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Review

Evolutionary and Epidemiological Implications of Multiple Infection in Plants

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Recent methodological advances have uncovered tremendous microbial diversity cohabiting in the same host plant, and many of these microbes cause disease. In this review we highlight how the presence of other pathogen species, or other pathogen genotypes, within a plant can affect key components of host–pathogen interactions: (i) within-plant virulence and pathogen accumulation, through direct and host-mediated mechanisms; (ii) evolutionary trajectories of pathogen populations, through virulence evolution, generation of novel genetic combinations, and maintenance of genetic diversity; and (iii) disease dynamics, with multiple infection likely to render epidemics more devastating. The major future challenges are to couple a community ecology approach with a molecular investigation of the mechanisms operating under coinfection and to evaluate the evolution and effectiveness of resistance within a coinfection framework.

Why Does Coinfection Matter?

During the growing season, plants in both agro- and natural ecosystems are likely to encounter a myriad of microbes, many of which are pathogenic. As a consequence, a pathogen strain entering a host plant will encounter not only the host's defenses but also a number of other microbial species or genotypes within the plant phytobiome (the entire microbial community associated with the various plant compartments including the rhizosphere, phyllosphere, and endophytic compartments, following the American Phytopathological Society). This diversity can fundamentally change the ability of a parasite strain to establish and grow on its host and dynamics under multiple infection have been suggested to be a major force driving pathogen evolution [1]. This has sparked a growing interest in the epidemiological and evolutionary consequences of coinfection in humans [2,3] and wild animal populations (e.g., [4,5]). By contrast, the impact of coinfection on disease dynamics and pathogen evolution in plant pathosystems has received comparatively less attention, with the significant exception of virus–virus interactions (see, for example, [6,7] and [8,9] for reviews). Plant pathologists have traditionally focused on two-way interactions within the 'single host–single pathogen' framework [10] and plant resistance is mostly considered in a 'unique pathogen genotype' framework. However, we are just beginning to understand the far-reaching consequences of the microbial diversity associated with plants. In this review we show how sensitive epidemiological and evolutionary dynamics of pathogens are to coinfection. We consider the methodology and current knowledge regarding coinfection levels in natural and agricultural plant systems and review the within-host mechanisms that mediate interactions between pathogen strains or species. We also consider the consequences, in terms of both the epidemiology and the evolution of the pathogen populations, highlighting the current challenges in this field.

Trends

Molecular tools are becoming readily available for the study of parasites. These technical advances have shown that multiple infection is common in the wild and in agriculture, with the same host individual simultaneously infected by several pathogen genotypes or species (i.e., coinfection).

Under coinfection, pathogens may interact either directly (mechanical or chemical interactions) or indirectly through host resources or defenses.

These direct or host-mediated interactions under coinfection can change virulence, within-host pathogen accumulation, and transmission.

Such plant-level effects can also be seen at the population level, with coinfection rendering epidemics more devastating.

Coinfection may drive the evolutionary trajectories of pathogen populations through its effects on virulence evolution and on the generation and maintenance of genetic diversity in pathogen populations.

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 http://dx.doi.org/10.1016/j.tplants.2015.10.014

Levels of Coinfection in Natural and Agricultural Plant Systems

The study of coinfection was for a long time hindered by methodological constraints, as symptom-based detection is rarely a reliable means of identifying coinfection. Hence, molecular or serological markers are generally a mandatory tool for the study of multiple infection (Box 1). As these tools are becoming available for a wider range of pathogens and next-generation sequencing opens new possibilities for the characterization of microbial communities, it is becoming increasingly clear that coinfection is common in both wild plants and agricultural crops and for both systemic and local diseases [11–14]. Levels of coinfection can be very high in some pathosystems. For example, multiple infection was found in a majority (16 of 21) of weed species tested for five viruses [15]. Up to 76% of plants were found to be infected by more than one pathogen genotype of the barley scald pathogen *Rhynchosporium secalis* [16] and up to 16 distinct genotypes were detected within a single lesion of a *Eucalyptus* leaf-infecting fungus, *Teratosphaeria nubilosa* [12]. In the Central African Republic, cassava was frequently affected by cassava mosaic disease (CMD) (85% incidence), with up to 58% of diseased plants infected by various virulent geminiviruses [13]. In addition, high spatiotemporal variability in coinfection levels was found when investigated, with, for example, 20–100% (average 35.3%) of infected plants harboring more than one of the six tested viruses within six *Arabidopsis thaliana* Spanish wild populations followed over 4 years [17].

Both plant genetics and environmental variation can shape the prevalence of coinfection in space and time. Host population resistance is a key determinant of pathogen population and community structure, with plant resistance genes determining whether a pathogen is capable of infecting a given host genotype (at one extreme gene-for-gene interactions, but also true for resistance controlled by multiple loci). As a consequence, a mismatch between host resistance and pathogen infectivity profiles may prevent coexistence. Host community structure is also likely to impact coinfection, as revealed by the negative relationship between plant diversity and coinfection levels found in grassland experimental plots [18]. In wild *Plantago* populations in the Aland archipelago, coinfection by multiple genotypes was found in approximately half of the populations infected by the powdery mildew *Podosphaera plantaginis* [11]. In this system, the prevalence of coinfection was higher in well-connected pathogen populations [19], suggesting that the dispersal rate among pathogen populations may be an important determinant of the multiplicity of infections. Moreover, host genotype was a key determinant of coinfection in common garden populations of *Plantago lanceolata*, with the lowest levels of coinfection

Box 1. Detecting and Quantifying Pathogens for the Study of Coinfection

The study of coinfection requires: (i) distinguishing coinfection from single infection; (ii) distinguishing coinfecting genotypes/species from each other; and (iii) quantifying each coinfecting pathogen. Given that pathogens are typically small and clonally reproducing, morphological identification is often impossible and symptom expression can be different under coinfection than under single infection (see, for example, [85], where unexpected symptoms are observed in dual viral infection), so that molecular or serological tools are required in most cases (but see [86]). One commonly used method to detect coinfection in a field sample comprises purification of several pathogen strains/species from a single plant (e.g., [77]). Genetic characterization can then be performed using various molecular markers, such as micro-satellites [12,22], or restriction length polymorphisms [77].

Alternatively, when researchers are interested only in the presence/absence of coinfection, molecular work can be performed directly on the sample without the purification step. Multipathogen molecular detection methods that rely on species- or genotype-specific primers have been developed in a few pathosystems (e.g., [87]). For haploid species, coinfection may be inferred from SNP genotyping [11]. In addition, recent next-generation sequencing technology allows the characterization of entire microbial communities [88,89] and consequently the determination of coinfection levels within each pathogen group (viral, bacterial, or fungal).

Finally, the prevalence of the different strains forming the coinfection can be quantified using quantitative PCR methods. These have been developed for a few pathogens, such as *Phytophthora infestans* [75], as well as some viruses (e.g., [90,91]). High-throughput sequencing has been used to monitor viruses [92], while genetic engineering and the use of fluorescent proteins allow the visualization of competition of genotypes in real time [93].