Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Plant Biology

Plastic potential: how the phenotypes and adaptations of pathogens are influenced by microbial interactions within plants

Kayleigh R O'Keeffe[1], Ignazio Carbone[2], Corbin D Jones[1,3] and Charles E Mitchell[1,4]

Predicting the effects of plant-associated microbes on emergence, spread, and evolution of plant pathogens demands an understanding of how pathogens respond to these microbes at two levels of biological organization: that of an individual pathogen and that of a pathogen population across multiple individual plants. We first examine the plastic responses of individual plant pathogens to microbes within a shared host, as seen through changes in pathogen growth and multiplication. We then explore the limited understanding of how within-plant microbial interactions affect pathogen populations and discuss the need to incorporate population-level observations with population genomic techniques. Finally, we suggest that integrating across levels will further our understanding of the ecological and evolutionary impacts of within-plant microbial interactions on pathogens.

Addresses
[1] Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
[2] Center for Integrated Fungal Research, Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, USA
[3] Integrative Program for Biological & Genome Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
[4] Curriculum for the Environment and Ecology, University of North Carolina, Chapel Hill, NC, USA

Corresponding author: O'Keeffe, Kayleigh R (kokeeffe@live.unc.edu)

Current Opinion in Plant Biology 2017, **38**:78–83

This review comes from a themed issue on **Biotic interactions**

Edited by **Silke Robatzek** and **Sarah Lebeis**

For a complete overview see the Issue and the Editorial

Available online 12th May 2017

http://dx.doi.org/10.1016/j.pbi.2017.04.014

Introduction

Plants are host to a diverse set of mutualistic, commensal, and parasitic microbes, referred to collectively as the phytobiome. The microbes (bacteria, fungi, and viruses) of the phytobiome live in, on and around their plant hosts. Historically difficult to interrogate – largely because work was limited to identifying microbial species by symptoms or by using culture-based methods – the composition, dynamics, and functional biology of the phytobiome are now being revealed by next-generation sequencing technologies [1].

Within the phytobiome, plant pathogens are among the best-studied microbes due to their negative impacts on their hosts. Infectious disease has historically been studied in a one host-one pathogen framework, but the field – thanks in part to next-generation sequencing [2] – is now appreciating the role and complexity of within-host microbial interactions. Within a shared host, pathogens may interact by directly facilitating or interfering with each other, or indirectly, mainly via host resources or the host's immune system [3]. Different plant pathogens may stimulate different signaling pathways, and the cross talk between such pathways can affect overall disease. Additionally, plants can attain bacteria-induced systemic acquired resistance (SAR), induced systemic resistance (ISR) or induced systemic susceptibility (ISS), in which prior infection or treatment results in increased resistance or susceptibility to subsequent invasions by pathogens [4,5]. Pathogens may also interact with non-parasitic microbes within the plant [6,7].

A key question in infection biology is how do these interactions shape pathogens in both the near and long term? Do the plastic responses of individual pathogens affect pathogen population genetic structure, altering their adaptability? Phenotypic plasticity is the ability of one genotype to express different phenotypes in different environmental contexts—an ability that is under genetic control and heritable [8]. For example, fungal pathogens such as *Mycosphaerella graminicola* and *Rhynchosporium commune* have been shown to exhibit phenotypic plasticity in growth rate and response to different temperatures [52,53]. Evolutionary theory juxtaposes genetic responses against plastic responses; however, there is conflict among both theoretical and empirical studies as to the direction of the connection between adaptive plasticity and evolution [9].

Studies that offer insight into a system on multiple levels of biological organization can help resolve this tension. Here we consider how microbial interactions shape plant pathogen growth and multiplication within individual plants and subsequent transmission between plants.

We emphasize that an understanding of plastic and genetic responses is critical at two key levels: the pathogen individual and the pathogen population. With a focus on fungal pathogens, we examine what we have learned about within-plant microbial interactions and disease from each of these levels while stressing the plastic responses of individual pathogens and genetic responses of pathogen populations to microbial interactions. Finally, we emphasize the novel insights that can be gained by integrating studies across levels.

Individual-level plasticity

Microbes are known to be major drivers of shifts in plant phenotype [10]. As noted above, pathogens induce complex defensive signaling pathways in plants [11,12], and beneficial microbes such as arbuscular mycorrhizal fungi facilitate increased nutrient uptake in plants [13•], which in turn alters the plant's physiological components. Less attention has been given to the plastic responses of plant pathogens to other components of the phytobiome, and how those responses, in turn, affect the infection process and consequent disease progression.

The best data on responses of pathogens to microbial interactions is from agriculturally important systems. This work suggests that specific interactions can alter the phenotypes of pathogens. For example, one fungus infecting maize, *Fusarium verticillioides*, modulates the growth of a maize fungal pathogen, *Ustilago maydis*, consequently decreasing the aggressiveness of *U. maydis* towards its host [14]. Transcriptomic analyses of *F. verticillioides* and *U. maydis* grown together in liquid culture suggests that *U. maydis* responds to *F. verticillioides* through the overexpression of siderophore biosynthetic genes and genes involved in toxin synthesis [15]. The interaction also results in the shutdown of antifungal metabolites like ustilagic acid in *U. maydis*. The phenotypic response of *U. maydis* to *F. verticillioides* is, therefore, genetically controlled, although its heritability has yet to be estimated. These findings suggest that *U. maydis* responds plastically to coinfection with *F. verticillioides*.

Interactions also occur indirectly between coinfecting pathogens through the defense pathways of the plant host. Plants respond to enemies with different infection strategies through different defense pathways, and cross talk between these pathways when two different pathogens infect a plant simultaneously can result in antagonism or synergism between the pathogens [16]. Necrotrophic pathogens, which kill host cells to feed and reproduce, induce defenses mediated by jasmonic acid. Biotrophic pathogens, which feed and reproduce on live host cells, induce defenses mediated by salicylic acid. Spoel *et al.*, [17] showed that salicylic acid-mediated defenses induced by the bacterium *Pseudomonas syringae* made *Arabidopsis thaliana* more susceptible to infection by the necrotrophic fungal pathogen *Alternaria brassicola* by suppressing the jasmonic acid signaling pathway. Disease symptoms from *A. brassicola* progressed more quickly, and *A. brassicola* produced more spores when the salicylic acid pathway suppressed the jasmonic acid pathway. Plastic responses to microbial interactions can therefore occur through indirect means. Although genes expressed by *A. brassicola* during infection of *A. thaliana* have been characterized [18], the genes that specifically respond to the presence/absence of jasmonic acid still need to be characterized to determine if this phenotypic response is genetically controlled [19].

To date, studies investigating the role of within-plant microbial interactions on plant pathogens primarily focus on phenotypic effects. In order for plasticity to be fully understood, the underlying genetic mechanisms controlling these differences need to be considered. As seen with the *Ustilago—Fusarium* example, gene expression studies are a powerful tool for assessing if phenotypic changes in response to biotic or abiotic factors are genetically controlled and give insight into what the controlling genetic mechanisms are. Characterizing gene expression in *in vitro* confrontation assays, though informative, can miss interactions that are mediated by the host. Adame-Álvarez *et al.* [7], for instance, investigated the effect asymptomatic endophytic fungal strains had on pathogens both *in vitro* and *in planta*. While *in vitro* confrontation assays between endophytes and three pathogens consistently showed symmetric reciprocal effects, *in planta* experiments revealed that these effects varied depending on the order of the arrival of the endophyte and pathogen on their shared host. One solution to the challenges of characterizing molecular mechanisms of plastic responses is to simultaneously characterize gene expression of both organisms *in situ*. Dual RNA-seq, sequencing host and pathogen transcriptomes in parallel, has the potential to meet this need [20] and can be applied to the frequent cases of host tissue infected with more than one microbe.

Pathogen population

In contrast to near-term plastic responses in pathogen individuals, long-term changes within pathogen *populations* are thought to demand evolved responses. The evolutionary forces shaping plant pathogen populations can determine a pathogen's evolutionary potential and predict its ability to shift hosts, expand its range, and emerge as a disease-causing agent [21,22]. Within-host microbial interactions may impact the evolutionary dynamics of pathogen populations, but few population genetic studies have focused on how interspecific microbial interactions affect the evolution of plant pathogens. Application of population genetic techniques to plant pathogen populations can address other questions related to the evolutionary and demographic processes shaping population structure. For example, these techniques can elucidate the processes driving or constraining the