MEETING REPORT

When green and red mycology meet: Impressions from an interdisciplinary forum on virulence mechanisms of phyto- and human-pathogenic fungi

Yidong Yu a, Bernhard Hube b, Jörg Kämper c, Vera Meyer d, and Sven Krappmann a

a Institute for Clinical Microbiology, Immunology and Hygiene, University Hospital Erlangen and Friedrich-Alexander University (FAU) Erlangen-Nürnberg, Erlangen, Bavaria, Germany; b Department of Microbial Pathogenicity Mechanisms, Hans Knöll Institute, Jena, Thuringia, Germany; c Department of Genetics, Institute of Applied Biosciences, Karlsruhe Institute of Technology, Karlsruhe, Baden-Württemberg, Germany; d Institute of Biotechnology, Department of Applied and Molecular Microbiology, Technische Universität Berlin, Berlin, Germany

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ABSTRACT
Fungal infections pose a constant threat to plants and humans, but detailed knowledge about pathogenesis, immunity, or virulence is rather scarce. Due to the fact that a certain overlap in the armoury of infection exists between plant- and human-pathogenic fungi, an interdisciplinary forum was held in October 2016 at the Institute for Clinical Microbiology, Immunology and Hygiene in Erlangen under the organisational umbrella from two special interest groups of German microbial societies. Scientific exchange and intense discussion of this timely topic was fostered by bringing together renowned experts in their respective fields to present their thoughts and recent findings in the course of a plenary lecture and six themed sessions, accompanied by oral and poster contributions of young researchers. By targeting the topic of fungal virulence mechanisms from various angles and in the context of plant and human hosts, some common grounds and exciting perspectives could be deduced during this vibrant scientific event.

KEYWORDS
cross-kingdom pathogens; effectors; fungal infection; IFoFun; pathogenicity

Setting the stage

Interdisciplinary research is of vital significance for modern science to understand complex systems and to draw valid conclusions from appropriate model systems. By linking several scientific disciplines and, most importantly, joining their forces and strengths in one particular aspect of research, unforeseeable insights and innovative strategies may emerge in a synergistic manner. 1 Given this promising and inspiring approach, an Interdisciplinary Forum on Virulence Mechanisms of Phyto- and Human-Pathogenic Fungi took place in October 2016, hosted at the Microbiology Institute of the University Hospital Erlangen and the Friedrich-Alexander University Erlangen-Nürnberg in Germany. This IFoFun-2016 was the most recent event emerging from a series of interdisciplinary meetings initiated in 1999 by a special interest group of the German Society of Microbiology and Hygiene (DGHM) and that is devoted to Eukaryotic Pathogens, among them human-pathogenic fungi. Together with the special interest group focusing on Fungal Biology and Biotechnology of the German Association for General and Applied Microbiology (VAAM), the above topic was addressed in several sessions during which renowned speakers and experts in their respective fields shared their recent insights and thoughts on specific aspects of fungal virulence with the interested participants.

Members of the fungal kingdom have a significant impact on numerous known and unknown processes on a global scale, among them fundamental issues such as ecology or health and nutrition of mankind. Among the estimated 6 million fungal species, 2 several have been identified regularly to be harmful for animals and human individuals, 3 while an apparently distinct group can act as plant pathogens and therefore affect human well-being in an indirect fashion. 4 With respect to the latter, several crop diseases described as rust, smut, blast, blotch, or mildew caused by plant-pathogenic fungi account for annual food losses that would feed about 8.5% of the world’s population. 5 Besides this direct impact on global agriculture, indirect consequences affecting staple crops can emerge from ecological perturbations caused by fungi - a scenario seen for the epidemic of the white-nose syndrome among bats that is caused by a fungal pathogen, given the agroecological impact of these insectivorous animals. 6, 7 Furthermore,
the loss of carbon dioxide-absorbing trees due to fungal damage impacts the global climate and therefore plants and humans likewise.

Human-pathogenic fungi have massively attracted attention in recent decades due to the significantly rising incidence of mycoses. This development is mainly based on the increasing number of susceptible individuals, predominantly immune-compromised patients in distinct clinical settings, supported by extended means of immunosuppressive regimens. Recent efforts have impressively highlighted the extent of deaths caused fungal infections, with fatalities reaching numbers assigned to malaria and tuberculosis on a global scale. This development is accompanied by a diagnostic as well as therapeutic dilemma: fungal infections are often underdiagnosed and may then be treated rather late and unsatisfactorily with only a limited set of antifungicals available. With the pharmaceutical pipeline lagging behind the demand for novel antifungal substances, accompanied by emerging resistance against some of the most established compounds, such as azoles, the need for novel starting points in antifungal therapy is evident and urgent. One key aspect in the identification of such targets lies in the comprehensive understanding of the pathogenesis of fungal infections. The outcome of a pathogen-host encounter relies on virulence of the former and susceptibility of the latter. Susceptibility is determined by the host’s immunity, and virulence as a means of aggressiveness has evolved in various contexts, may it be the primary ecological niche in the case of opportunistic fungal agents or the host environment for obligate pathogens. Detailed knowledge about the means of fungal virulence may not only help in understanding aspects of infection and pathogenesis but can assist in identifying virulence determinants that are relevant in the context of either host system, like means of iron sequestration, extracellular proteolytic activities, or potent secondary metabolites. Besides this, pathogenicity factors of plant invaders, such as toxins, small proteins, or secreted effectors may find a counterpart with a conserved or further evolved function in human pathogenic fungi and vice versa. Given the similarities, but also distinct differences of the host systems and the corresponding virulence mechanisms, the topics addressed during the IFOFun-2016 were well suited for an interdisciplinary exchange addressing the impact of fungal pathogens on agriculture and human health. The IFOFun-2016 covered a variety of aspects to gain valuable insights about common themes of fungal pathogenicity by combining the expertise of established researchers with the enthusiasm of younger researchers in 6 scientific sessions, headed by a plenary talk and accompanied by poster presentations and intense discussions (Fig. 1).

To start with…

The IFOFun-2016 commenced with a plenary talk of Nancy Keller from Madison, Wisconsin (USA), a renowned expert in the field of fungal secondary metabolism on the regulatory, biosynthetic, and product level. With her expertise in “crossover fungal pathogens,” Professor Keller gave a brilliant and vivid opening lecture on how to translate biosynthetic gene clusters into fungal armor and weaponry. While giving an illustrative overview on the variety of natural compounds of fungal origin, including antibiotics, toxins, or pigments, she highlighted the chromosomal arrangements of gene clusters being typical for the encoding entities. After this historical detour, she shared her thoughts on the interesting questions why natural compounds are made and to what extent their involvement in virulence can be predicted. As illustrative example, she presented recent data on “crossing path(ogen)s” of the filamentous fungus Aspergillus flavus and the bacterial plant pathogen Ralstonia solanacearum, both being able to infect potato plants. Either pathogen interferes with its competing partner during pathogenesis by an inter-kingdom dialog between two plant pathogens: the bacteria induce the formation of chlamydospore-like structures via the action of the lipopeptide ralsolamycin, followed by bacterial entry into tissues of A. flavus; this in turn is able to synthesize a non-ribosomal peptide for counteraction. She finally gave an overview on natural product clusters as deduced from the genome sequence of the opportunistic saprobe A. fumigatus, together with concluding remarks about their conservation in pathogenic fungi. These highly valuable insights set the stage for the series of scientific presentations during the following sessions that were dedicated to relevant aspects of fungal virulence.

Feeding from the host - nutrition & metabolism (Session 1)

In the course of infection, every pathogen must propagate and replicate in the host environment. Correspondingly, fungal growth sustained by a versatile primary...
metabolism is vital for pathogenesis. For biosyntheses, the fungal pathogen needs to mobilize and assimilate nutrients from the infected tissue, which is typically executed by osmotrophy. The host can therefore be seen as a substrate that provides metabolic building blocks, but that may also have evolved means of restricting access to essential metabolites, a strategy that has recently been termed as nutritional immunity.\textsuperscript{18} This aspect of fungal pathogenicity was addressed by a series of presentations during the opening session of the IFoFun-2016 that was chaired by Joachim Morschhäuser from Würzburg, Germany, and launched by a talk of Matthias Brock from Nottingham, UK. He described the interplay of \textit{A. terreus} with banana tissue as mediated by the action of a fruit rot toxin that is further supported by the phytotoxin terrein.\textsuperscript{19} From expression analyses, regulatory mechanisms that go beyond primary metabolism were identified, demonstrating an intertwined relationship between global regulators and gene cluster-specific transcription factors in this fungal pathogen of plants and humans.\textsuperscript{20}

Jörg Kämper from the Karlsruhe Institute of Technology in Germany presented his recent insights on pathogenic development of \textit{Ustilago maydis} in planta that is regulated by two transcription factors, Biz1 and Hdp2, identified by their relationship to the mating-type locus-dependent class I gene \textit{rbf1}.\textsuperscript{21} Notably, two independent promoter regions were mapped for the \textit{hdp2} gene that respond to distinct transcriptional regulators in axenic culture or in planta, respectively. The session was closed by Jim Kronstad from Vancouver, Canada, who demonstrated nutritional and proteostatic regulation of capsule formation in the opportunistic fungal pathogen \textit{Cryptococcus neoformans}. Capsule size and morphology are influenced by a variety of factors, among

\textbf{Figure 1}. People and topics of the IFoFun-2016. Incomplete gathering of invited speakers, session chairs and young investigators contributing to the IFoFun-2016; in total, the forum attracted 82 attendees from 11 countries that vividly discussed 28 presentations during 6 themed sessions and acknowledged the data on 26 scientific posters. The lower illustration depicts fungal cells infiltrating a cluster of human or plant host cells to indicate the various aspects of virulence in this context of fungal invasion that were covered during the forum; see text for details.
them iron availability, surface polyphosphates, or cAMP signaling,\textsuperscript{22,23} which underscores flexibility of this dynamic cellular shield.

**The host is a special place - sensing the environment & tolerating stress (Session 2)**

Infection of a susceptible host, either plant or human, commonly poses a stressful condition to the invading pathogen due to specific environmental parameters. Changing the environment during invasion relies on adaptation processes, and the host response may trigger additional stressors such as elevated temperature or increased levels of reactive oxygen and nitrogen species. Both adaptation and stress response are initiated by the sensing of environmental conditions, which are then transduced and translated into a cellular read-out to cope with the given external circumstances. Although the plant and mammalian body environments are significantly different, there may be common means of response among plant- and human-pathogenic fungi, which were addressed in the second IFoFun-2016 session hosted by Vera Göhre from Cologne, Germany. As first speaker, Elaine Bignell, deputy director of the recently established Manchester Fungal Infection Group (MFIG), provided insights into the transcriptional response of *A. fumigatus* under host-imposed stress.\textsuperscript{24,25} Moreover, she highlighted the presumed role of secreted factors for epithelial damage and introduced a mechanistic model on fungal persistence in the host lung environment.\textsuperscript{26} The contribution of alveolar epithelial cells in fungicidal defense was demonstrated by *in vitro* studies as well as in murine infections, thereby affecting the immunotolerant disposal of inhaled *A. fumigatus* conidia. Matthias Hahn from the University of Kaiserslautern, Germany, next gave an overview about the spread and fitness of multi-resistant strains of the necrotrophic plant pathogen *Botrytis cinerea*\textsuperscript{27} and described a role of a sensor protein, Sho1, related to BMP1 MAPK signaling\textsuperscript{28,29} in pH modulation. Interestingly, *sho1* mutants phenocopy the lack of velvet complex components such as Vel1 or Lai1;\textsuperscript{30} however, transcriptional profiling studies in *planta* revealed similarities but also significant differences, leaving the connection between Sho1 and the velvet complex an open issue. Introducing a true cross-kingdom pathogen, Antonio Di Pietro from Cordoba, Spain, presented details on the interaction of *Fusarium oxysporum* with its infected plant host.\textsuperscript{31} The fungal ortholog of a plant factor triggers rapid extracellular alkalization and root growth arrest, presumably mediated by a receptor-like kinase,\textsuperscript{32} which implies that *Fusarium* is able to hijack plant mechanisms for its own benefits. Working on *Candida albicans*, Janet Quinn from Newcastle, UK, highlighted the role of stress resistance for virulence of this fungal commensal and pathogen.\textsuperscript{33,34} Moreover, the established role of the stress-activated protein kinases (SAPKs) as global mediators of fungal virulence was emphasized,\textsuperscript{35} accompanied by an in-depth characterization of the *C. albicans* Hog1 kinase in its interplay with the phosphorelay protein Ypd1.\textsuperscript{36} This identified the Ssk1 response regulator as scaffold protein and master regulator of the Hog1 SAPK in *C. albicans* to support antifungal drug discovery.

**A holistic view on infection - systems biology (Session 3)**

The tremendous progress in experimental methodologies to scrutinize living systems has enabled fungal researchers to gain deeper insights about the pathogen-host interplay at a comprehensive scale. Most prominently, -omics approaches accompanied by high-throughput techniques allow data acquisition in an extensive and fast way, and by means of sophisticated bioinformatics, patterns and key points of virulence may be hypothesized to be further investigated in suitable models of infection. In the light of these timely approaches, a Systems Biology session was chaired by Vera Meyer from Berlin, Germany, and opened by Axel Brakhage from the Hans Knöll Institute (HKI) in Jena, the German hotspot of fungal infection biology and natural product research. Focusing on virulence of the opportunistic fungus *A. fumigatus*, his team had characterized histones formed during neutrophil extracellular trap release (NETosis) against this human pathogenic fungus\textsuperscript{37} by means of proteomic approaches. Furthermore, he highlighted the role of conidial cell surface proteins and pigments upon interaction with host immune cells\textsuperscript{38} and presented the successful application of a CRISPR/Cas9-based approach for gene reconstitution, thereby underscoring the potential of this versatile tool for advanced molecular genome editing studies in filamentous fungi.\textsuperscript{39,40} Christophe D’Enfert from the Institute Pasteur in Paris, France, gave an update on regulatory networks of *C. albicans* morphogenesis, focusing on heat shock responsive transcription factors that play apparently antagonistic regulatory roles in hyphae formation despite binding to overlapping regions of target gene promoters; among these, a gene product essential for chlamydospore formation could be identified.\textsuperscript{41} Furthermore, the function of a heat shock factor type DNA binding protein as master regulator for transcriptional regulators of morphology was highlighted. Finally in this session, Stephanie Diezmann from Bath, UK, shared her findings about the Hsp90 heat shock protein of *C. albicans* and how it affects fungal genome ploidy as well as stress responses.\textsuperscript{42,43}
The next generation of fungal infection research - presentations from young scientists (Session 4)

In a session of short presentations, nine upcoming researchers dedicated to fungal infection biology had been selected from submitted abstracts to give insights on their recent results. Sascha Brunke from Jena, Germany, had the kick-off in this vivid session that was chaired by Nir Osherov from Tel Aviv, Israel, by presenting his latest insights about the experimental evolution of pathogenic fungi, to be followed by Kai Heimel from the University of Göttingen, Germany, who revealed that the unfolded protein response of U. maydis coordinates development and virulence of this maize smut pathogen. Falk Hillmann from Jena spoke about the virulence determinants of pathogenic fungi that contribute to the defense against mycophtagous amoeba. Vera Göhre from Cologne described her efforts to establish a novel pathosystem of the smut-fungus Thecaphora thlaspeos and the model plant Arabidopsis thaliana. Next, Annika Franke from Jena outlined the recently discovered lytic peptide toxin of C. albicans – candidalysin – in its action on macrophage cell damage and the inflammatory response. From Erlangen, Johannes Schmidtperter presented data on a nuclear Dbf2-related (NDR) kinase binding protein from Colletotrichum higginsianum and its influence on virulence by affecting appressorium morphology. Michael Blatzer from Innsbruck in Austria shared novel insights about amphothericin B resistance in A. fumigatus, and Daniela Nordzieke joining from Cordoba, Spain, presented interesting data on the NOX complex from F. graminearum and its role in chemo-tropic sensing of plant peroxides. The session was closed by Sascha Jung from Berlin, Germany, proposing a novel biologic role of the A. niger antifungal protein AnAFP. All contributions, together with the ones that had not been nominated for oral presentation, were also on display as posters throughout the entire event to support their scientific debate. This was further promoted by a dedicated lunch break poster session sponsored by BIOMED CENTRAL and the open access journal Fungal Biology and Biotechnology.

Manipulating the host - extracellular effectors & host-pathogen interactions (Session 5)

One of the most exciting topics of current fungal research relies to the intimate interplay between a fungal invader and the infected host. Bacterial pathogens have evolved strategies to manipulate the host response on the cellular level by means of effector molecules that are secreted and may be translocated into infected cells by secretion systems of various types that serve as molecular injection needles or nanomachines. A variety of cellular processes have been identified on the host side to be altered by the concerted action of such effectors, such as apoptosis, second messenger signaling, mitochondrial function, or immunity. Extracellular effectors of plant-pathogenic fungi, including oomycetes, had been identified some time ago, and recent efforts of the Ustilago research community have revealed a plethora of fungal factors that are secreted into the apoplastic space and taken up by plant host cells, which are then manipulated in their response toward the invading pathogen. For human pathogenic fungi, however, the existence of genuine secreted extracellular effectors remains to be demonstrated, although orthologues have been identified and it is therefore highly likely that similar modes of action are executed by this group of fungal pathogens. Covering this emerging topic in fungal infection research, four experts’ talks on effector biology were coordinated by Bernhard Hube from Jena, Germany: Armin Djamei, a pivotal researcher in the field of fungal effector biology, presented his recent efforts to dissect effector functions in the biotrophic maize-U. maydis pathosystem, exemplified by the action of fungal effectors on plant auxin production. Correspondingly, Julian Naglik from London, UK, reported research progress on the first characterized effector of a human pathogenic fungus, candidalysin of C. albicans, which is generated by processing the ECE1 gene product, and provided an overview on C. albicans-epithelial interactions. Gunther Döhlemann from the Cologne-based Cluster of Excellence on Plant Sciences (CEPLAS) in Germany described cell type-specific functions of secreted U. maydis effectors, predominantly relating to tumor formation in the maize host. The session was closed by Markus Künzler from the ETH Zürich in Switzerland, who impressively demonstrated the systemic intracellular defense response of Coprinopsis cinerea after fungivorous nematode attack to produce nematotoxic lectins and the secretion of extracellular effectors presumably acting against bacterial competitors.

The basic unit of virulence - cell biology of infection (Session 6)

Virulence expressed during infection of a susceptible host is a trait that is executed by the action of various cellular components, some of them being specific for the fungal nature of the pathogen. Accordingly, several aspects of fungal cell biology that are relevant in the context of infection were addressed in the final IFoFun-2016 session that was hosted by Frank Ebel from Munich, Germany. Lauren Ryder from Exeter, UK, focused on the turgor pressure modulation of the rice blast fungus
Magnaporthe oryzae\textsuperscript{63} by an osmosensing histidine kinase that supports pathogenicity but also acts as negative regulator of melanin synthesis. Holger Deising from Halle, Germany, presented data on evasion of the model pathogen *Colletotrichum graminicola* from plant PAMP-triggered immunity by surface modulations in biotrophic hyphae\textsuperscript{66} and how to use host-induced silencing of essential fungal genes to improve plant health. The expert in fungal RNA biology Michael Feldbrügge from Düsseldorf, Germany, gave an update on the RNA-binding protein Rrm4 of *U. maydis* and presented a regulator important for early endosome function in delivering *de novo* synthesized proteins together with endosomal mRNA.\textsuperscript{65} In a concluding presentation, Neil Gow from Aberdeen, Scotland, spoke about the characteristics of the fungal cell wall to serve as immunological and drug target. He focused on N-mannan, which supports binding of human immune cells to *C. albicans*, and its synthesis that is mediated by a highly redundant gene family;\textsuperscript{66} furthermore, he shared interesting data on the role of chitin in drug resistance and immune cell polarization.\textsuperscript{67}

**Where are we now and where do we go from here?**

The interdisciplinary forum on virulence mechanisms of phyto- and human-pathogenic fungi had brought together experts on various topics of fungal pathogenicity by covering some of the most relevant aspects of pathogenesis, among them fundamental (metabolism, stress, cell biology) and also specific (systems, effectors) ones. Virulence determinants of fungal pathogens have been shaped to some degree during co-evolution upon close interaction with the host, may it be plant or human, and are complemented by general traits characteristic for the fungal lifestyle. With respect to the latter, a certain degree of overlap is likely to exist between plant- and human-pathogenic fungi and some of these dual use virulence determinants\textsuperscript{68} could be translated from insights gained in one pathosystem to the other. Such common strategies and shared mechanisms of fungal virulence imply the chance to identify pan-antifungal targets, and the fruitful discussion of such aspects as during the IFoFun-2016 event holds the promise of gaining further and deeper insights into fungal virulence and its counteraction.

Several contributions to the IFoFun-2016 touched such connections in greater detail, while others presented relevant aspects of virulence in one pathosystem that have not been addressed so far in the other (Table 1). Among the former, the seminal works on *Fusarium oxysporum* performed in the Di Pietro laboratory clearly stands out by describing the influence of ambient pH and its modulation on virulence, an aspect that has been addressed for *Aspergillus* infections before extensively.\textsuperscript{24,32} Also the UPR system as presented by Kai Heimel in his short talk on *U. maydis* has been recognized to support pathogenicity of human pathogenic fungi as well,\textsuperscript{44,69} thereby representing a common determinant of fungal virulence. The same is obviously true for fungal-specific traits, such as RNA biology and transport elucidated by the Feldbrügge group,\textsuperscript{65} or the unique composition and structure of the fungal cell wall, as it has been studied by N. Gow and several others in the field.\textsuperscript{70} An apparently more specific trait of plant-pathogenic fungi seems to be related to apoplastic effectors,\textsuperscript{50,71} as they were presented by the IFoFun-2016 speakers A. Djamai and G. Döhlemann. Yet, given the vast amount of secreted molecules and factors produced by human pathogenic fungi, it can be assumed that overlapping aspects in the patho-effectorome of either fungal class might be revealed in the future. Such common grounds became clearly evident during the plenary lecture of N. Keller by her historical perspective, providing an overview on fungal secondary metabolites serving as myco- or phytotoxins. Also in this regard, melanins that act as spore pigments had been demonstrated to influence fungal fitness and virulence in either pathosystem.\textsuperscript{72-74} The fact that genes of secondary metabolism are generally clustered assists in the identification of encoding entities, as it had been exemplified for the human pathogenic fungus *A. fumigatus*.\textsuperscript{16} The knowledge gained there is likely to support comparative studies in

### Table 1. Selected highlights presented during the IFoFun-2016.

| Take Home Message                                                                 | Presenter \(^*\)          | Reference |
|----------------------------------------------------------------------------------|---------------------------|-----------|
| Crossing pathogen(s) reveal the role for a natural product                       | N. Keller (P)             | 15        |
| The Cryptococcus neoformans capsule is a highly dynamic structure                | J. Kronstad (S)           | 22,23     |
| *Fusarium oxysporum* acts as a true cross-kingdom pathogen                       | A. Di Pietro (S)          | 33,32     |
| Fungal spore components determine the interaction with immune cells              | A. Brakhage (S)           | 38        |
| Regulatory networks of *C. albicans* morphogenesis are complex                   | C. D’Enfert (S)           | 41        |
| Experimental evolution reveals unexpected factors of fungal virulence            | S. Brunke (Y)             | 75,76     |
| *Candida albicans* produces a toxic surprise: Candidalysin                        | J. Naglik (S)             | 56        |
| Coprinopsis cinerea produces potent fungal defense effectors                      | M. Künzler (S)            | 59-61     |
| The fungal cell wall acts as immunological & drug target                          | N. Gow (S)                | 70        |
| Copper detoxification supports *A. fumigatus* virulence                          | N. Osherov (Po)           | 77        |

Note. \(^*\) P: plenary lecture, S: session talk, Y: young researcher presentation, Po: poster
plant pathogenic fungi and might reveal natural products that are active in either host, be it plant or human.

Pathogenicity factors that specifically interfere with host cell functions are of great interest when defining mechanisms of virulence. In this respect, less conservation between human and phyto pathogens might exist due to differing target characteristics; yet, the degree of conservation among some validated determinants might indicate common grounds even for these elaborated systems. For instance, orthologues can be identified in the genomes of human-pathogenic fungi for several extracellular effector molecules acting on plant cells, and the recent identification of the first cytolytic peptide toxin of the clinically important fungus *C. albicans* suggests converging strategies among pathogenic fungi for cell manipulation during host invasion. Furthermore, the majority of human pathogenic fungi secrete a wealth of highly active molecules, be it secondary metabolites or disruptive enzymatic activities. Initially evolved for competition and osmotrophy, respectively, the role of such putative effectors during infection and pathogenesis remains to be scrutinized, and insights gained from the plant pathosystem are likely to be instructive for this promising research perspective.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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**ORCID**

Bernhard Hube http://orcid.org/0000-0002-6028-0425

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