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A multi-layered mechanistic modelling approach to understand how effector genes extend beyond phytoplasma to modulate plant hosts, insect vectors and the environment

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Members of the *Candidatus* genus Phytoplasma are small bacterial pathogens that hijack their plant hosts via the secretion of virulence proteins (effectors) leading to a fascinating array of plant phenotypes, such as witch's brooms (stem proliferations) and phyllody (retrograde development of flowers into vegetative tissues). Phytoplasma depend on insect vectors for transmission, and interestingly, these insect vectors were found to be (in)directly attracted to plants with these phenotypes. Therefore, phytoplasma effectors appear to reprogram plant development and defence to lure insect vectors, similarly to social engineering malware, which employs tricks to lure people to infected computers and webpages. A multi-layered mechanistic modelling approach will enable a better understanding of how phytoplasma effector-mediated modulations of plant host development and insect vector behaviour contribute to phytoplasma spread, and ultimately to predict the long reach of phytoplasma effector genes.

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Introduction

Social engineering malware is designed to be spread by people via attracting them to infected websites, social media posts, or email links [1]. The ability to spread relies heavily on human psychology, something that cannot be deduced from studying the computer code alone. Similarly, the insect transmitted plant pathogenic bacteria, phytoplasma, use methods to modulate the behaviours of their insect vectors, which are sap-feeding hemipteran insects, enabling the spread of these bacteria in the environment. Phytoplasma secrete specific virulence proteins, named effectors, into their host plants to accomplish this. Intriguingly, two phytoplasma effector proteins, SAP11 and SAP54, influence leafhopper fecundity and plant preference. Here, we describe how these two effectors have a crucial role in re-programming plant cellular processes leading to the induction of specific plant phenotypes. We then summarise how these effectors modulate the behaviours of phytoplasma insect vectors. Finally, we argue that a modelling approach will enable the dissection of the mechanisms involved in phytoplasma epidemics. Indeed, using experimental data of how phytoplasma effectors change plant development and plant interactions with insect vectors, we can now model the impacts of phytoplasma effector genes on the wider environment, including how the changes in insect vector behaviours may contribute to spread of phytoplasma to other plants within the field, to neighbouring fields and across longer distances. This can be achieved through multi-layered mechanistic modelling. In mechanistic models, relationships between the variables in the data set are specified in terms of the biological processes that are thought to have given rise to the data, in contrast to phenomenological and statistical models, where the relationship seeks to best describe the data [2]. The interactions and feedbacks between different layers of organization calls for multilevel modelling, the layers allowing to include the modelling of: effector-mediated changes within the plant; effector-triggered modifications of leafhopper behaviours; the impact of leafhopper behaviours on phytoplasma acquisition and transmission; and the impact of spatial dimensions and plant species occurrences within a field and its neighbouring fields on phytoplasma spread.

Phytoplasma have small repeat-rich genomes

Phytoplasma possess the smallest genome of any plant pathogenic bacteria and have limited metabolic pathways [3–6] and hence are completely reliant on plant host cells for essential nutrients. While phytoplasma genomes sizes are only 600–900 kb [5,7], a large portion of their genomes consist of repeats that resemble composite transposable elements, called potential mobile units (PMUs),

sequence variable mosaics (SVMs) or mobile unit genes (MUGs) [3,8,9]. The repeats are pathogenicity islands, as these tend to cluster together in phytoplasma genomes, form extrachromosomal units, are horizontally transferred among phytoplasma and carry the majority of effector genes [3,10–12]. Genomic synteny between closely related phytoplasma is low, particularly in PMU-rich regions, indicating that these regions are prone to frequent recombination events in which effector genes may be lost or gained [3,13,14•,15,16]. Comparing the complete genome sequences of three phytoplasmas to those of *Acholeplasma* species, the closest free-living relatives of phytoplasma, revealed that the former are enriched in genes for host-interactions and virulence proteins [17].

Phytoplasma secrete effectors that migrate systematically in plants

Phytoplasma-infected plants often show dramatic symptoms, such as extensive stem proliferations, creating a witch's broom phenotype, the retrograde development of flowers into leaf-like flowers, called phyllody, and greening of flower organs, called virescence (Figure 1a). These symptoms are now known to be caused by effector proteins secreted by the phytoplasma into the cytoplasm of phloem sieve cells and that unload from the phloem to other cells and migrate systemically [18–21]. Phytoplasma have a functional Sec-dependent pathway [22] and candidate effectors were identified via the presence of cleavable signal peptides. For example, the genome of aster yellows witches' broom (AYWB; *Ca.* P. asteris) phytoplasma encodes genes for 56 secreted AY-WB proteins (SAPs) that are candidate effectors [18], whereas fewer effectors genes were found in the genomes of maize bushy stunt phytoplasma (MBSP; *Ca.* P. asteris) and *Ca.* P. aurantifolia strains [14•,23]. SAP11 and SAP54 of AYWB (Figure 1b,c) and TENGU from onion yellows phytoplasma (OY; *Ca.* P. asteris) and homologs of these effectors from other phytoplasmas were demonstrated to be genuine virulence factors. Here we describe how these effectors contribute to the symptoms induction of phytoplasma-infected plants.

Phytoplasma effector SAP11 induces stem proliferations and alters leaf development

Transgenic *Arabidopsis thaliana* plants that stably express $SAP11_{AYWB}$ under control of a *35S* promoter produce more stems with leaves that have altered shapes, resembling the witch's broom symptoms of infected plants (Figure 1a,b) [24–26]. $SAP11_{AYWB}$ transgenic plants also exhibit altered root architectures, higher expression of genes involved in phosphate (Pi) starvation and reduced defence responses mediated by suppression of jasmonic acid (JA) synthesis and JA and salicylic acid (SA) responses [24,26,27••]. $SAP11_{AYWB}$ interacts with and destabilizes proteins of the plant-specific TEOSINTE BRANCHED 1, CYCLOIDEA, PCF1 (TCP) transcription factor family [25,26] (Figure 1c). This family consists of class I and II members that at the cellular level antagonistically regulate cell proliferation and senescence, including the JA signalling pathway [28–31]. $SAP11_{AYWB}$ interacts and destabilizes class II TCPs specifically, thereby inhibiting senescence, including reducing JA synthesis, but promoting cell proliferation leading to leaf crinkling and stem proliferation phenotypes (Figure 1c) [25,26]. $SAP11_{AYWB}$ targeting of plant cell nuclei, via it is nuclear localization signal (NLS), contributes to TCP destabilization [18,25] and in AYWB-infected plants, $SAP11_{AYWB}$ unloads from the phloem to migrate to adjacent tissues and was detected in nuclei of trichomes [18]. Interestingly, $SAP11_{AYWB}$ undergoes proteolytic cleavage in plants [32], but how this affects its function, including nuclear localization and interactions with TCPs, is not yet clear.

A SAP11 homolog identified from apple proliferation phytoplasma (AP; Ca. *P. mali*) [33] interacts with TCPs as well [34], and so does SWP11 of wheat blue dwarf phytoplasma [35]. *Nicotiana benthamiana* plants that express $SAP11_{AP}$ show defects in the development of glandular trichomes and reduced expression of a gene encoding an o-methyltransferase involved in the biosynthesis of the volatile organic compound (VOC) 3-isobutyl-2-methoxypyrazine (IBMP) [27••]. This is in agreement with the class II TCP4 being involved in the regulation of trichrome branching [36] and AP-infected apple trees emitting altered VOCs resulting in the attraction of AP psyllid vectors [37]. Therefore, SAP11 effectors of diverse phytoplasmas interact with plant TCP transcription factors and induce a range of phenotypes in plants.

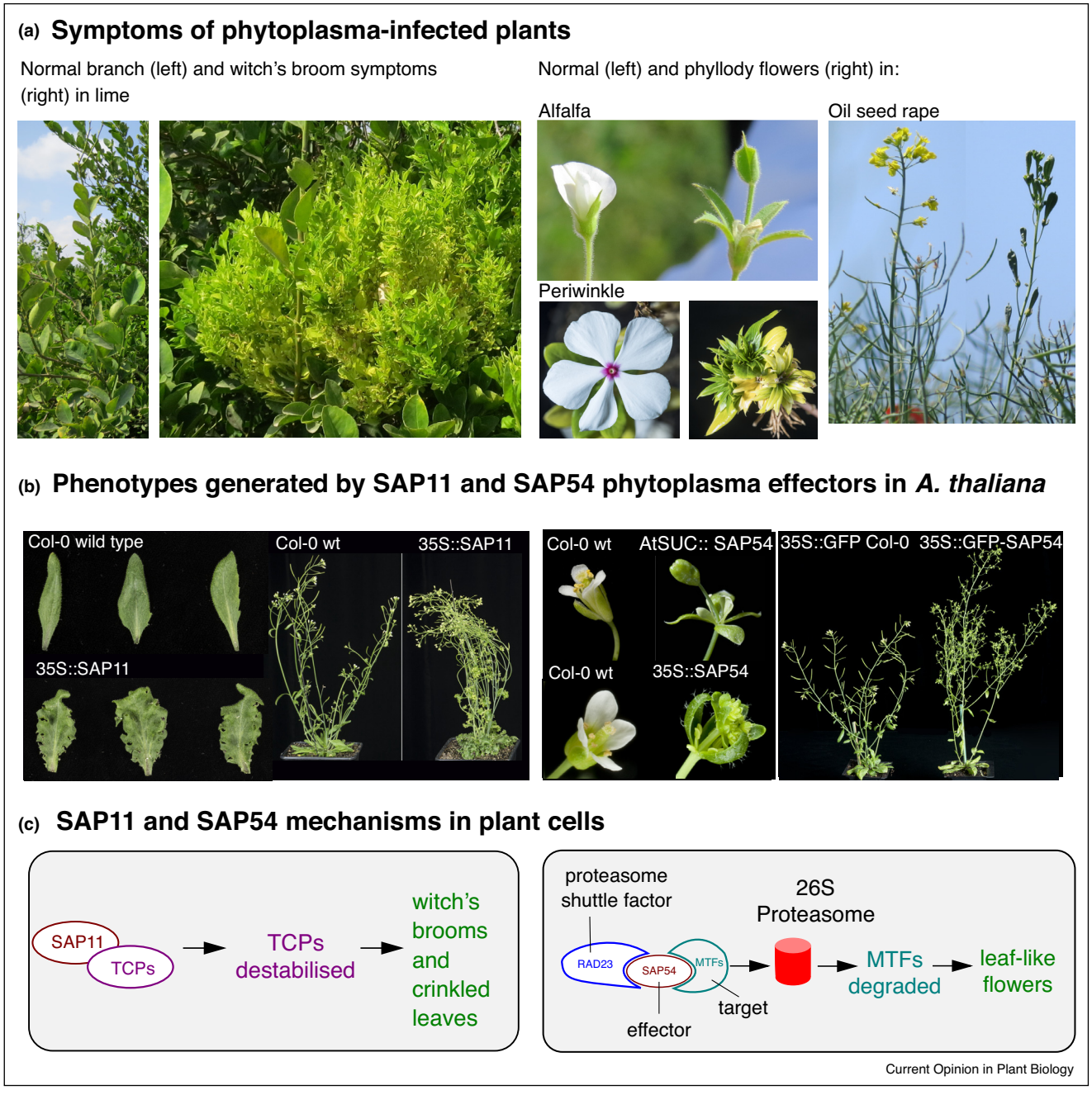
The phytoplasma effector TENGU induces transcriptional changes in plants

Plants that stably produce TENGU display witches' brooms, dwarfism and flower sterility [19,38]. TENGU is highly expressed during OY infection [19] and is cleaved into shorter peptides of 19-21 amino acid long, of which an 11 amino acid domain induces the changes in plant phenotype [39]. Plants stably expressing TENGU show misregulation of transcripts involved in auxin and JA signalling leading to the conclusion that TENGU suppresses the expression of auxin response factor 6 (ARF6) and ARF8 genes [38].

Phytoplasma effector SAP54 induces the development of leaf-like flowers

A. thaliana plants that stably express $SAP54_{AYWB}$ phytoplasma under control of the ubiquitous *35S* and the phloem-specific *AtSuc2* promoters produce leaf-like flowers, often with new stems emerging from within the flower, resembling phytoplasma-induced phyllody and virescence symptoms (Figure 1a,b) [20]. SAP54 and homologs of this effector, named phyllogen, degrade MADS-box transcription factors (MTFs), including those involved in flower development (Figure 1c) [40,41]. For $SAP54_{AYWB}$, this degradation process requires the 26S

Figure 1



(a) Phytoplasma-infected plants often display an array of fascinating changes in plant development, including stem proliferations (witch's brooms) and the retrograde development of flowers into leaves (phyllody). **(b)** Single phytoplasma virulence proteins (effectors) produced by phytoplasma and secreted into plants during infection alter *Arabidopsis thaliana* development; SAP11 of Aster Yellows Witches Broom phytoplasma (AY-WB) changes leaf development and the production of more branches that resemble witch's broom symptoms and AY-WB SAP54 the formation of leaf-like indeterminate flower-like tissues that look like phyllody symptoms. **(c)** The molecular mechanisms of how these virulence factors induce plant developmental changes are known, that is: SAP11 destabilizes plant TEOSINTE BRANCHED 1, CYCLOIDEA, PCF1 (TCP) transcription factors, generating witch's brooms and crinkled leaves; and SAP54 degrades plant MADS-box transcription factors (MTFs) by interacting with the plant proteasome shuttle factor RAD23 (susceptibility factor) and via 26S proteasome, generating leaf-like flowers.

proteasome shuttle factors RAD23, particularly RAD23C and D (Figure 1c) [40]. $SAP54_{AYWB}$ binds the MTF K-domains [40], which facilitates dimerization of MTFs and is characterized by a coiled-coil structure [42]. It was proposed that SAP54 may fold into a structure similar to that of the K-domain, and as such, SAP54 could be useful for studying the contributions of MTFs to flowering of genetically intractable plants [43•]. SAP54/phyllogen homologs were identified in at least 17 diverse phytoplasma species, and, for those tested, interactions with MTFs were shown [20,41,44•,45••].

Phytoplasma colonize multiple organs within their insect vectors

Phytoplasma are transmitted to plants by sap-feeding insects of the order Hemiptera, primarily leafhoppers, planthoppers and psyllids [46]. Transmission of phytoplasma through insects involves invasion of gut cells, migration throughout the haemolymph, colonization of various organs, including the salivary glands, and passage to the saliva from where the phytoplasma are introduced into the phloem sieve cytoplasm when the insects feed [15]. Phytoplasma can have broad or narrow plant host ranges, often coinciding with the host ranges of their predominant insect vectors [47]. Phytoplasma adjust to their plant and insect hosts via differential regulation of gene expression, including effector genes [48,49]. For AYWB phytoplasma, approximately 30% of the 56 effector genes are upregulated in AYWB-infected leafhoppers, and 60% in AYWB-infected plants [40]. Specific sigma factors are likely to regulate this gene expression transition [48,49].

So far, only a few researchers have focused on dissection of the molecular mechanisms involved in phytoplasma–insect interactions. The abundant cell-surface phytoplasma membrane protein antigenic membrane protein (Amp) of *Ca*. P. asteris OY forms a complex with actin and myosin heavy and light chains in the guts of OY vector insects, but not in those of non-vectors, indicating that Amp binds specific gut receptors of insect vectors only [50]. The Amp proteins of another *Ca*. P. asteris also form complexes with actin and ATP synthases located in the gut and salivary glands of insect vectors only [51,52]. Moreover, the Amp proteins of stolbur phytoplasmas (named StAmp) show signatures of diversifying selection [53]. Therefore, Amp is likely to have a crucial role in determining the specificity of phytoplasma–vector interactions. *Ca*. P. asteris also produce Amp in the plant phloem [54] and this is likely important, as insect vectors feed from the phloem and acquire the phytoplasma from there. Interestingly, *Ca*. P. mali and related phytoplasmas produce a different type of immunodominant membrane protein, called immunodominant membrane protein (Imp), which are under strong diversifying selection [55]. By contrast, the *Ca*. P. mali immunodominant protein Idp specifically binds plant actin and appears to have a role in phytoplasma mobility within the plant [56].

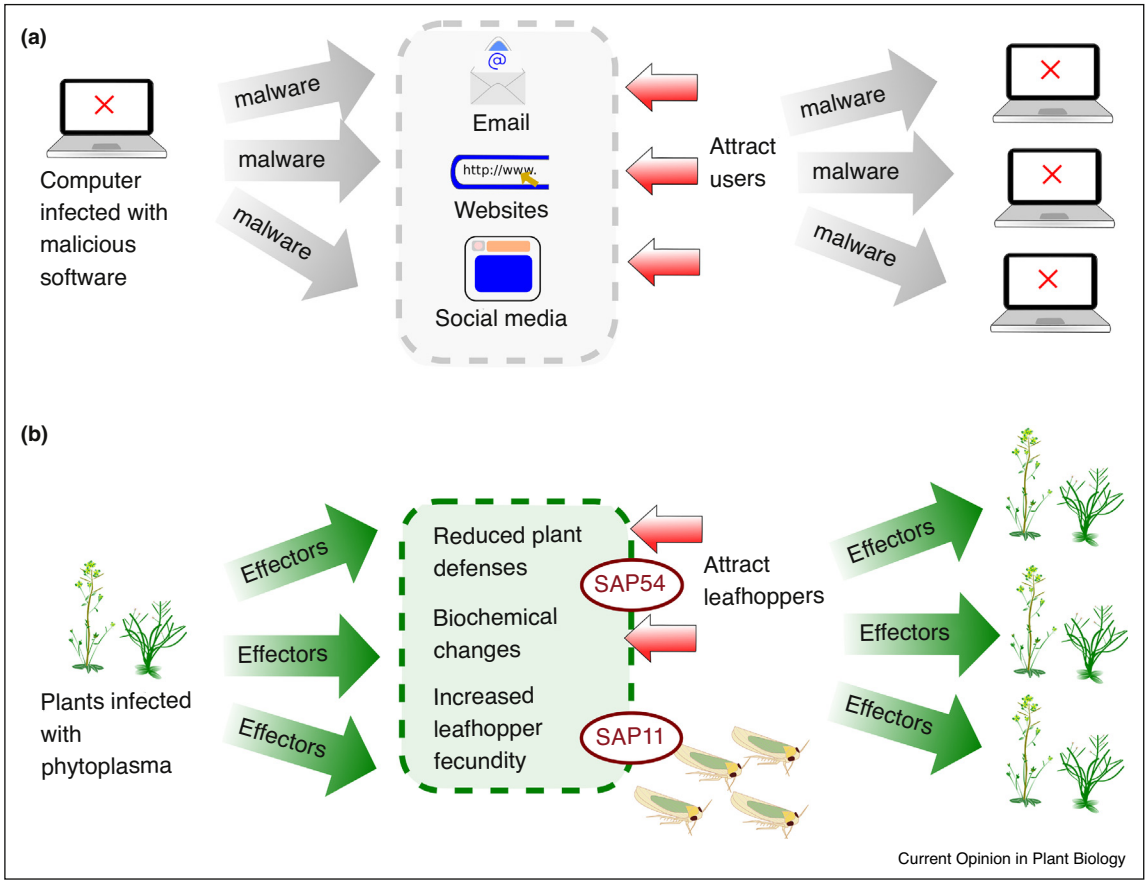
Phytoplasma versus malware, an analogy

Predicting spread of vector-borne disease agents requires an understanding of the mechanisms by which these agents are transferred among hosts. Following up on our analogy, malware is unknowingly transmitted by computer users, often when visiting infected websites or opening infected email attachments (Figure 2a). Social engineering malware makes use of social disguises, cultural ploys and psychological tricks to lure users to infected servers [1], for example through the promise of prizes or financial gain, or by pretending to be sent by friends or family. Another characteristic of such malware is its ability to combat existing technical countermeasures, again typically involving the user voluntarily removing or turning off existing protection [1]. Once a new computer host becomes infected, the malicious software can spread rapidly, infecting other software or resources, or modifying core functions, potentially leaving the system open to further attacks. The spread of phytoplasma can be viewed in a comparable manner. Insect vectors, such as leafhoppers, are attracted to infected plants, contract the pathogen, and then transmit it to new host plants (Figure 2b). Similar to malware, effector proteins modify the physiology of infected plants triggering modulations in leafhopper behaviours, which in turn increase the number of infected vectors. The same core strategies are involved, namely luring in more insects and reducing countermeasures, together increasing both the number of leafhopper visits and the fecundity of those visiting leafhoppers. Note, however, also the limitation of such an analogy, because unlike computer users, leafhoppers get colonized by the phytoplasma and transmit these bacteria, adding an additional level of complexity. In the next sections, we will describe the mechanisms by which this impact on the leafhoppers is achieved.

How phytoplasma virulence effectors contribute to insect vector transmission

Phytoplasma is a biotrophic bacterial pathogen that depends on insect vectors for transmission and colonization of plants. In this regard, it is interesting that phytoplasma SAP11 suppresses JA synthesis via targeting class II TCPs, because plants compromised in JA synthesis and signalling are more susceptible to insects, including leafhoppers that vector phytoplasma [26,57]. Therefore, phytoplasma effectors appear to have evolved to promote insect vector colonization. By contrast, biotrophic pathogens that do not depend on insect vectors for transmission often produce effectors that activate the JA pathway leading, via phytohormone crosstalk, to SA pathway inhibition, thereby promoting colonization of these pathogens [58]. Consistent with this, *P. syringae* HopBB1 targets class I TCPs to de-repress JA signalling [59] and some effectors from biotrophic fungi and oomycetes were found to interact with class I TCPs too [60–62].

Figure 2



Comparing the spread of phytoplasma between plants to that of social engineering malware between computers. **(a)** Malware is transmitted between computers by unsuspecting users. The software employs mechanisms to attract users, through false promises or by impersonating as a known contact, and to combat technical countermeasures, again typically involving the user, by voluntarily removing or turning off existing protection. **(b)** The phytoplasma are spread by hemipteran vectors (in the picture: leafhoppers). Their spread is promoted by biochemical changes made by phytoplasma effector proteins. The changes they induce both attracts the insects to the plants and reduces the defence of the plants against the insects, making them better hosts.

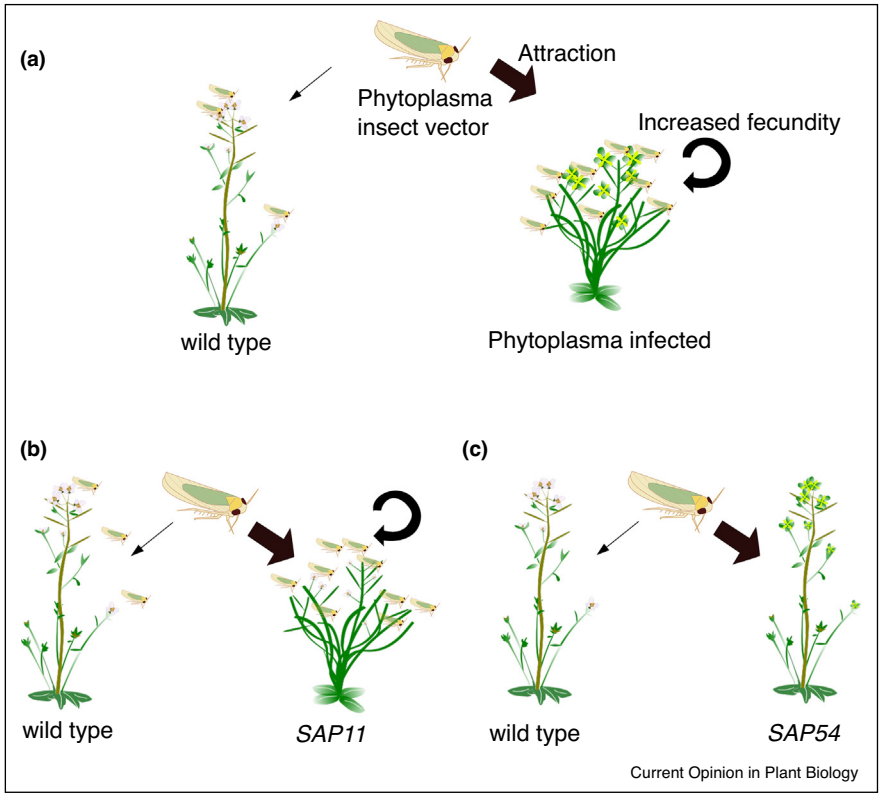
Insect vectors reproduce more on phytoplasma-infected plants in nature and the laboratory in choice and no-choice tests [26,40,63,64] and the SAP11 and SAP54 effectors are likely to play a role in this (Figure 3). $SAP11_{AYWB}$ promotes egg laying of the AYWB leafhopper vector *Macrosteles quadrilineatus* on *A. thaliana* plants in no-choice tests (Figure 3), in agreement with increased egg production of these insects on plants that produce less JA [26]. Leafhoppers also show a preference for colonization of *Nicotiana attenuata* plants that are compromised in JA synthesis, in both nature and the glasshouse [57]. Given that $SAP11_{AP}$ modulates VOCs of plants [27••], it is likely that SAP11 effectors also promote leafhopper vector attraction in choice tests, though this has not yet been examined.

$SAP54_{AYWB}$ promotes *M. quadrilineatus* colonization of *A. thaliana* in choice tests, but not in no-choice tests (Figure 3) [40]. The leafhoppers are also more attracted to AYWB-infected plants in a manner that is dependent on RAD23, which is involved in the SAP54-mediated degradation of MADS-box transcription factors (Figure 1c) [40]. Interestingly this attraction also occurs before flowering and to single leaves of the transgenic plants, indicating that the leaf-like flowers are not required for the leafhopper choice [63]. Nonetheless, inhibition of flower development may have other advantages for phytoplasma by, for instance, delaying plant senescence and death, which often occurs in herbaceous plants upon seed production.

A multi-layered mechanistic approach for predicting phytoplasma epidemics

We have described phytoplasma infection at two different levels: firstly, the mechanisms acting within the plant, and secondly, how the changes they induce modulate

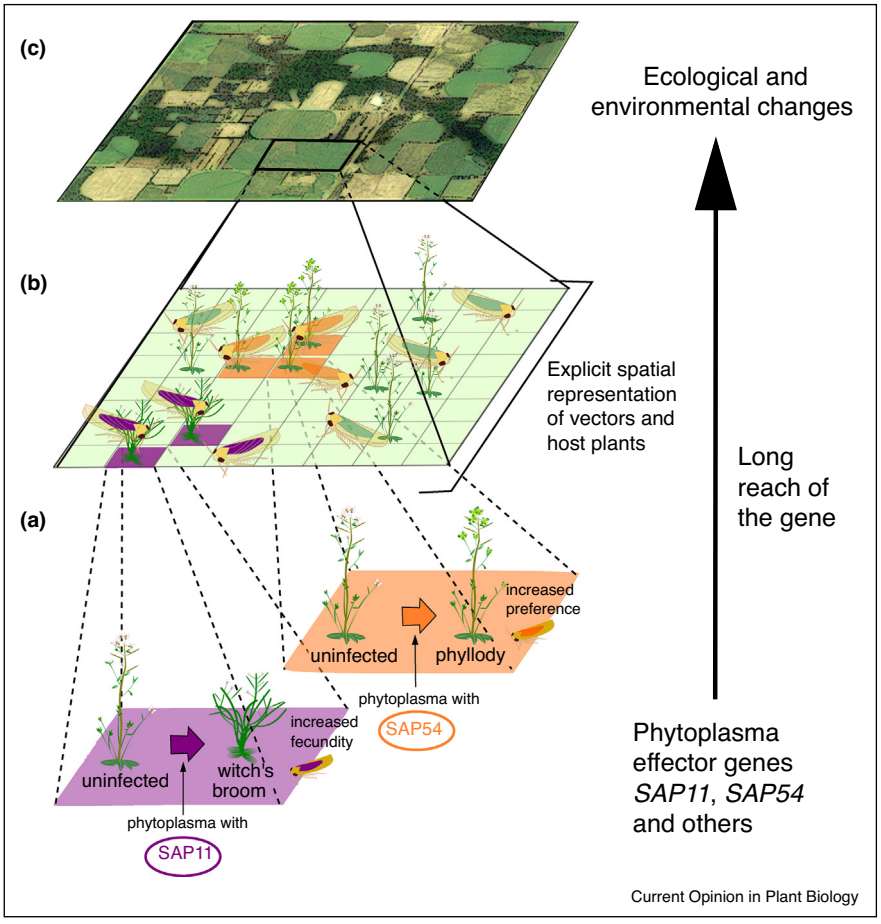
Figure 3



M. quadrilineatus colonizes AYWB phytoplasma-infected *A. thaliana* better in choice and no-choice tests that involves the actions of the AYWB effectors SAP11 and SAP54. **(a)** Leafhoppers are more attracted to (choice tests) and have increased fecundity on (no-choice tests) phytoplasma-infected plants. **(b)** Leafhoppers have increased fecundity on and are more attracted to $SAP11_{AYWB}$ transgenic plants. **(c)** Leafhoppers are more attracted to $SAP54_{AYWB}$ transgenic plants.

leafhopper behaviour. The final step is to develop an understanding of how leafhopper behaviour influences outbreaks of phytoplasma infection. The spread of social engineering malware cannot be understood by looking at the source code alone, but requires, at a completely different level, understanding of human psychology as well. Likewise, we argue that understanding the spread of phytoplasma requires a comparable multi-level mechanistic approach, designed to tackle some of the challenges of predicting plant disease propagation, as pointed out by [65], for example, capturing host spatial structure, realistic vector dispersal, vector preference and the inclusion of multiple pathogen strains, along with their evolution. We envision such a model to be designed to integrate each of the three levels we have described (Figure 4). The lowest layer comprises the phytoplasma and their effector genes driven processes acting within the plants, phytoplasma titre, effector protein-driven modifications in plant development and the consequential plant phenotypes and their impact on the insect vectors. The next layer captures the field setting, taking the spatial structure of both hosts and vectors into account [66,67]. It describes realistic leafhopper dispersal, including their natural movement as well as their preference for specific (modified) hosts, which can take the form of either directed movement towards specific plants or increased residence time [68]. One possible choice would be to use individual-based models (IBMs), to straightforwardly capture the individual behaviour of insect vectors under different conditions [69]. The top layer takes environmental conditions and crop and weed distribution into account, as well as their impact on dispersal, which can directly include geographical information systems (GIS)-based data [70,71]. Such an approach would allow us to link changes in external forcing, such as climate change, to phytoplasma outbreak. Moreover, through the modelling of competition between multiple phytoplasma effectors, as displayed in the figure, potentially including their evolution, one could address how impact propagates through the diverse biological scales in order to be able to answer how the presence or absence of a single effector gene in the genome of a phytoplasma population can contribute to large-scale changes in phytoplasma infection dynamics.

Figure 4



A multi-layered, mechanistic modelling approach to understand the processes involved in phytoplasma epidemics, integrating between multiple levels of infection dynamics, namely: (i) effector-mediated changes within the plant, and the resulting modifications in leafhopper behaviours; (ii) the field setting, capturing host spatial structure, realistic vector dispersal, and vector preference; and (iii) the environmental setting, taking both climate and geographical information into account. The actions of phytoplasma effectors SAP11 and SAP54, and possibly other effectors, are likely to impact all these layers. Hence, this multi-layered approach is in-effect modelling the long reach of the effector gene.

Conclusions

Two distinctive features of social engineering malware are its pervasiveness and persistence. It has been proposed that these cannot be combatted by the pursuit of purely technical solutions alone, but require a multi-pronged attack [1]. Data discussed here suggests the same to be true of phytoplasma infections. Phytoplasma effector proteins not only modulate plant development, but also promote colonisations of insect vectors that are crucial for phytoplasma spread. However, it is not yet clear to what extent an increase in vector colonization helps phytoplasma spread, and whether some effectors are more advantageous to spread than others. A multi-layered modelling approach, with the ability to incorporate all levels of phytoplasma infection, would provide the missing link between insect vector behaviours and pathogen spread, and, in particular, how these are influenced by specific effector proteins.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Abraham S, Chengalur-Smith I: **An overview of social engineering malware: trends, tactics, and implications**. *Technol Soc* 2010, **32**:183-196.

2. Hilborn R, Mangel M: *The Ecological Detective: Confronting Models with Data*. Princeton University Press; 1997.

3. Bai X, Zhang J, Ewing A, Miller SA, Radek AJ, Shevchenko DV, Tsukerman K, Walunas T, Lapidus A, Campbell JW *et al.*: **Living with genome instability: the adaptation of phytoplasmas to diverse environments of their insect and plant hosts**. *J Bacteriol* 2006, **188**:3682-3696.

4. Kube M, Mitrovic J, Duduk B, Rabus R, Seemüller E: **Current view on phytoplasma genomes and encoded metabolism**. *Sci World J* 2012:2012.

5. Kube M, Schneider B, Kuhl H, Dandekar T, Heitmann K, Migdoll AM, Reinhardt R, Seemüller E: **The linear chromosome of the plant–pathogenic mycoplasma'Candidatus Phytoplasma mali'**. *BMC Genom* 2008, **9**:306.

6. Oshima K, Kakizawa S, Nishigawa H, Jung H-Y, Wei W, Suzuki S, Arashida R, Nakata D, Miyata S, Ugaki M *et al.*: **Reductive evolution suggested from the complete genome sequence of a plant–pathogenic phytoplasma**. *Nat Genet* 2004, **36**:27.

7. Tran-Nguyen LT, Kube M, Schneider B, Reinhardt R, Gibb KS: **Comparative genome analysis of "Candidatus Phytoplasma australiense" (subgroup tuf-Australia I; rp-A) and "Ca. Phytoplasma asteris" strains OY-M and AY-WB**. *J Bacteriol* 2008, **190**:3979-3991.

8. Arashida R, Kakizawa S, Ishii Y, Hoshi A, Jung H-Y-Y, Kagiwada S, Yamaji Y, Oshima K, Namba S: **Cloning and characterization of the antigenic membrane protein (Amp) gene and in situ detection of Amp from malformed flowers infected with Japanese hydrangea phyllody phytoplasma**. *Phytopathology* 2008, **98**:769-775.

9. Wei W, Davis RE, Jomantiene R, Zhao Y: **Ancient, recurrent phage attacks and recombination shaped dynamic sequence-variable mosaics at the root of phytoplasma genome evolution**. *Proc Natl Acad Sci* 2008, **105**:11827-11832.

10. Chung W-C, Chen L-L, Lo W-S, Lin C-P, Kuo C-H: **Comparative analysis of the peanut witches'-broom phytoplasma genome reveals horizontal transfer of potential mobile units and effectors**. *PLoS One* 2013, **8**:e62770.

11. Ku C, Lo W-S-S, Kuo C-H-H: **Horizontal transfer of potential mobile units in phytoplasmas**. *Mob Genet Elem* 2013 http://dx.doi.org/10.4161/mge.26145.

12. Toruño TY, Seruga Musić M, Simi S, Nicolaisen M, Hogenhout SA: **Phytoplasma PMU1 exists as linear chromosomal and circular extrachromosomal elements and has enhanced expression in insect vectors compared with plant hosts**. *Mol Microbiol* 2010, **77**:1406-1415.

13. Andersen MT, Liefting LW, Havukkala I, Beever RE: **Comparison of the complete genome sequence of two closely related isolates of 'Candidatus Phytoplasma australiense' reveals genome plasticity**. *BMC Genom* 2013, **14**:529.

14. • Orlovskis Z, Canale MC, Haryono M, Lopes JRS, Kuo C-H, Hogenhout SA: **A few sequence polymorphisms among isolates of Maize bushy stunt phytoplasma associate with organ proliferation symptoms of infected maize plants**. *Ann Bot* 2017, **119**:869-884.

This study shows that the effector SAP11 of MBSP is conserved in distinct isolates in Mexico and Brazil, and is one of the first whole genome comparisons among multiple phytoplasma strains with a *Ca.* species.

15. Sugio A, Hogenhout SA: **The genome biology of phytoplasma: modulators of plants and insects**. *Curr Opin Microbiol* 2012, **15**:247-254.

16. Hogenhout SA, Musić M: **Phytoplasma genomics, from sequencing to comparative and functional genomics — what have we learnt?** *Phytoplasmas — Genomes, Plant Hosts and Vectors*. CABI; 2009.

17. Kube M, Siewert C, Migdoll AM, Duduk B, Holz S, Rabus R, Seemüller E, Mitrovic J, Müller I, Büttner C: **Analysis of the complete genomes of Acholeplasma brassicae, A. palmae and A. laidlawii and their comparison to the obligate parasites from 'Candidatus Phytoplasma".'**. *J Mol Microbiol Biotechnol* 2014, **24**:19-36.

18. Bai X, Correa VR, Toruño TY, Ammar E-D-D, Kamoun S, Hogenhout SA: **AY-WB phytoplasma secretes a protein that targets plant cell nuclei**. *Mol Plant Microbe Interact* 2009, **22**:18-30.

19. Hoshi A, Oshima K, Kakizawa S, Ishii Y, Ozeki J, Hashimoto M, Komatsu K, Kagiwada S, Yamaji Y, Namba S: **A unique virulence factor for proliferation and dwarfism in plants identified from a phytopathogenic bacterium**. *Proc Natl Acad Sci* 2009, **106**:6416-6421.

20. MacLean AM, Sugio A, Makarova OV, Findlay KC, Grieve VM, Tóth R, Nicolaisen M, Hogenhout SA: **Phytoplasma effector SAP54 induces indeterminate leaf-like flower development in Arabidopsis plants**. *Plant Physiol* 2011, **157**:831-841.

21. Sugio A, MacLean AM, Kingdom HN, Grieve VM, Manimekalai R, Hogenhout SA: **Diverse targets of phytoplasma effectors: from plant development to defense against insects**. *Annu Rev Phytopathol* 2011, **49**:175-195.

22. Kakizawa S, Oshima K, Kuboyama T, Nishigawa H, Jung H, Sawayanagi T, Tsuchizaki T, Miyata S, Ugaki M, Namba S: **Cloning and expression analysis of phytoplasma protein translocation genes**. *Mol Plant Microbe Interact* 2001, **14**:1043-1050.

23. Anabestani A, Izadpanah K, Abbà S, Galetto L, Ghorbani A, Palmano S, Siampour M, Veratti F, Marzachì C: **Identification of putative effector genes and their transcripts in three strains related to 'Candidatus Phytoplasma aurantifolia'**. *Microbiol Res* 2017, **199**:57-66.

24. Lu Y-T, Li M-Y, Cheng K-T, Tan CM, Su L-W, Lin W-Y, Shih H-T, Chiou T-J, Yang J-Y: **Transgenic plants that express the phytoplasma effector SAP11 show altered phosphate starvation and defense responses**. *Plant Physiol* 2014, **164**:1456-1469.

25. Sugio A, MacLean AM, Hogenhout SA: **The small phytoplasma virulence effector SAP11 contains distinct domains required for nuclear targeting and CIN-TCP binding and destabilization**. *New Phytol* 2014, **202**:838-848.

26. Sugio A, Kingdom HN, MacLean AM, Grieve VM, Hogenhout SA: **Phytoplasma protein effector SAP11 enhances insect vector reproduction by manipulating plant development and defense hormone biosynthesis**. *Proc Natl Acad Sci* 2011, **108**:E1254-E1263.

27. •• Tan CM, Li C-H-H, Tsao N-W-W, Su L-W-W, Lu Y-T-T, Chang SH, Lin YY, Liou J-C-C, Hsieh L-C-C, Yu J-Z-Z *et al.*: **Phytoplasma SAP11 alters 3-isobutyl-2-methoxypyrazine biosynthesis in *Nicotiana benthamiana* by suppressing NbOMT1**. *J Exp Bot* 2016, **67**:4415-4425.

This publication shows that SAP11 is altering the expression of a gene involved in the production of volatiles.

28. Li S: **The *Arabidopsis thaliana* TCP transcription factors: a broadening horizon beyond development**. *Plant Signal Behav* 2015, **10**:e1044192.

29. Nicolas M, Cubas P: **TCP factors: new kids on the signaling block**. *Curr Opin Plant Biol* 2016, **33**:33-41.

30. Danisman S, Van der Wal F, Dhondt S, Waites R, de Folter S, Bimbo A, van Dijk AD, Muino JM, Cutri L, Dornelas MC: **Arabidopsis class I and class II TCP transcription factors regulate jasmonic acid metabolism and leaf development antagonistically**. *Plant Physiol* 2012, **159**:1511-1523.

31. Schommer C, Palatnik JF, Aggarwal P, Chételat A, Cubas P, Farmer EE, Nath U, Weigel D: **Control of jasmonate biosynthesis and senescence by miR319 targets**. *PLoS Biol* 2008, **6**:e230.

32. Lu Y-T-T, Cheng K-T-T, Jiang S-Y-Y, Yang J-Y-Y: **Post-translational cleavage and self-interaction of the phytoplasma effector SAP11**. *Plant Signal Behav* 2014 http://dx.doi.org/10.4161/psb.28991.

33. Siewert C, Luge T, Duduk B, Seemüller E, Büttner C, Sauer S, Kube M: **Analysis of expressed genes of the bacterium 'Candidatus Phytoplasma mali'highlights key features of virulence and metabolism**. *PLoS One* 2014, **9**:e94391.

34. Janik K, Mithöfer A, Raffeiner M, Stellmach H, Hause B, Schlink K: **An effector of apple proliferation phytoplasma targets TCP transcription factors — a generalized virulence strategy of phytoplasma?** *Mol Plant Pathol* 2017, **18**:435-442.

35. Wang N, Li Y, Chen W, Yang HZ, Zhang PH, Wu YF: **Identification of wheat blue dwarf phytoplasma effectors targeting plant proliferation and defence responses**. *Plant Pathol* 2017 http://dx.doi.org/10.1111/ppa.12786.

36. Vadde B, Challa KR, Nath U: **The TCP4 transcription factor regulates trichome cell differentiation by directly activating GLABROUS INFLORESCENCE STEMS in *Arabidopsis thaliana***. *Plant J* 2017 http://dx.doi.org/10.1111/tpj.13772.

37. Mayer CJ, Vilcinskas A, Gross J: **Phytopathogen lures its insect vector by altering host plant odor**. *J Chem Ecol* 2008, **34**:1045-1049.

38. Minato N, Himeno M, Hoshi A, Maejima K, Komatsu K, Takebayashi Y, Kasahara H, Yusa A, Yamaji Y, Oshima K *et al.*: **The phytoplasmal virulence factor TENGU causes plant sterility by downregulating of the jasmonic acid and auxin pathways**. *Sci Rep* 2014, **4**:7399.

39. Sugawara K, Honma Y, Komatsu K, Himeno M, Oshima K, Namba S: **The alteration of plant morphology by small peptides released from the proteolytic processing of the bacterial peptide TENGU**. *Plant Physiol* 2013, **162**:2005-2014.

40. MacLean AM, Orlovskis Z, Kowitwanich K, Zdziarska AM, Angenent GC, Immink RGH, Hogenhout SA: **Phytoplasma effector SAP54 hijacks plant reproduction by degrading MADS-box proteins and promotes insect colonization in a RAD23-dependent manner**. *PLoS Biol* 2014, **12**:e1001835.

41. Maejima K, Iwai R, Himeno M, Komatsu K, Kitazawa Y, Fujita N, Ishikawa K, Fukuoka M, Minato N, Yamaji Y *et al.*: **Recognition of floral homeotic MADS domain transcription factors by a phytoplasmal effector, phyllogen, induces phyllody**. *Plant J* 2014, **78**:541-554.

42. Puranik S, Acajjaoui S, Conn S, Costa L, Conn V, Vial A, Marcellin R, Melzer R, Brown E, Hart D: **Structural basis for the oligomerization of the MADS domain transcription factor SEPALLATA3 in Arabidopsis**. *Plant Cell* 2014, **26**:3603-3615.

43. • Rümpler F, Gramzow L, Theißen G, Melzer R: **Did convergent protein evolution enable phytoplasmas to generate 'zombie plants'?** *Trends Plant Sci* 2015, **20**:798-806.
This paper provides compelling evidence that the SAP54 effector could have a similar fold to that of the K-domain of MADS-box transcription factors.

44. • Kitazawa Y, Iwabuchi N, Himeno M, Sasano M, Koinuma H, Nijo T, Tomomitsu T, Yoshida T, Okano Y, Yoshikawa N *et al.*: **Phytoplasma-conserved phyllogen proteins induce phyllody across the Plantae by degrading floral MADS domain proteins**. *J Exp Bot* 2017, **68**:2799-2811.
This study builds on previous evidence that SAP54-like effectors interact with MADS-box transcription factors making it relevant for a broad range of phytoplasma–plant interactions.

45. •• Yang C-Y, Huang Y-H, Lin C-P, Lin Y-Y, Hsu H-C, Wang C-N, Li-Yu DL, Shen B-N, Lin S-S: **MiR396-targeted short vegetative phase is required to repress flowering and is related to the development of abnormal flower symptoms by the PHYL1 effector**. *Plant Physiol* 2015 http://dx.doi.org/10.1104/pp.15.00307.
This elegant study shows the mechanisms by which an effector of a biotrophic bacterial pathogen degrades a class 1 TCP transcription factor thereby derepressing jasmonate responsive genes leading to higher pathogen virulence.

46. Weintraub PG, Beanland L: **Insect vectors of phytoplasmas**. *Annu Rev Entomol* 2006, **51**:91-111.

47. Foissac X, Wilson MR: **Current and possible future distributions of phytoplasma diseases and their vectors**. In *Phytoplasmas Genomes Plant Hosts Vectors.* Edited by Weintraub PG, Jones P. CBA International; 2009.

48. Ishii Y, Kakizawa S, Oshima K: **New ex vivo reporter assay system reveals that σ factors of an unculturable pathogen control gene regulation involved in the host switching between insects and plants**. *Microbiol Open* 2013, **2**:553-565.

49. Miura C, Komatsu K, Maejima K, Nijo T, Kitazawa Y, Tomomitsu T, Yusa A, Himeno M, Oshima K, Namba S: **Functional characterization of the principal sigma factor RpoD of phytoplasmas via an in vitro transcription assay**. *Sci Rep* 2015, **5**.

50. Suzuki S, Oshima K, Kakizawa S, Arashida R, Jung H-Y-Y, Yamaji Y, Nishigawa H, Ugaki M, Namba S: **Interaction between the membrane protein of a pathogen and insect microfilament complex determines insect-vector specificity**. *Proc Natl Acad Sci U S A* 2006, **103**:4252-4257.

51. Galetto L, Bosco D, Balestrini R, Genre A, Fletcher J, Marzachì C: **The major antigenic membrane protein of "Candidatus Phytoplasma asteris" selectively interacts with ATP synthase and actin of leafhopper vectors**. *PLoS ONE* 2011, **6**:e22571.

52. Rashidi M, Galetto L, Bosco D, Bulgarelli A, Vallino M, Veratti F, Marzachì C: **Role of the major antigenic membrane protein in phytoplasma transmission by two insect vector species**. *BMC Microbiol* 2015, **15**:193.

53. Fabre A, Danet J-L, Foissac X: **The stolbur phytoplasma antigenic membrane protein gene stamp is submitted to diversifying positive selection**. *Gene* 2011, **472**:37-41.

54. Kakizawa S, Oshima K, Nishigawa H, Jung H-Y, Wei W, Suzuki S, Tanaka M, Miyata S, Ugaki M, Namba S: **Secretion of immunodominant membrane protein from onion yellows phytoplasma through the Sec protein–translocation system in *Escherichia coli***. *Microbiology* 2004, **150**:135-142.

55. Kakizawa S, Oshima K, Jung H-Y, Suzuki S, Nishigawa H, Arashida R, Miyata S, Ugaki M, Kishino H, Namba S: **Positive selection acting on a surface membrane protein of the plant–pathogenic phytoplasmas**. *J Bacteriol* 2006, **188**:3424-3428.

56. Boonrod K, Munteanu B, Jarausch B, Jarausch W, Krczal G: **An immunodominant membrane protein (Imp) of 'Candidatus Phytoplasma mali' binds to plant actin**. *Mol Plant Microbe Interact* 2012, **25**:889-895.

57. Kallenbach M, Bonaventure G, Gilardoni PA, Wissgott A, Baldwin IT: **Empoasca leafhoppers attack wild tobacco plants in a jasmonate-dependent manner and identify jasmonate mutants in natural populations**. *Proc Natl Acad Sci* 2012, **109**:E1548-E1557.

58. Kazan K, Lyons R: **Intervention of phytohormone pathways by pathogen effectors**. *Plant Cell* 2014, **26**:2285-2309.

59. Yang L, Teixeira PJPL, Biswas S, Finkel OM, He Y, Salas-Gonzalez I, English ME, Epple P, Mieczkowski P, Dangl JL: ***Pseudomonas syringae* Type III effector HopBB1 promotes host transcriptional repressor degradation to regulate phytohormone responses and virulence**. *Cell Host Microbe* 2017, **21**:156-168.

60. Mukhtar MS, Carvunis A-R, Dreze M, Epple P, Steinbrenner J, Moore J, Tasan M, Galli M, Hao T, Nishimura MT: **Independently evolved virulence effectors converge onto hubs in a plant immune system network**. *Science* 2011, **333**:596-601.

61. Stam R, Motion G, Boevink PC, Huitema E: **A conserved oomycete CRN effector targets and modulates tomato TCP14-2 to enhance virulence**. *BioRxiv* 2013 http://dx.doi.org/10.1101/001248.

62. Weßling R, Epple P, Altmann S, He Y, Yang L, Henz SR, McDonald N, Wiley K, Bader KC, Gläßer C: **Convergent targeting of a common host protein-network by pathogen effectors from three kingdoms of life**. *Cell Host Microbe* 2014, **16**:364-375.

63. Orlovskis Z, Hogenhout SA: **A bacterial parasite effector mediates insect vector attraction in host plants independently of developmental changes**. *Front Plant Sci* 2016, **7**:885.

64. Queiroz RB, Donkersley P, Silva FN, Al-Mahmmoli IH, Al-Sadi AM, Carvalho CM, Elliot SL: **Invasive mutualisms between a plant pathogen and insect vectors in the Middle East and Brazil**. *Open Sci* 2016, **3**:160557.

65. Cunniffe NJ, Koskella BE, Metcalf CJ, Parnell S, Gottwald TR, Gilligan CA: **Thirteen challenges in modelling plant diseases**. *Epidemics* 2015, **10**:6-10.

66. Ostfeld RS, Glass GE, Keesing F: **Spatial epidemiology: an emerging (or re-emerging) discipline**. *Trends Ecol Evol* 2005, **20**:328-336.

67. Zhou X, Hoy CW, Miller SA, Nault LR: **Spatially explicit simulation of aster yellows epidemics and control on lettuce**. *Ecol Model* 2002, **2–3**:293-307.

68. Sisterson MS: **Effects of insect-vector preference for healthy or infected plants on pathogen spread: insights from a model**. *J Econ Entomol* 2008, **101**:1-8.

69. Willem L, Verelst F, Bilcke J, Hens N, Beutels P: **Lessons from a decade of individual-based models for infectious disease transmission: a systematic review (2006–2015)**. *BMC Infect Dis* 2017, **17**:612.

70. Jamison A, Tuttle E, Jensen R, Bierly G, Gonser R: **Spatial ecology, landscapes, and the geography of vector-borne disease: a multi-disciplinary review**. *Appl Geogr* 2015, **63**:418-426.

71. Arifin SMN, Arifin RR, Pitts D, de A, Rahman MS, Nowreen S, Madey GR, Collins FH: **Landscape epidemiology modeling using an agent-based model and a geographic information system**. *Land* 2015, **4**:378-412.