *Xanthomonas perforans* (Paret *et al.*, 2013). Interestingly, doping the TiO_2 NPs with Ag and Zn significantly increased the photocatalytic activity against *X. perforans*. Treatment of *X. perforans*-infected tomato plants with TiO_2/Zn NPs at approximately 500–800 ppm significantly reduced bacterial spot severity compared with untreated and copper controls (Paret *et al.*, 2013). ZnO and Ag NPs also exhibited promising antimicrobial activity against *E. amylovora* with minimum inhibitory concentrations of 83.3 and 108.3 ppm, respectively (Q. Zeng, unpublished information).

Some NPs can exert antimicrobial activity through the release of ions, such as Ag^+, Zn^{2+} and Cu^{2+}, which are toxic to bacteria. For example, the release of Ag^+ greatly contributed to the antimicrobial activity of Ag NPs (Lok *et al.*, 2007). Some modifications and improvements can also enhance the antimicrobial activities of NPs: for example, the association of NPs with a support material has recently shown promising antimicrobial effects. Bare NPs tend to form agglomerations that weaken the antimicrobial activity (Ocsoy *et al.*, 2013). Grapheme oxide (GO) has been used as support material to grow Ag NPs, significantly reducing agglomeration and enhancing the antimicrobial activity against the bacterial spot pathogen *X. perforans* (Ocsoy *et al.*, 2013). In a glasshouse assay, control of bacterial spot on infected tomato transplants treated with 100 ppm of the Ag@dsDNA@GO NPs occurred at a similar level as conventional copper treatment.

In addition to antimicrobial activities, NPs have also been suggested to be efficient delivery systems for many other antimicrobial compounds. Multiple NP delivery systems, such as hydrogel, dentrimers, liposomes, carbon nanotubes, micelles, and micro- and nanoemulsions, have been studied for the delivery of various active ingredients (De *et al.*, 2014). Among them, nanoemulsions have shown promising delivery of an agriculturally important herbicide (Lim *et al.*, 2012), and a nanoemulsion formulation was able to enhance the permeability of the antibiotic ampicillin

through the citrus cuticle into the phloem via a foliar spray targeted against Huanglongbing disease (Yang *et al.*, 2015). As the plant cuticle (wax, cutin and pectin) acts as the major barrier preventing antimicrobial compounds from penetrating into plant tissues, and as many plant-pathogenic bacteria infect the phloem and xylem tissues, the development of antimicrobial delivery technology which penetrates through the cuticle may have wide applications in the control of bacterial plant diseases.

In summary, NPs possess many desirable traits that may make them excellent antimicrobials for the management of bacterial plant diseases in the future. The application of antimicrobial NPs in agriculture could significantly enlarge our selection of antimicrobials. In addition, most NPs display antimicrobial activities through different mechanisms, which could possibly reduce the risk of resistance development. The application of NPs as delivery systems for pesticides also opens up new opportunities to enhance the efficacy of currently available antimicrobials targeting pathogens inside plants. However, limitations, such as cost, material preparation, particle agglomeration and efficient active ingredient delivery, still need to be resolved. Moreover, the environmental fate of NPs needs to be determined before large-scale application, and the potential for persistence of NPs in food products must be determined because of toxicity effects (for example, see Gaillet and Rouanet, 2015; Nel *et al.*, 2006).

INNOVATIONS IN DELIVERY AND OTHER OPPORTUNITIES FOR DISEASE MANAGEMENT

Since the early 1950s, the conventional delivery of bactericides and other pesticides in plant agriculture has been via air blast sprayers. This technology is fairly inefficient, in that up to 40%–70% of sprayed materials can be lost via drift (Steiner, 1969). In addition, these chemicals target surface bacterial populations, with only very limited potential effects on systemic bacterial pathogens. Opportunities for improvements in disease management will require more thinking 'outside the box' to develop new methods of delivery or new strategies to combat pathogens. For example, endotherapy approaches with fruit trees conferred reductions in the incidence of fire blight disease over longer time periods than conventional sprays (Acimovic *et al.*, 2015), and may prove to be an effective disease management strategy for systemic bacterial diseases. Likewise, a thermal therapy treatment for Huanglongbing disease, involving the continuous exposure of infected citrus seedlings to temperatures of 40–42°C for 7–10 days, significantly reduced the detectable titre of '*Candidatus* Liberibacter asiaticus' (Hoffmann *et al.*, 2013). The large-scale implementation of approaches such as these at the farm level with mature trees appears to be impractical and is currently not feasible; however, the systems are effective at a research scale and represent possible new practices that could benefit from interdisciplinary research input from agricultural engineers and others to improve the practicality and chances of future success. Perhaps new management methods for systemic bacterial pathogens could be developed following novel pathways similar to those described.

THE NEXT HAMMER: GENOME EDITING METHODS FOR THE DEVELOPMENT OF DURABLE RESISTANCE

The deciphering of the TALE code for specific DNA binding has opened up new avenues for the design of new and more powerful tools for genome modification. TALE nucleases (TALENs) are TALE-binding domains, fused to the *Fok*I nuclease cleavage domain, which result in a very precise sequence-directed molecular tool to introduce double-strand breaks (dsbs) at specific genomic locations (Christian *et al.*, 2010; Miller *et al.*, 2011). dsbs induce native DNA repair mechanisms, such as non-homologous end joining, an error-prone repair mechanism that introduces variable length insertions or deletions at the breaking point, generally resulting in frame shifts in the target gene, rendering it non-functional. The second mechanism is homology-directed repair that inserts homologous DNA templates at the targeted point, allowing the precise insertion or deletion of nucleotides in a specific locus (Joung and Sander, 2013). This technology has proven to be an efficient mechanism for genome editing, not only for model plant organisms, such as *Arabidopsis thaliana* and tobacco, but also for economically important crop plants, including soybean, rice and corn (Cermak *et al.*, 2011; Haun *et al.*, 2014; *Li et al.*, 2012; Liang *et al.*, 2014; Zhao *et al.*, 2013). A successful example of genome modification using TALEN to engineer bacterial blight-resistant rice cultivars has been reported by *Li et al.* (2012). In this study, TALEN technology was employed to target the *S* gene *Os11N3* (*OsSWEET14*), which encodes a member of the SWEET sucrose efflux transporter family, and is activated by *X. oryzae* pv. *oryzae* through at least four different TALE proteins (*AvrXa7*, *PthXo3*, *TalC* and *Tal5*) to divert sugar transport and favour pathogen nutrition and proliferation within the host (Streubel *et al.*, 2013). Sequence-specific TALEN editing of EBE in the promoter region of *OsSWEET14* rendered the promoter insensitive to AvrXa7 and PthXo3, abrogating the activation of the *S* gene, without compromising the normal function of the sucrose efflux system in the plant (Li *et al.*, 2012).

Another recently developed technology that has been widely exploited for genome editing is the CRISPR/Cas9 system. Clustered regularly interspaced short palindromic repeats (CRISPRs) are a family of DNA repeats present in most Archaea and in about 40% of bacterial species, which provide acquired immunity against foreign DNA from bacteriophages or plasmids (Horvath and Barrangou, 2010). These distinctive loci consist of repetitive palindromic sequences (21–47 bp), separated by hypervariable spacer sequences that exhibit homology to exogenous viral and plasmid sequences, ranging between 21 and 72 bp. These arrays are often

located adjacent to helper *cas* (CRISPR-associated) genes that encode polymerases, nucleases and helicases. When spacer sequences are transcribed, they generate small CRISPR-RNA (crRNA) fragments that hybridize with a small non-coding trans-activating crRNA (tracrRNA). This double-stranded RNA molecule is used as a guide to target invading DNA sequences as a result of complementarity, which directs the Cas protein complex to these sequences for DNA degradation by double-strand cleavage (Horvath and Barrangou, 2010). Three types (I, II and III) of CRISPR/Cas system have been described. In type II systems, the RNA-guided nuclease Cas9 is responsible for DNA cleavage as a result of its nuclease domains. A fusion of both crRNA and tracrRNA in a single small guide molecule (sgRNAs) of about 20 nucleotides has been demonstrated to be functional for the introduction of genome modifications with precise target specificity (Jinek *et al.*, 2012). As a result of their small size, multiple sgRNAs can be used together with Cas9 for the simultaneous targeting of multiple loci. This multiplex genome editing feature poses an advantage over other genome editing technologies, including TALENs. Moreover, a modification of the Cas9 endonuclease to induce nickase activity rather than double-strand cleavage has been used to target specific regulatory elements of a gene of interest, repressing its transcription in a mechanism referred to as CRISPR interference (CRISPRi; Qi *et al.*, 2013).

The CRISPR/Cas9 genome editing technology has been successfully employed for the genetic editing of single or multiple gene targets in several plants, such as *A. thaliana*, tobacco, rice and sweet orange (Gao *et al.*, 2015; Jia and Wang, 2014; Li *et al.*, 2013; Shan *et al.*, 2014). Moreover, this new technology has been shown to be efficacious for the generation of mutations in the *TaMLO-A1* allele of bread wheat, which encode a protein that represses plant defence against powdery mildew. This modification conferred resistance to the pathogen (Wang *et al.*, 2014), demonstrating that CRISPR/Cas editing can be successfully employed in the engineering of durable resistance, even at different levels of ploidy.

CONCLUSIONS

During the past two decades, a burst in research focused on the molecular biology and pathogenesis of plant-pathogenic bacteria, and the determinant factors in host–pathogen interactions, has opened the door to a new era in bacterial disease management, from targeting virulence factors to molecular breeding for durable broad-spectrum resistance. The continuously increasing amount of data has resulted in a greater understanding of the function of type III effector proteins and their targets in specific plant hosts and has broadened the potential of what applications can be considered as new possibilities for the management of disease with host resistance. Novel genome editing technologies, such as the TALEN and CRISPR/Cas9 systems, provide plant breeders with tools for the generation of new sources of resistance, not only for bacterial diseases, but also at a much broader level, without introducing foreign DNA material into the host genome. Moreover, the increased precision of these new methodologies and the possibility of resistance stacking offer a very promising scenario for multi-resistance development, with a reduced effort and time framework compared with traditional breeding strategies. However, we are currently within an interim time period in bacterial plant disease management. All of the new innovative strategies described here are still in various stages of development. Thus, growers will continue to rely on existing methods, including the use of commercially available bactericides and biological controls and plant cultivars bred for resistance.

One aspect of the novel management tools under development, which, to our knowledge, has not been widely considered, is that the deployment of these novel tools may not be via currently existing technologies. Are current spray technologies for crops the best method to deliver disease management chemicals? Other considerations include the way in which crops are grown, i.e. does the use of crop monocultures represent the best strategy to deploy novel cultivars developed with genome editing technology and predicted to harbour durable resistance? In addition, will the public accept food crops engineered for disease resistance using genome editing technology? It is clear that the genetic and genomic advancements in plant bacteriology research that are predicted to protect food supplies and provide consistency in increasing crop yields must also be accompanied by similar scale advancements addressing engineering and sociological questions, as these ancillary topics will be just as critical. Indeed, if advances in genetics and genomics represent a second generation in terms of bacterial disease management, the 'implementation of innovation', including effective delivery of innovative management strategies and successes in consumer acceptance of these strategies, will probably represent a third generation of advances needed for the realization of sustainable disease management.

ACKNOWLEDGEMENTS

Work in the authors' laboratories is funded by the Agriculture and Food Research Initiative competitive grants 2013-51106-20944 (GWS), 2015-67013-23068 (GWS) and 2016-67030-24856 (QZ) from the US Department of Agriculture National Institute of Food and Agriculture, Michigan State University AgBioResearch, Project GREEEN, a Michigan plant agriculture initiative at Michigan State University, the Northeastern IPM Partnership Grant (QZ) and USDA-NIFA Hatch grant (QZ). We thank Adriana Bernal, Ana Maria Bocanczy, Megan Botti-Marino, Martin Chilvers, Cesdar Medina and Nian Wang for the use of disease symptom photographs.

REFERENCES

Acimovic, S.G., Zeng, Q., McGhee, G.C., Sundin, G.W. and Wise, J.C. (2015) Control of fire blight (*Erwinia amylovora*) on apple trees with trunk-injected plant resistance inducers and antibiotics and assessment of induction of pathogenesis-related protein genes. *Front. Plant Sci.* **6**, 16.

Bauer, W.D. and Mathesius, U. (2004) Plant responses to bacterial quorum sensing signals. *Curr. Opin. Plant Biol.* **7**, 429–433.

Behlau, F., Canteros, B.I., Minsavage, G.V., Jones, J.B. and Graham, J.H. (2011) Molecular characterization of copper resistance genes from *Xanthomonas citri* subsp. *citri* and *Xanthomonas alfalfae* subsp. *citrumelonis*. *Appl. Environ. Microbiol.* **77**, 4089–4096.

Behlau, F., Hong, J.C., Jones, J.B. and Graham, J.H. (2013) Evidence for acquisition of copper resistance genes from different sources in citrus-associated xanthomonads. *Phytopathology*, **103**, 409–418.

Blair, J.M.A., Webber, M.A., Baylay, A.J., Ogbolu, D.O. and Piddock, L.J.V. (2015) Molecular mechanisms of antibiotic resistance. *Nat. Rev. Microbiol.* **13**, 42–51.

Boch, J., Scholze, H., Schornack, S., Landgraf, A., Hahn, S., Kay, S., Lahaye, T., Nickstadt, A. and Bonas, U. (2009) Breaking the code of DNA binding specificity of TAL-type III effectors. *Science*, **326**, 1509–1512.

Boch, J., Bonas, U. and Lahaye, T. (2014) TAL effectors–pathogen strategies and plant resistance engineering. *New Phytol.* **204**, 823–832.

von Bodman, S.B., Bauer, W.D. and Coplin, D.L. (2003) Quorum sensing in plant-pathogenic bacteria. *Annu. Rev. Phytopathol.* **41**, 455–482.

Brackman, G., Cos, P., Maes, L., Nelis, H.J. and Coenye, T. (2011) Quorum sensing inhibitors increase the susceptibility of bacterial biofilms to antibiotics *in vitro* and *in vivo*. *Antimicrob. Agents Chemother.* **55**, 2655–2661.

Breen, S., Solomon, P.S., Bedon, F. and Vincent, D. (2015) Surveying the potential of secreted antimicrobial peptides to enhance plant disease resistance. *Front. Plant Sci.* **6**, 900.

Brogden, A. (2005) Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat. Rev. Microbiol.* **3**, 238–250.

Brown, E.D. and Wright, G.D. (2016) Antibacterial drug discovery in the resistance era. *Nature*, **529**, 336–343.

Cermak, T., Doyle, E.L., Christian, M., Wang, L., Zhang, Y., Schmidt, C., Baller, J.A., Somia, N.V., Bogdanove, A.J. and Voytas, D.F. (2011) Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic Acids Res.* **39**, e82.

Chiou, C.S. and Jones, A.L. (1993) Nucleotide sequence analysis of a transposon (Tn*5393*) carrying streptomycin resistance genes in *Erwinia amylovora* and other gram-negative bacteria. *J. Bacteriol.* **175**, 732–740.

Choi, O. and Hu, Z. (2008) Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. *Environ. Sci. Technol.* **42**, 4583–4588.

Christian, M., Cermak, T., Doyle, E.L., Schmidt, C., Zhang, F., Hummel, A., Bogdanove, A.J. and Voytas, D.F. (2010) Targeting DNA double-strand breaks with TAL effector nucleases. *Genetics*, **186**, 757–761.

Chua, S.L., Hultqvist, L.D., Yuan, M., Rybtke, M., Nielsen, T.E., Givskov, M., Tolker-Nielsen, T. and Yang, L. (2015) *In vitro* and *in vivo* generation and characterization of *Pseudomonas aeruginosa* biofilm-dispersed cells via c-di-GMP manipulation. *Nat. Protoc.* **10**, 1165–1180.

Cooksey, D.A. (1990) Genetics of bactericide resistance in plant pathogenic bacteria. *Annu. Rev. Phytopathol.* **28**, 201–219.

Danhorn, T. and Fuqua, C. (2007) Biofilm formation by plant-associated bacteria. *Annu. Rev. Microbiol.* **61**, 401–422.

De, A., Bose, R., Kumar, A. and Mozumdar, S. (2014) Targeted delivery of pesticides using biodegradable polymeric nanoparticles. *Antimicrob. Agents Chemother.* **49**, 249–255.

Dixon, R.A. and Paiva, N.L. (1995) Stress-induced phenylpropanoid metabolism. *Plant Cell*, **7**, 1085.

Dodds, P.N. and Rathjen, J.P. (2010) Plant immunity: towards an integrated view of plant–pathogen interactions. *Nat. Rev. Genet.* **11**, 539–548.

Dong, Y.H., Wang, L.H., Xu, J.L., Zhang, H.B., Zhang, X.F. and Zhang, L.H. (2001) Quenching quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase. *Nature*, **411**, 813–817.

Doyle, E.L., Stoddard, B.L., Voytas, D.F. and Bogdanove, A.J. (2013) TAL effectors: highly adaptable phytobacterial virulence factors and readily engineered DNA-targeting proteins. *Trends Cell. Biol.* **23**, 390–398.

Dudler, R. and Eberl, L. (2006) Interactions between bacteria and eukaryotes via small molecules. *Curr. Opin. Biotechnol.* **17**, 268–273.

Durrant, W.E. and Dong, X. (2004) Systemic acquired resistance. *Annu. Rev. Phytopathol.* **42**, 185–209.

Feys, B.J. and Parker, J.E. (2000) Interplay of signaling pathways in plant disease resistance. *Trends Genet.* **16**, 449–455.

Forsberg, K.J., Reyes, A., Wang, B., Selleck, E.M., Sommer, M.O.A. and Dantas, G. (2012) The shared antibiotic resistome of soil bacteria and human pathogens. *Science*, **337**, 1107–1111.

Förster, H., McGhee, G.C., Sundin, G.W. and Adaskaveg, J.E. (2015) Characterization of streptomycin resistance in isolates of *Erwinia amylovora* from California. *Phytopathology*, **105**, 1302–1310.

Gaillet, S. and Rouanet, J.M. (2015) Silver nanoparticles: their potential toxic effects after oral exposure and underlying mechanisms – a review. *Food Chem. Toxicol.* **77**, 58–63.

Gao, J., Wang, G., Ma, S., Xie, X., Wu, X., Zhang, X., Wu, Y., Zhao, P. and Xia, Q. (2015) CRISPR/Cas9-mediated targeted mutagenesis in *Nicotiana tabacum*. *Plant Mol. Biol.* **87**, 99–110.

Ghareeb, H., Bozso, Z., Ott, P.G., Repenning, C., Stahl, F. and Wydra, K. (2011) Transcriptome of silicon-induced resistance against *Ralstonia solanacearum* in the silicon non-accumulator tomato indicates priming effect. *Physiol. Mol. Plant Pathol.* **75**, 83–89.

Graham, J.H. and Myers, M.E. (2011) Soil application of SAR inducers imidacloprid, thiamethoxam, and acibenzolar-*S*-methyl for citrus canker control in young grapefruit trees. *Plant Dis.* **95**, 725–728.

Gutierrez-Barranquero, J.A., de Vicente, A., Carrion, V.J., Sundin, G.W. and Cazorla, F.M. (2013) Recruitment and rearrangement of three different genetic determinants into a conjugative plasmid increase copper resistance in *Pseudomonas syringae*. *Appl. Environ. Microbiol.* **79**, 1028–1033.

Hall-Stoodley, L., Costerton, J.W. and Stoodley, P. (2004) Bacterial biofilms: from the natural environment to infectious diseases. *Nat. Rev. Microbiol.* **2**, 95–108.

Haun, W., Coffman, A., Clasen, B.M., Demorest, Z.L., Lowy, A., Ray, E., Retterath, A., Stoddard, T., Juillerat, A., Cedrone, F., Mathis, L., Voytas, D.F. and Zhang, F. (2014) Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family. *Plant Biotechnol. J.* **12**, 934–940.

Helman, Y. and Chernin, L. (2015) Silencing the mob: disrupting quorum sensing as a means to fight plant disease. *Mol. Plant Pathol.* **16**, 316–329.

Hert, A.P., Marutani, M., Momol, M.T., Roberts, P.D., Olson, S.M. and Jones, J.B. (2009) Suppression of the bacterial spot pathogen *Xanthomonas euvesicatoria* on tomato leaves by an attenuated mutant of *Xanthomonas perforans*. *Appl. Environ. Microbiol.* **75**, 3323–3330.

Hoffmann, M.T., Doud, M.S., Williams, L., Zhang, M.Q., Ding, F., Stover, E., Hall, D., Zhang, S., Jones, L., Gooch, M., Fleites, L., Dixon, W., Gabriel, D. and Duan, Y.D. (2013) Heat treatment eliminates '*Candidatus* Liberibacter asiaticus' from infected citrus trees under controlled conditions. *Phytopathology*, **13**, 15–22.

van der Hoorn, R.A. and Kamoun, S. (2008) From guard to decoy: a new model for perception of plant pathogen effectors. *Plant Cell*, **20**, 2009–2017.

Horvath, P. and Barrangou, R. (2010) CRISPR/Cas, the immune system of Bacteria and Archaea. *Science*, **327**, 167–170.

Hummel, A.W., Doyle, E.L. and Bogdanove, A.J. (2012) Addition of transcription activator-like effector binding sites to a pathogen strain-specific rice bacterial blight resistance gene makes it effective against additional strains and against bacterial leaf streak. *New Phytol.* **195**, 883–893.

Jia, H. and Wang, N. (2014) Targeted genome editing of sweet orange using Cas9/sgRNA. *PLoS One*, **9**, e93806.

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A. and Charpentier, E. (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, **337**, 816–821.

Johnson, K.B. (2010) Pathogen refuge: a key to understanding biological control. *Annu. Rev. Phytopathol.* **48**, 141–160.

Johnson, K.B. and Stockwell, V.O. (1998) Management of fire blight: a case study in microbial ecology. *Annu. Rev. Phytopathol.* **36**, 227–248.

Jones, J.B., Jackson, L.E., Balogh, B., Obradovic, A., Iriarte, F.B. and Momol, M.T. (2007) Bacteriophages for plant disease control. *Annu. Rev. Phytopathol.* **45**, 245–262.

Jones, J.D.G. and Dangl, J.L. (2006) The plant immune system. *Nature*, **444**, 323–329.

Joung, J.K. and Sander, J.D. (2013) TALENs: a widely applicable technology for targeted genome editing. *Nat. Rev. Mol. Cell. Biol.* **14**, 49–55.

Kerr, A. (2016) Biological control of crown gall. *Australas. Plant Pathol.* **45**, 15–18.

Khokhani, D., Zhang, C., Li, Y., Wang, Q., Zeng, Q., Yamazaki, A., Hutchins, W., Zhou, S.S., Chen, X. and Yang, C.H. (2013) Discovery of plant phenolic compounds that act as type III secretion system inhibitors or inducers of the fire blight pathogen, *Erwinia amylovora*. *Appl. Environ. Microbiol.* **79**, 5424–5436.

Kleitman, F., Shtienberg, D., Blachinsky, D., Oppenheim, D., Zilberstaine, M., Dror, O. and Manulis, S. (2005) *Erwinia amylovora* populations resistant to oxolinic acid in Israel: prevalence, persistence and fitness. *Plant Pathol.* **54**, 108–115.

Kloepper, J.W., Ryu, C.M. and Zhang, S. (2004) Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology*, **94**, 1259–1266.

Koczan, J.M., McGrath, M.J., Zhao, Y. and Sundin, G.W. (2009) Contribution of *Erwinia amylovora* exopolysaccharides amylovoran and levan to biofilm formation: implications in pathogenicity. *Phytopathology*, **99**, 1237–1244.

Kolodkin-Gal, I., Romero, D., Cao, S., Clardy, J., Kolter, R. and Losick, R. (2010) D-amino acids trigger biofilm disassembly. *Science*, **328**, 627–629.

Kostakioti, M., Hadjifrangiskou, M. and Hultgren, S.J. (2013) Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. *Cold Spring Harb. Perspect. Med.* **3**, a010306.

Leach, J.E., Vera Cruz, C.M., Bai, J. and Leung, H. (2001) Pathogen fitness penalty as a predictor of durability of disease resistance genes. *Annu. Rev. Phytopathol.* **39**, 187–224.

Lee, Y.A., Hendson, M., Panopolous, N.J. and Schroth, M.N. (1994) Molecular cloning, chromosomal mapping, and sequence analysis of copper resistance genes from *Xanthomonas campestris* pv. *juglandis* – homology with small blue copper proteins and multicopper oxidase. *J. Bacteriol.* **176**, 173–188.

Li, J. and Wang, N. (2014) Foliar application of biofilm formation-inhibiting compounds enhances control of citrus canker caused by *Xanthomonas citri* subsp. *citri*. *Phytopathology*, **104**, 134–142.

Li, J.F., Norville, J.E., Aach, J., McCormack, M., Zhang, D., Bush, J., Church, G.M. and Sheen, J. (2013) Multiplex and homologous recombination-mediated genome editing in *Arabidopsis* and *Nicotiana benthamiana* using guide RNA and Cas9. *Nat. Biotechnol.* **31**, 688–691.

Li, J.Y., Trivedi, P. and Wang, N. (2016) Field evaluation of plant defense inducers for the control of citrus huanglongbing. *Phytopathology*, **106**, 37–46.

Li, T., Liu, B., Spalding, M.H., Weeks, D.P. and Yang, B. (2012) High-efficiency TALEN-based gene editing produces disease-resistant rice. *Nat. Biotechnol.* **30**, 390–392.

Li, Y., Peng, Q., Selimi, D., Wang, Q., Charkowski, A.O., Chen, X. and Yang, C.H. (2009) The plant phenolic compound p-coumaric acid represses gene expression in the *Dickeya dadantii* type III secretion system. *Appl. Environ. Microbiol.* **75**, 1223–1228.

Li, Y., Zhang, W., Niu, J. and Chen, Y. (2012) Mechanism of photogenerated reactive oxygen species and correlation with the antibacterial properties of engineered metal-oxide nanoparticles. *ACS Nano*, **6**, 5164–5173.

Li, Y., Hutchins, W., Wu, X., Liang, C., Zhang, C., Yuan, X., Khokhani, D., Chen, X., Che, Y., Wang, Q. and Yang, C.H. (2015) Derivative of plant phenolic compound inhibits the type III secretion system of *Dickeya dadantii* via HrpX/HrpY two-component signal transduction and Rsm systems. *Mol. Plant Pathol.* **16**, 150–163.

Liang, Z., Zhang, K., Chen, K. and Gao, C. (2014) Targeted mutagenesis in *Zea mays* using TALENs and the CRISPR/Cas system. *J. Genet. Genomics*, **41**, 63–68.

Lim, C.J., Basria, M., Omarc, D., Rahman, M.B.A., Salleh, A.B. and Rahman, R.N.Z.R.A. (2012) Physicochemical characterization and formation of glyphosate-laden nano-emulsion for herbicide formulation. *Ind. Crop. Prod.* **36**, 607–613.

Lindow, S., Newman, K., Chatterjee, S., Baccarai, C., Iavarone, A.T. and Ionescu, M. (2014) Production of *Xylella fastidiosa* diffusible signal factor in transgenic grape causes pathogen confusion and reduction in severity of Pierce's disease. *Mol. Plant–Microbe Interact.* **27**, 244–254.

Lok, C.N., Ho, C.M., Chen, R., He, Q.Y., Yu, W.Y., Sun, H., Tam, P.K., Chiu, J.F. and Che, C.M. (2007) Silver nanoparticles: partial oxidation and antibacterial activities. *J. Biol. Inorg. Chem.* **12**, 527–534.

Macho, A.P. and Zipfel, C. (2015) Targeting of plant pattern recognition receptor-triggered immunity by bacterial type-III secretion system effectors. *Curr. Opin. Microbiol.* **23**, 14–22.

Manke, A., Wang, L. and Rojanasakul, Y. (2013) Mechanisms of nanoparticle-induced oxidative stress and toxicity. *Biomed. Res. Int.* **2013**, 942916.

Massart, S., Perazzolli, M., Hofte, M., Pertot, I. and Jijakli, M.H. (2015) Impact of the omic technologies for understanding the modes of action of biological control agents against pathogens. *BioControl*, **60**, 725–746.

Maxson-Stein, K., He, S.Y., Hammerschmidt, R. and Jones, A.L. (2002) Effect of treating apple trees with acibenzolar-*S*-methyl on fire blight and expression of pathogenesis-related protein genes. *Plant Dis.* **86**, 785–790.

McGhee, G.C. and Sundin, G.W. (2011) Evaluation of kasugamycin for fire blight management, effect on nontarget bacteria, and assessment of kasugamycin resistance potential in *Erwinia amylovora*. *Phytopathology*, **101**, 192–204.

McManus, P.S., Stockwell, V.O., Sundin, G.W. and Jones, A.L. (2002) Antibiotic use in plant agriculture. *Annu. Rev. Phytopathol.* **40**, 443–465.

Mellano, M.A. and Cooksey, D.A. (1988) Nucleotide sequence and organization of copper resistance genes from *Pseudomonas syringae* pv. *tomato*. *J. Bacteriol.* **170**, 2879–2883.

Miller, J.C., Tan, S., Qiao, G., Barlow, K.A., Wang, J., Xia, D.F., Meng, X., Paschon, D.E., Leung, E., Hinkley, S.J., Dulay, G.P., Hua, K.L., Ankoudinova, I., Cost, G.J., Urnov, F.D., Zhang, H.S., Holmes, M.C., Zhang, L., Gregory, P.D. and Rebar, E.J. (2011) A TALE nuclease architecture for efficient genome editing. *Nat. Biotechnol.* **29**, 143–148.

Molina, L., Constantinescu, F., Michel, L., Reinmann, C., Duffy, B. and Defago, G. (2003) Degradation of pathogen quorum-sensing molecules by soil bacteria: a preventive and curative biological control mechanism. *FEMS Microbiol. Ecol.* **45**, 71–81.

Moller, W.J., Schroth, M.N. and Thomson, S.V. (1981) The scenario of fire blight and streptomycin resistance. *Plant Dis.* **65**, 563–568.

Montesano, M., Brader, G., Ponce de Leon, I. and Palva, E.T. (2005) Multiple defence signals induced by *Erwinia carotovora* ssp. *carotovora* elicitors in potato. *Mol. Plant Pathol.* **6**, 541–549.

Montesinos, E. (2007) Antimicrobial peptides and plant disease control. *FEMS Microbiol. Lett.* **270**, 1–11.

Moss, W.P., Byrne, J.M., Campbell, H.L., Ji, P., Bonas, U., Jones, J.B. and Wilson, M. (2007) Biological control of bacterial spot of tomato using *hrp* mutants of *Xanthomonas campestris* pv. *vesicatoria*. *Biol. Cont.* **41**, 199–206.

Muranaka, L.S., Giorgiano, T.E., Takita, M.A., Forim, M.R., Silva, L.F.C., Coletta-Filho, H.D., Machado, M.A. and de Souza, A.A. (2013) N-acetylcysteine in agriculture, a novel use for an old molecule: focus on controlling the plant pathogen *Xylella fastidiosa*. *PLoS One*, **8**, e72937.

Nadal, A., Montero, M., Company, N., Badosa, E., Messeguer, J., Montesinos, L., Montesinos, E. and Pla, M. (2012) Constitutive expression of transgenes encoding derivatives of the synthetic antimicrobial peptide BP100: impact on rice host plant fitness. *BMC Plant Biol.* **12**, 159.

Nel, A., Xia, T., Madler, L. and Li, N. (2006) Toxic potential of materials at the nanolevel. *Science*, **311**, 622–627.

Ocsoy, I., Paret, M.L., Ocsoy, M.A., Kunwar, S., Chen, T., You, M.X. and Tan, W.H. (2013) Nanotechnology in plant disease management: DNA-directed silver nanoparticles on graphene oxide as an antibacterial against *Xanthomonas perforans*. *ACS Nano*, **7**, 8972–8980.

Parachin, N.S., Mulder, K.C., Viana, A.A.B., Dias, S.C. and Franco, O.L. (2012) Expression systems for heterologous production of antimicrobial peptides. *Peptides*, **38**, 446–456.

Paret, M.L., Vallad, G.E., Averett, D.R., Jones, J.B. and Olson, S.M. (2013) Photocatalysis: effect of light-activated nanoscale formulations of $TiO_{(2)}$ on *Xanthomonas perforans* and control of bacterial spot of tomato. *Phytopathology*, **103**, 228–236.

Perry, J.A. and Wright, G.D. (2013) The antibiotic resistance "mobilome": searching for the link between environment and clinic. *Front. Microbiol.* **4**, 138.

Pieterse, C.M.J., Zamioudis, C., Berensen, R.L., Weller, D.M., Van Wees, S.C.M. and Bakker, P.A.H.M. (2014) Induced systemic resistance by beneficial microbes. *Annu. Rev. Phytopathol.* **52**, 347–375.

Pradhanang, P.M., Ji, P., Momol, M.T., Olson, S.M., Mayfield, J.L. and Jones, J.B. (2005) Application of acibenzolar-*S*-methyl enhances host resistance in tomato against *Ralstonia solanacearum*. *Plant Dis.* **89**, 989–993.

Qi, L.S., Larson, M.H., Gilbert, L.A., Doudna, J.A., Weissman, J.S., Arkin, A.P. and Lim, W.A. (2013) Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell*, **152**, 1173–1183.

Redondo, C., Sena-Velez, M., Gell, I., Ferragud, E., Sabuquillo, P., Graham, J.H. and Cubero, J. (2015) Influence of selected bactericides on biofilm formation and viability of *Xanthomonas citri* subsp. *citri*. *Crop Prot.* **78**, 204–213.

Roberts, P.D., Momol, M.T., Ritchie, L., Olson, S.M., Jones, J.B. and Balogh, B. (2008) Evaluation of spray programs containing famoxadone plus cymoxanil, acibenzolar-*S*-methyl, and *Bacillus subtilis* compared to copper sprays for management of bacterial spot of tomato. *Crop Prot.* **27**, 1519–1526.

Rodrigues, C.M., Takita, M.A., Coletta-Filho, H.D., Olivato, J.C., Caserta, R., Machado, M.A. and de Souza, A.A. (2008) Copper resistance of biofilm cells of the plant pathogen *Xylella fastidiosa*. *Appl. Microbiol. Biotechnol.* **77**, 1145–1157.

Rodriguez, C., Lang, L., Wang, A., Altendorf, K., Garcia, F. and Lipski, A. (2006) Lettuce for human consumption in Costa Rica contains complex communities of culturable oxytetracycline- and gentamicin-resistant bacteria. *Appl. Environ. Microbiol.* **72**, 5870–5876.

Romling, U., Galperin, M.Y. and Gomelsky, M. (2013) Cyclic di-GMP: the first 25 years of a universal bacterial second messenger. *Microbiol. Mol. Biol. Rev.* **77**, 1–52.

Rutherford, S.T. and Bassler, B.L. (2012) Bacterial quorum sensing: its role in virulence and possibilities for its control. *Cold Spring Harb. Perspect. Med.* **2**, a012427.

Sarojini, V., Washington, V., Lewis, G., Mitchell, R., De Zoysa, G. and Mistry, M. (2010) A mechanistic investigation into the inhibition of growth and biofilm formation in *Erwinia amylovora* by non-protein amino acids. *Acta Hortic.* **896**, 541–545.

Schornack, S., Moscou, M.J., Ward, E.R. and Horvath, D.M. (2013) Engineering plant disease resistance based on TAL effectors. *Annu. Rev. Phytopathol.* **51**, 383–406.

Sen, Y., van der Wolf, J., Visser, R.G.F. and van Heusden, S. (2015) Bacterial canker of tomato: current knowledge of detection, management, resistance, and interactions. *Plant Dis.* **99**, 4–13.

Servin, A., Elmer, W., Mukherjee, A., De la Torre-Roche, R., Hamdi, H., White, J.C., Bindraban, P. and Dimka, C. (2015) A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. *J. Nanopart. Res.* **17**, 92.

Shan, Q., Wang, Y., Li, J. and Gao, C. (2014) Genome editing in rice and wheat using the CRISPR/Cas system. *Nat. Protoc.* **9**, 2395–2410.

Shtienberg, D., Zilberstaine, M., Oppenheim, D., Herzog, Z., Manulis, S., Shwartz, H. and Kritzman, G. (2001) Efficacy of oxolinic acid and other bactericides in suppression of *Erwinia amylovora* in pear orchards in Israel. *Phytoparasitica*, **29**, 143–154.

Silver, L.L. (2011) Challenges of antimicrobial discovery. *Clin. Microbiol. Rev.* **24**, 71–109.

Smillie, C.S., Smith, M.B., Friedman, J., Cordero, O.X., David, L.A. and Alm, E.J. (2011) Ecology drives a global network of gene exchange connecting the human microbiome. *Nature*, **480**, 241–244.

Stavrinides, J., McCann, H.C. and Guttman, D.S. (2008) Host–pathogen interplay and the evolution of bacterial effectors. *Cell Microbiol.* **10**, 285–292.

Steiner, P.W. (1969) The distribution of spray material between target and nontarget areas of a mature apple orchard by airblast equipment. MS Thesis, Cornell University, Ithaca, NY.

Storz, G. and Imlay, J.A. (1999) Oxidative stress. *Curr. Opin. Microbiol.* **2**, 188–194.

Streubel, J., Pesce, C., Hutin, M., Koebnik, R., Boch, J. and Szurek, B. (2013) Five phylogenetically close rice SWEET genes confer TAL effector-mediated susceptibility to *Xanthomonas oryzae* pv. *oryzae*. *New Phytol.* **200**, 808–819.

Sundin, G.W. and Bender, C.L. (1995) Expression of the *strA-strB* streptomycin resistance genes in *Pseudomonas syringae* and *Xanthomonas campestris* and characterization of IS*6100* in *Xanthomonas campestris*. *Appl. Environ. Microbiol.* **61**, 2891–2897.

Sundin, G.W. and Bender, C.L. (1996) Dissemination of the *strA-strB* streptomycin resistance genes among commensal and pathogenic bacteria from humans, animals, and plants. *Mol. Ecol.* **5**, 133–143.

Sundin, G.W., Werner, N.A., Yoder, K.S. and Aldwinckle, H.S. (2009) Field evaluation of biological control of fire blight in the eastern United States. *Plant Dis.* **93**, 386–394.

Vidal, S., deLeon, I.P., Denecke, J. and Palva, E.T. (1997) Salicylic acid and the plant pathogen *Erwinia carotovora* induce defense genes via antagonistic pathways. *Plant J.* **11**, 115–123.

Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C. and Qiu, J.L. (2014) Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nat. Biotechnol.* **9**, 947–951.

Worthington, R.J., Rogers, S.A., Huigens, R.W., Melander, C. and Ritchie, D.F. (2012) Foliar-applied small molecule that suppresses biofilm formation and enhances control of copper-resistant *Xanthomonas euvesicatoria* on pepper. *Plant Dis.* **96**, 1638–1644.

Yamazaki, A., Li, J., Zeng, Q., Khokhani, D., Hutchins, W.C., Yost, A.C., Biddle, E., Toone, E.J., Chen, X. and Yang, C.H. (2012) Derivatives of plant phenolic compound affect the type III secretion system of *Pseudomonas aeruginosa* via a GacS-GacA two-component signal transduction system. *Antimicrob. Agents Chemother.* **56**, 36–43.

Yang, C., Powell, C.A., Duan, Y., Shatters, R. and Zhang, M. (2015) Antimicrobial nanoemulsion formulation with improved penetration of foliar spray through citrus leaf cuticles to control citrus huanglongbing. *PLoS One*, **10**, e0133826.

Yang, F., Korban, S.S., Pusey, P.L., Elofsson, M., Sundin, G.W. and Zhao, Y. (2014) Small-molecule inhibitors suppress the expression of both type III secretion and amylovoran biosynthesis genes in *Erwinia amylovora*. *Mol. Plant Pathol.* **15**, 44–57.

Yang, S., Peng, Q., San Francisco, M., Wang, Y., Zeng, Q. and Yang, C.H. (2008) Type III secretion system genes of *Dickeya dadantii* 3937 are induced by plant phenolic acids. *PLoS One*, **3**, e2973.

Yuan, X., Khokhani, D., Wu, X., Yang, F., Biener, G., Koestler, B.J., Raicu, V., He, C.Y., Waters, C.M., Sundin, G.W., Tian, F. and Yang, C.H. (2015) Cross-talk between a regulatory small RNA, cyclic-di-GMP signalling and flagellar regulator FlhDC for virulence and bacterial behaviours. *Environ. Microbiol.* **17**, 4745–4763.

Zaumeyer, W.J. (1958) Antibiotics in the control of plant diseases. *Annu. Rev. Microbiol.* **12**, 415–440.

Zeng, X., Tian, D., Gu, K., Zhou, Z., Yang, X., Luo, Y., White, F.F. and Yin, Z. (2015) Genetic engineering of the *Xa10* promoter for broad-spectrum and durable resistance to *Xanthomonas oryzae* pv. *oryzae*. *Plant Biotechnol. J.* **13**, 993–1001.

Zhang, J., Yin, Z. and White, F. (2015) TAL effectors and the executor R genes. *Front. Plant Sci.* **6**, 641.

Zhang, L., Gu, F.X., Chan, J.M., Wang, A.Z., Langer, R.S. and Farokhzad, O.C. (2008) Nanoparticles in medicine: therapeutic applications and developments. *Clin. Pharmacol. Ther.* **83**, 761–769.

Zhao, J., Wang, Z., Dai, Y. and Xing, B. (2013) Mitigation of CuO nanoparticle-induced bacterial membrane damage by dissolved organic matter. *Water Res.* **47**, 4169–4178.