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VIROPLANT in a Nutshell

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SINCE 2018, VIROLOGY RESEARCHERS from universities and industry across eight countries in Europe have joined forces with academics in the field of social and economic sciences as well as stakeholders. Together they study the feasibility of applying viruses to control pests and pathogens in crop production within the research program VIROPLANT (https://viroplant.eu/).

This Horizon 2020 project aims to develop new environmentally friendly virus-based control strategies against bacterial, fungal, and insect vector-transmitted diseases as well as insect pests. Such integrated biocontrol strategies based on viruses would aspire to reduce pesticide usage and, therefore, limit their negative impact on the environment. The project also takes into account the economic feasibility and the societal perception of using viruses as biocontrol agents in agriculture.

On one hand, the project focuses on the discovery and potential exploitation of phytopathogen-relevant viruses, such as mycoviruses infecting fungi and oomycetes that cause disease in grapevine, or viruses associated with the vector of grapevine Flavescence dorée phytoplasma,[1] as well as insect pests of horticultural importance and insect vectors of tomato, pepper, and onion viral diseases.[2,3] On the other hand, the emphasis lies with bacterial diseases associated with the production of kiwifruit, bean, stone fruit, tomato, and cucumber. For these pathogens, a phage-based biocontrol strategy is envisioned that takes into account the bacterial infection avenues and tailored delivery of new phage cocktails, the development of relevant bioassays, and trials to challenge efficacy and an overall safety appraisal. All of this linked to studies to assess social and economic acceptability of these virus-based biocontrol strategies, which in times of a COVID pandemic, has become more difficult to translate to a lay audience.

One key example toward the development of a phage biocontrol cocktail is bacterial canker in cherry (*Prunus avium*). *Prunus* canker is caused by *Pseudomonas syringae* pv. syringae, a major phytopathogen for hundreds of economically relevant plant species showing widespread resistance to traditional disease management based on copper treatment. Rabiey et al. set up an extensive library of >70 phages, isolated from soil, leaf, and bark of cherry trees, from which a phage cocktail was selected and characterized. An established *in planta* bioassay in beans and an assay on cherry leaves and twigs indicated the phage biocontrol cocktail significantly reduced bacterial infections.[4] A delivery based on injection in the tree bark would present an appealing avenue to pursue in this regard.

Combinatorial treatments including phages and silica nanoparticles are also being evaluated whether they act synergistically to combat *Ralstonia solanacearum*, a diverse phytopathogen causing bacterial wilt in tomato plants, for which a phage cocktail has been established.[5]

Although basic principles have been defined in terms of assaying efficacy, only limited information is available concerning the ecological impact of the widespread application of phages in the field. From this perspective, a key question arises: which changes are introduced into the target environment after phage treatment, or does phage introduce a restoration of a healthy microbiome limiting the effect of the bacterial pathogen on the microbial community? An answer to this question can only be provided by a comprehensive Environmental Risk Assessment, which studies the environmental footprint of the agricultural system by phage biocontrol. This includes alterations to the microbiome, the spread of phage, resistance development, the killing of off-target organisms, as well as hazards and risks associated with horizontal gene transfer.

In this regard, Holtappels et al. argue for the integration of phage-based biocontrol in smart farming, based on Internet of Things as well as research needed to assess the biosafety of phages within a context of biopesticide regulation in Europe.[6] This integration may also be of key importance toward economic feasibility and the impact of how patents in this field are set up. Indeed, it appears that patent filings remain limited, despite a growing interest in phage biocontrol. An analysis did show that overall, research articles and patent documents share a common focus in terms of pathogens targeted. The emphasis lies with efforts by groups within Asia to protect biocontrol preparations, with granted patents that contain relatively broad claims.[7]

Currently, only a limited number of phage biocontrol products have reached the market, primarily in the United States, where the Environmental Protection Agency has granted license to four phage products. Within Europe, additional hurdles will still need to be overcome to implement phage-based biocontrol. Indeed, pesticide registration will require key insights into specific pathosystems, assessment of phage resistance development, and tailored production and application methods. In addition, no specific European Union

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legislation or guideline exists with regard to environmental safety assessment, requiring interpretations of existing regulations to bring phage biocontrol into the fold.

Taken together, these opportunities and hurdles represent a unique challenge that the VIROPLANT consortium remains eager to tackle and enthusiastically aims to engage new opportunities for collaboration with external interested parties within academia and industry.

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References

1. Ottati S, Chiapello M, Galetto L, et al. New viral sequences identified in the flavescence dorée phytoplasma vector scaphoideus titanus. Viruses. 2020;12:1–20.
2. Ottati S, Persico A, Rossi M, et al. Biological characterization of *Euscelidius variegatus* iflavirus 1. J Invertebr Pathol. 2020;173:107370.
3. Nerva L, Vigani G, Di Silvestre D, et al. Biological and molecular characterization of chenopodium quinoa mitovirus 1 reveals a distinct small RNA response compared to those of cytoplasmic RNA viruses. J Virol. 2019;93:1–17.
4. Rabiey M, Roy SR, Holtappels D, et al. Phage biocontrol to combat *Pseudomonas syringae* pathogens causing disease in cherry. Microb Biotechnol. 2020;13:1428–1445.
5. Wang X, Wei Z, Yang K, et al. Phage combination therapies for bacterial wilt disease in tomato. Nat Biotechnol. 2019;37: 1513–1520.
6. Holtappels D, Fortuna K, Lavigne R, et al. The future of phage biocontrol in integrated plant protection for sustainable crop production. Curr Opin Biotechnol. 2021;68:60–71.
7. Holtappels D, Lavigne R, Huys I, et al. Protection of phage applications in crop production: A patent landscape. Viruses. 2019;11:277.

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