

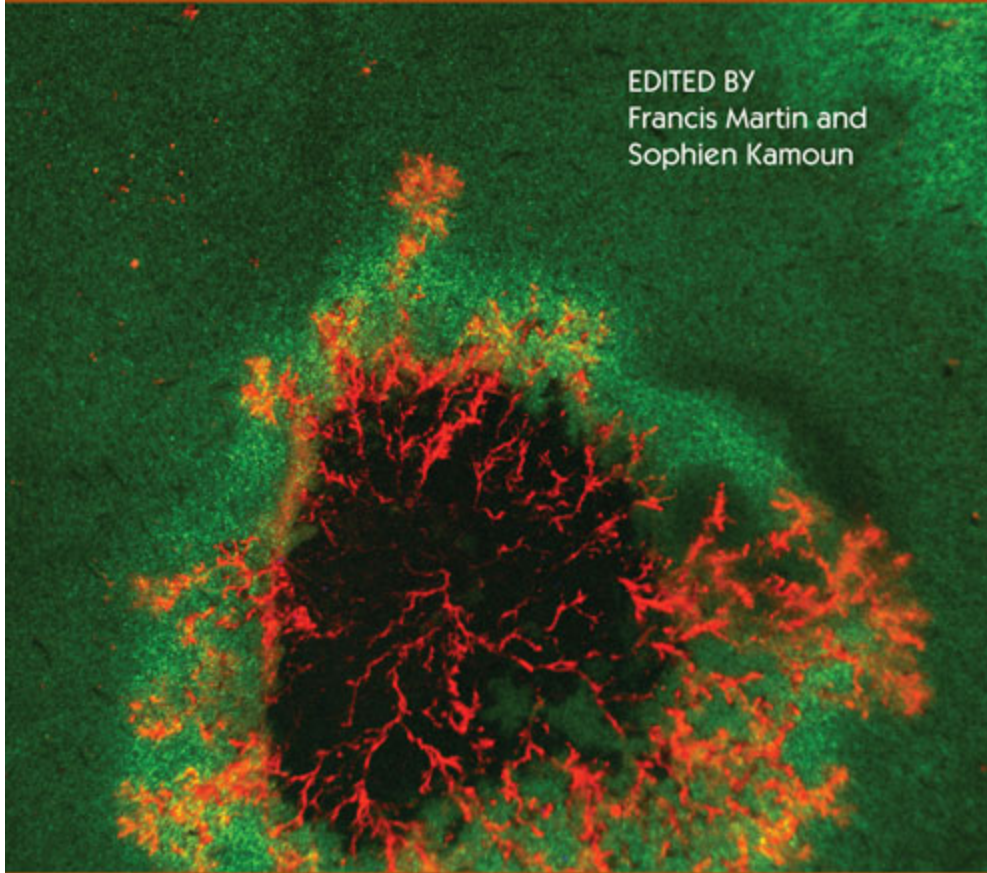
EFFECTORS IN PLANT-MICROBE INTERACTIONS

EDITED BY
Francis Martin and
Sophien Kamoun

 WILEY-BLACKWELL

EFFECTORS IN PLANT-MICROBE INTERACTIONS

EDITED BY
Francis Martin and
Sophien Kamoun



 WILEY-BLACKWELL

Contents

Cover

Title Page

Copyright

Contributors

Foreword

References

Preface

Section 1: Plant Immune Response Pathways

*1: Innate Immunity: Pattern
Recognition in Plants*

*1.1 Pattern Recognition through MAMPs
(Microbe-Associated Molecular Patterns)*

1.2 Some Classical MAMP-Receptor Pairs

*1.3 Physiological Responses and Signaling
Events Induced by Elicitors*

1.4 The Biological Relevance of PTI

References

2: Microbial Effectors and Their Role in Plant Defense Suppression

2.1 The Gene-for-Gene Concept and the Emergence of Effectors

2.2 Diversity of Effectors

2.3 Effector Targets

2.4 Models to Explain Recognition of Effectors by R-gene Products

2.5 Synthesis and Discussion

References

Section 2: Genome-Wide Analyses of Microbial Effectors and Effector Evolution

3: Comparative Genomics and Evolution of Bacterial Type III Effectors

3.1 Introduction

3.2 Effector Structure

3.3 Effector Acquisition

3.4 Effector Change and Loss

3.5 Effector Repertoire Evolution

3.6 Future Prospects

References

4: The Effectors of Smut Fungi

4.1 Introduction

4.2 Plant Responses to *U. maydis*

4.3 The effectors of *U. maydis*

4.4 Regulation of *U. maydis* Effector Genes

4.5 Stage and Organ Specificity of *U. maydis* Effectors

4.6 The Effectors of Smut Fungi Related to *U. maydis*

4.7 Outlook

4.8 Acknowledgements

References

5: Evolutionary and Functional Dynamics of Oomycete Effector Genes

5.1 Introduction

5.2 Oomycete Effectors Target Different Sites in Host Plant Tissue

5.3 Oomycete Effectors have a Modular Architecture

5.4 Oomycete Effector Genes Show Distinct Patterns of Expression During Plant Colonization

5.5 Effector Genes Populate Plastic Regions of Oomycete Genomes

5.6 Evolution of *P. infestans* Genome and Effector Genes Following Host Jumps

5.7 Several Oomycete Effectors Suppress Plant Immunity

5.8 Effectors Are Useful in Breeding and Deployment of Disease Resistance

5.9 Outlook
References

Section 3: Microbial Effector
Functions: Virulence and
Avirulence

6: Suppression and Activation of the
Plant Immune System by
Pseudomonas syringae Effectors
AvrPto and AvrPtoB

6.1 Pseudomonas syringae pv. tomato
Interactions with Plants

6.2 AvrPto and AvrPtoB Have Both
Redundant and Unique Activities in Plants

6.3 AvrPto is a Small Effector with Two PTI-
Suppressing Domains Both of Which Can
Activate ETI in Certain Solanaceous Plants

6.4 AvrPtoB is a Large Modular Effector
with Domains that Suppress PTI and ETI but
Which Also Activate ETI in Certain Tomato
Genotypes

6.5 AvrPtoB Virulence Activity

6.6 An Evolutionary Model of the Tomato-
Pseudomonas Interaction

6.7 Summary

6.8 Acknowledgments

References

7: Rust Effectors

7.1 General Introduction to Rusts

7.2 Identification of Effectors in Bean Rust and Flax Rust as Haustorial Secreted Proteins

7.3 Genome-Wide Effector Prediction in the Poplar Rust and Wheat Stem Rust Genomes

7.4 Comparative Genomics of Effectors

7.5 Function of Rust Effectors

7.6 Conclusions

References

8: Dothideomycete Effectors Facilitating Biotrophic and Necrotrophic Lifestyles

8.1 Introduction to Dothideomycetes

8.2 Pregenome Identification of Avirulence and Effector Genes in Dothideomycetes

8.3 Pregenomic Identification of Host-Selective Proteinaceous Toxins of Dothideomycetes

8.4 Whole-Genome Searches for Effectors

8.5 Translocation of Fungal Effectors

8.6 Effector Diversification and Avoidance of R Protein-Mediated Resistance

8.7 Concluding Remarks

8.8 Acknowledgements

References

Section 4: Effector Trafficking: Processing/Uptake by Plants and Secretion/Delivery by Microbes

9: Effector Translocation and Delivery by the Rice Blast Fungus Magnaporthe oryzae

9.1 Introduction

9.2 The Fungus Magnaporthe oryzae

9.3 Hyphal Tip Secretion in Filamentous Fungi

9.4 Identification of Magnaporthe oryzae Effectors

9.5 To BIC or Not to BIC—That Is the Question

9.6 Effector Translocation into Host Rice Cells by M. oryzae

9.7 Concluding Remarks

References

10: Entry of Oomycete and Fungal Effectors into Host Cells

10.1 Effector Entry into Host Cells

10.2 Mechanisms of Entry

10.3 Summary, Perspective, and Conclusions

10.4 Acknowledgments

Note

References

Section 5: Emerging Effectors- Symbionts, Nematodes, Insects, Metabolites

11: Roles of Effector Proteins in the Legume-Rhizobia Symbiosis

**11.1 Introduction to the Legume-Rhizobia
Symbiosis**

11.2 Nodule Formation

**11.3 Rhizobial Molecular Signals Required
for Nodule Formation**

11.4 Regulation of Rhizobial T3SS and T4SS

**11.5 Effects of T3SS and T4SS on
Nodulation**

**11.6 Secretion System Substrates—
Rhizobial Effectors**

11.7 Conclusions

References

12: Mutualistic Effectors: Architects of Symbiosis

12.1 The Concept of Mutualism

**12.2 Restructuring of Plant Signaling
Pathways**

12.3 Restructuring of Plant and Fungal Cell Wall Properties

12.4 Fungal Effectors Divert and Reprogram Plant Defenses

12.5 Fungal Effectors Restructure Nutrient Fluxes

12.6 Concluding Thoughts and Future Directions

References

13: Nematode Effector Proteins: Targets and Functions in Plant Parasitism

13.1 Introduction

13.2 Cell Wall-Modifying Effectors

13.3 Nematode Effectors Manipulating Plant Cell Biology

13.4 Nematode Effectors Manipulating Plant Defenses

13.5 Perspectives

References

14: Effectors in Plant-Insect Interactions

14.1 Introduction

14.2 Herbivore Behavior and Feeding Styles

14.3 Similarities between Plant-Microbe and Plant-Insect Interactions

[14.4 Evidence for Effectors That Elicit Plant Defenses in Plant-Insect Interactions](#)

[14.5 Evidence for Effectors That Promote Plant-Insect Interactions](#)

[14.6 Functional Genomics Approaches for Identification of Insect Effectors](#)

[14.7 Future Perspectives](#)

[Acknowledgments](#)

[References](#)

[15: Fungal Secondary Metabolites: Ancient Toxins and Novel Effectors in Plant-Microbe Interactions](#)

[15.1 Introduction to Fungal Secondary Metabolism](#)

[15.2 Fungal SMs as Effectors](#)

[15.3 Fungal Secondary Metabolism: A Genomic Perspective](#)

[15.4 Fungal SMs as Effectors Involved in Plant Infection](#)

[15.5 Next Challenges about Effector SMs](#)

[References](#)

[Index](#)

Effectors in Plant–Microbe Interactions

Edited by
FRANCIS MARTIN
SOPHIEN KAMOUN

 **WILEY-BLACKWELL**
A John Wiley & Sons, Ltd., Publication

This edition first published 2012 © 2012 by John Wiley & Sons, Inc.

Wiley-Blackwell is an imprint of John Wiley & Sons, formed by the merger of Wiley's global Scientific, Technical and Medical business with Blackwell Publishing.

Registered office:

John Wiley & Sons Ltd, The Atrium, Southern Gate,
Chichester, West Sussex, PO19 8SQ, UK

Editorial offices:

2121 State Avenue, Ames, Iowa 50014-8300, USA
The Atrium, Southern Gate, Chichester, West Sussex, PO19
8SQ, UK

9600 Garsington Road, Oxford, OX4 2DQ, UK

For details of our global editorial offices, for customer services and for information about how to apply for permission to reuse the copyright material in this book please see our website at www.wiley.com/wiley-blackwell.

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Blackwell Publishing, provided that the base fee is paid directly to the Copyright Clearance Center, 222 Rosewood Drive, Danvers, MA 01923. For those organizations that have been granted a photocopy license by CCC, a separate system of payments has been arranged. The fee codes for users of the Transactional Reporting Service are ISBN-13: 978-0-4709-5822-3/2012.

Designations used by companies to distinguish their products are often claimed as trademarks. All brand names and product names used in this book are trade names, service marks, trademarks or registered trademarks of their respective owners. The publisher is not associated with any product or vendor mentioned in this book. This publication is designed to provide accurate and authoritative information

in regard to the subject matter covered. It is sold on the understanding that the publisher is not engaged in rendering professional services. If professional advice or other expert assistance is required, the services of a competent professional should be sought.

Library of Congress Cataloging-in-Publication Data

Effectors in plant-microbe interactions / edited by Francis Martin, Sophien Kamoun.

p. cm.

Includes bibliographical references and index.

ISBN 978-0-4709-5822-3 (hard cover : alk. paper)

1. Plant-microbe relationships - Molecular aspects. I. Martin, Francis, 1954-II. Kamoun, Sophien.

QR351.E34 2012

579'.178-dc23

2011028322

A catalogue record for this book is available from the British Library.

This book is published in the following electronic formats:

ePDF 9781119949107; Wiley Online Library

9781119949138; ePub 9781119949114; Mobi

9781119949121

Disclaimer

The publisher and the author make no representations or warranties with respect to the accuracy or completeness of the contents of this work and specifically disclaim all warranties, including without limitation warranties of fitness for a particular purpose. No warranty may be created or extended by sales or promotional materials. The advice and strategies contained herein may not be suitable for every situation. This work is sold with the understanding that the publisher is not engaged in rendering legal, accounting, or other professional services. If professional assistance is required, the services of a competent professional person

should be sought. Neither the publisher nor the author shall be liable for damages arising herefrom. The fact that an organization or Website is referred to in this work as a citation and/or a potential source of further information does not mean that the author or the publisher endorses the information the organization or Website may provide or recommendations it may make. Further, readers should be aware that Internet Websites listed in this work may have changed or disappeared between when this work was written and when it is read.

1 2012

Contributors

Pierre Abad

INRA UMR 1301, CNRS UMR 6243
UNSA 400 route des Chappes
F-06903 Sophia-Antipolis
France

Silvia Ardissonne

Laboratoire de Biologie Moléculaire des Plantes Supérieures
Université de Genève
30 Quai Ernest-Ansermet
Sciences III
1211 Genève 4
Switzerland

Thomas J. Baum

Department of Plant Pathology and Microbiology
Iowa State University
Ames, IA 50011
USA

Thomas Boller

Botanisches Institut
Universität Basel
Hebelstrasse 1
4056 Basel
Switzerland

Jorunn I.B. Bos

Cell and Molecular Sciences
James Hutton Institute
Invergowrie

Dundee, DD2 5DA
UK

Liliana M. Cano

The Sainsbury Laboratory
Norwich, NR4 7UH
UK

Delphine Chinchilla

Botanisches Institut
Universität Basel
Hebelstrasse 1
4056 Basel
Switzerland

Jérôme Collemare

Wageningen University
Laboratory of Phytopathology
Droevendaalsesteeg 1
6708 PB Wageningen
The Netherlands

Mireille van Damme

The Sainsbury Laboratory
Norwich, NR4 7UH
UK

Present address:

Wageningen University
Laboratory of Phytopathology
Droevendaalsesteeg 1
6708 PB Wageningen
The Netherlands

Eric L. Davis

Department of Plant Pathology
North Carolina State University
Raleigh, NC 27607
USA

William James Deakin

Laboratoire de Biologie Moléculaire des Plantes Supérieures
Université de Genève
30 Quai Ernest-Ansermet
Sciences III
1211 Genève 4
Switzerland

Peter N. Dodds

CSIRO Plant Industry
GPO Box 1600
Canberra, ACT 2601
Australia

Sébastien Duplessis

UMR INRA-UHP 1136 Interactions Arbres/Micro-organismes
Centre INRA de Nancy
54280 Champenoux
France

Gunther Doehlemann

Max Planck Institute for Terrestrial Microbiology
Department of Organismic Interactions
Karl-von-Frisch Strasse 10
D-35043 Marburg
Germany

Dagmar Hann

Botanisches Institut

Universität Basel
Hebelstrasse 1
4056 Basel
Switzerland

Saskia A. Hogenhout

Department of Disease and Stress Biology
John Innes Centre
Norwich Research Park
Norwich, NR4 7UH
UK

Richard S. Hussey

Department of Plant Pathology
University of Georgia
Athens, GA 30602
USA

David L. Joly

Agriculture and Agri-Food Canada
Pacific Agri-Food Research Centre
Summerland, BC V0H 1Z0
Canada

Regine Kahmann

Max Planck Institute for Terrestrial Microbiology
Dept. Organismic Interactions
Karl-von-Frisch-Strasse 10
D-35043 Marburg
Germany

Sophien Kamoun

The Sainsbury Laboratory
Norwich, NR4 7UH

UK

Ralf Koebnik

Institut de recherche pour le développement
UMR 'Résistance des Plantes aux Bioagresseurs'
911 Avenue Agropolis
34394 Montpellier
France

Thomas Kroj

UMR Biologie et Génétique des Interactions Plante-Parasite
Campus International de Baillarguet
F-34398 Montpellier
France

Marc-Henri Lebrun

UR 1290 INRA BIOGER
Campus AgroParisTech
Thiverval-Grignon
France and UMR 5140 CNRS UCB BCS Microbiologie
Adaptation Pathogénie
Bayer Cropscience
Lyon, France

Magdalen Lindeberg

Department of Plant Pathology and Plant-Microbe Biology
Plant Science Building
Cornell University
Ithaca, NY 14853
USA

Francis Martin

UMR INRA-UHP 1136 Interactions Arbres/Micro-organismes
Centre INRA de Nancy
54280 Champenoux

France

Gregory Martin

Boyce Thompson Institute for Plant Research and
Department of Plant Pathology and Plant-Microbe Biology
Cornell University
Ithaca, NY 14853
USA

Thomas Mentlak

School of Biosciences
University of Exeter
Geoffrey Pope Building
Exeter, EX4 4QG
UK

Melissa G. Mitchum

Division of Plant Sciences and Bond Life Sciences Center
University of Missouri
Columbia, MO 65211
USA

Ricardo Oliva

The Sainsbury Laboratory
Norwich, NR4 7UH
UK

Jonathan M. Plett

UMR INRA-UHP 1136 Interactions Arbres/Micro-organismes
Centre INRA de Nancy
54280 Champenoux
France

Sylvain Raffaele

The Sainsbury Laboratory

Norwich, NR4 7UH
UK

Marie-Noëlle Rosso

INRA UMR 1301
CNRS UMR 6243
UNSA 400 route des Chappes
F-06903 Sophia-Antipolis
France

Thierry Rouxel

INRA-Bioger
Campus AgroParisTech
BP 01
78850 Thiverval-Grignon
France

Kerstin Schipper

Max Planck Institute for Terrestrial Microbiology
Department of Organismic Interactions
Karl-von-Frisch-Strasse 10
D-35043 Marburg
Germany

Sebastian Schornack

The Sainsbury Laboratory
Norwich, NR4 7UH
UK

María Eugenia Segretin

The Sainsbury Laboratory
Norwich, NR4 7UH
UK

Present address:

Laboratorio de Biotecnología Vegetal
INGEBI-CONICET
Vta. Obligado 2490 2do. piso
(C1428ADN) Ciudad de Buenos Aires
Argentina

Geert Smant

Laboratory of Nematology
Wageningen University
Binnenhaven 5
6709PD Wageningen
The Netherlands

Nicholas J. Talbot

School of Biosciences
University of Exeter
Geoffrey Pope Building
Exeter, EX4 4QG
UK

Brett M. Tyler

Virginia Bioinformatics Institute and Department of Plant
Pathology
Physiology and Weed Science
Virginia Polytechnic Institute and State University
Washington Street
Blacksburg, VA 24061
USA

Present address:

Center for Genome Research and Biocomputing
and Department of Botany and Plant Pathology
3021 Agriculture and Life Sciences Building
Oregon State University
Corvallis, Oregon, 97331-7303

USA

Pierre J.G.M. de Wit
Wageningen University
Laboratory of Phytopathology
P.O. Box 6798PB
Wageningen, The Netherlands

Foreword

Effectors in Plant-Microbe Interactions: Past to Present

Brian Staskawicz

Department of Plant and Microbial Biology, University of California Berkeley, Berkeley, CA 94720, USA

The basic understanding of why a phytopathogen can cause disease on only a few species of any particular plant has long intrigued plant pathologists. In fact, if one looks at all the potential disease-causing agents of plants, the ability of a pathogen to cause disease is often the exception as most plants are able to recognize and actively defend themselves against most pathogens in nature. Early work by E.C. Stakman at the University of Minnesota in early twentieth century established the concept of the “physiological race” of a single species of rust (Stakman, 1914). He demonstrated that physiological races derived from the sexual cycle of *Puccinia graminis* gave rise to distinct strains that varied in their ability to cause disease when inoculated on various wheat varieties. This observation was critical to the concept that resistance to cereal rust pathogens was race specific and that knowledge of the genetic variation in rusts was essential to the successful breeding for disease resistance. It was then Harold Flor in the 1940s with his work on flax rust who provided a genetic explanation for Stakman's “physiological race” concept (Flor, 1942). His work established that single gene differences in both the host and pathogen controlled whether a flax rust strain caused disease on a particular

cultivar of flax. Building on these prior observations and work by Al Ellingboe along with the discovery of recombinant DNA and gene cloning, I set out with Douglas Dahlbeck and Noel Keen in the early 1980s to clone a gene that defined the “physiological race” that Stakman and Flor had previously described and genetically characterized. The cloning of an “avirulence” gene from a *Pseudomonas syringae* pv. *glycinea* race established that a single gene in the pathogen controlled whether this bacterium caused disease on a particular cultivar of soybean (Staskawicz et al., 1984). In this case, the avirulence gene was recognized as a single resistance gene in soybean. However, it was not until several years later that it was established that these so-called avirulence genes also played a major role in the virulence of the pathogen. This was accomplished once methods had been established for performing site-directed gene mutations in phytopathogenic bacteria such that isogenic strains could be constructed and evaluated on hosts that did not contain the cognate resistance gene. Mutations in the *avrBs2* gene resulted in lower bacterial growth populations on pepper plants that did not contain the cognate *Bs2* gene (Kearney and Staskawicz, 1990). Once it was established that avirulence genes could be isolated in this manner, it was not long before several more examples were published. The concept that avirulence genes also had a role in virulence was further strengthened by the discovery that the “*Hrp*” gene in *Xanthomonas*, *Ralstonia*, and *Pseudomonas* turned out to be highly homologous to the type three secretion systems genes that had been earlier established in animal bacterial pathogens (Fenselau et al., 1992; Gough et al., 1992). Since the medical field used the term “effector protein” to describe proteins that were delivered via the bacterial type three secretion systems, phytopathologists also adopted this term to be consistent with the medical field. Since the original discovery of phytopathogenic effectors, it has become

apparent that all classes of plant pathogens employ effectors to either modulate or suppress plant innate immune functions (Dodds and Rathjen, 2010). Since the field has rapidly expanded over the last 5 years, the publishing of this book is timely as it brings together a wealth of information and points of view on a wide range of pathogen effectors. There is no question that we have learned a great deal about the mode of action of pathogen effectors to date, but this field is in its infancy and surely will flourish in the years to come. The combination of molecular, cellular, genomic, and structural studies will be paramount to this effort. As for the future, the sequencing of field isolates of naturally occurring pathogens will shed new light on pathogen diversity and will provide novel insights into the evolution and function of pathogen effectors in agricultural systems. This, in turn, will greatly benefit the deployment of durable disease-resistance strategies to control disease in an environmentally sustainable manner. One can only hope that translational approaches will be employed to solve important disease problems that are currently present and for new diseases that will emerge in the future.

References

- 1.** Stakman, E. (1914) A Study in cereal rusts, physiological races. *University of Minnesota Agricultural Experiment Station Technical Bulletin* 138, 1-56.
- 2.** Flor, H.H. (1942) Inheritance of pathogenicity in *Melampsora lini*. *Phytopathology* 32, 653-669.
- 3.** Staskawicz, B., Dahlbeck, D., & Keen, N. (1984) Cloned avirulence gene of *Pseudomonas syringae* pv. *glycinea* determines race-specific incompatibility on Glycine max (L.) Merr (Translated from ENG). *Proceedings of the National Academy of Sciences USA* 81(19), 6024-6028.

- 4.** Kearney, B. & Staskawicz, B.J. (1990) Widespread distribution and fitness contribution of *Xanthomonas campestris* avirulence gene *avrBs2*. *Nature* 346, 385–386.
- 5.** Fenselau, S., Balbo, I., & Bonas, U. (1992) Determinants of pathogenicity in *Xanthomonas campestris* pv. *vesicatoria* are related to proteins involved in the secretion in bacterial pathogens of animals. *Molecular Plant-Microbe Interactions* 5, 390–396.
- 6.** Gough, C.L., Genin, S., Zischek, C., & Boucher, C.A. (1992) *hrp* genes of *Pseudomonas solanacearum* are homologous to pathogenicity determinants of animal pathogenic bacteria and are conserved among plant pathogenic bacteria. *Molecular Plant-Microbe Interactions* 5(5), 384–389.
- 7.** Dodds, P.N. & Rathjen, J.P. (2010) Plant immunity: towards an integrated view of plant-pathogen interactions. *Nature Reviews Genetics* 11(8), 539–548.

Preface

Every single plant in nature is closely associated with mutualistic microbes, particularly fungi and bacteria. In addition, plants are repeatedly attacked by a multitude of pathogens and pests, including bacteria, fungi, oomycetes, nematodes, and insects. Deciphering how plants interact with both mutualistic and parasitic microbes is central to understanding their biology. One could almost argue that plant biology should be viewed as a subdiscipline of plant-microbe interactions. Identifying the plant-microbe cross talks is also crucial for a better understanding of the processes regulating the complex interactions between entangled plant and microbial communities in ecosystems.

The field of plant-microbe interactions has significantly matured in recent years. All major classes of molecular players both from plants (surface and intracellular immune receptors) and microbes (microbial pattern molecules and effectors) have now been revealed. This book focuses on effectors, secreted microbial molecules that alter plant processes and facilitate colonization. Effectors are central to our newly integrated view of plant-microbe interactions. Effectors have evolved to facilitate parasitism, for example, by suppressing host immunity in a variety of ways. However, they can also “trip on the wire” and activate plant immune receptors, a response known as effector-triggered immunity. These are complex interactions and the coevolutionary dynamics between plants and microbes have left striking marks in their genomes. Our goal was to take stock of current knowledge on effectors of plant-associated organisms and illustrate the diverse and complex ways in which effectors interact with their host plants.

The book opens with general reviews on plant immunity and how it is targeted by microbial effectors (Chapters 1

and 2). The field of effector biology has greatly benefited from genome-wide analyses, which result in complete catalogs of effector genes. Chapters 3–5 report on genome-wide analyses and evolution of effector genes. These chapters nicely illustrate how comparative genomics greatly contributed to our understanding of effector evolution. Chapters 6–8 describe how effectors function in suppressing host immunity and how they are perceived by plant immune receptors. How effectors traffic inside plant cells is covered by Chapters 9 and 10. Finally, the closing Chapters 11–15 cover emerging topics. Effectors have been reported in a number of plant-microbe systems, including bacterial and fungal symbioses, as well as nematode and insect pests.

Effector biology is a new and fast-paced field of research. As with all emerging fields of science, consensus among researchers has not always been reached and some topics remain controversial. Readers will surely notice more than one example throughout the book. We elected to keep such “inconsistencies” rather than enforce an arbitrarily sanitized version. We hope that such differences between authors will be informative of the current dynamic state of our science.

Books may have become less fashionable in the age of tweeting and microblogging. However, we hope that there is value in a document that summarizes the current state of the field of effector biology and provides a handy complement to the literature for both novice and experienced scientists.

Francis Martin and Sophien Kamoun