

Commentary

When plants are Trojan horses for viruses

Like the wooden horse left by the Greeks that entered Troy, and was apparently innocuous, new plant genotypes often incorporated by researchers into their laboratories and most new plants migrating into already existing populations look healthy. In this issue of *New Phytologist*, Verhoeven *et al.* (2023; pp. 1146–1153) show that, as in the Aeneid story where the horse hides Odysseus and his soldiers, asymptomatic *Arabidopsis thaliana* (*Arabidopsis*) plants may harbor latent viruses with potential significant effects on the biology of the plant. In particular, the authors identify *Arabidopsis* latent virus 1 (ArLV1) as being present in laboratory collections of *Arabidopsis* genotypes and in wild plant populations at high prevalence causing asymptomatic infections. This striking observation raises two interesting questions: how was this latent virus so successful in invading *Arabidopsis* populations? And what are the consequences of ArLV1 presence for the plant even if infection is asymptomatic? (Fig. 1).

‘... this work makes plant biologists aware of the potential risk of asymptomatic virus infections in their lab seed stocks.’

The most accepted theory on the evolution of parasite virulence states that a negative effect of infection on the host fitness is an unavoidable consequence of the parasite multiplication. Because multiplication is positively associated with between-host transmission, a certain level of virulence is necessary for the parasite to invade the host population (Anderson & May, 1982). However, there is increasing evidence that asymptomatic plant–virus infections are frequent in nature (Zhang *et al.*, 2018; Tabara *et al.*, 2021). Indeed, it is thought that scientists know of only a small proportion of the latent and persistent plant viruses existing in nature (Takahashi *et al.*, 2019; Chofong *et al.*, 2021). Several nonmutually exclusive explanations have been proposed for the high prevalence of asymptomatic infections, which may also account for the ArLV1 success:

(1) Accumulating evidence indicates that virus infections may provide beneficial effects for plants by protecting them against herbivores or more virulent viruses (Safari *et al.*, 2019), by improving pollination and seed production (Groen *et al.*, 2016), and/or by conferring tolerance to abiotic stresses (Roossinck, 2015; Aguilar & Lozano-Duran, 2022). This is changing how scientists understand plant viruses, from considering them as strict parasites to acknowledging that they often establish mutualistic interactions

with plants. Verhoeven *et al.*, support this view as they report improved drought tolerance in ArLV1-infected plants. It has been shown that the same virus may confer tolerance to different abiotic stresses (Montes & Pagán, 2022). Hence, these results open the door to studying other positive effects of ArLV1 for *Arabidopsis* in suboptimal conditions, which may explain the ubiquitous nature of the virus.

(2) Viral asymptomatic infections are host- and environment-dependent, and changes in these factors might result in pathogenic rather than mutualistic interactions (Takahashi *et al.*, 2019; Ilyas *et al.*, 2022). Thus, a virus that infects a given plant species asymptotically may have a negative effect on other hosts, which may be competing for resources in the same habitat. This role of viruses as natural ‘biological weapons’, which has been shown to be linked to the success of certain invasive plant species (Malmstrom *et al.*, 2005), may explain at least in part the proliferation of persistent/latent viruses. In agreement, *Nicotiana benthamiana* plants developed symptoms of disease when Verhoeven *et al.*, mechanically inoculated them with ArLV1, even though infections in *Arabidopsis* were asymptomatic. *Arabidopsis* is considered a poor competitor. If the ArLV1 effect observed in *N. benthamiana* can be extended to other plant species co-occurring with *Arabidopsis* in nature, virus infection would have a significant impact on the plant’s competitive ability.

(3) The theory of the evolution of virulence largely assumes that parasites are horizontally transmitted from host to host. In the case of plant viruses, this is achieved by direct contact, mechanical means (lawn and farm equipment, grazing animals, etc.), or by vectors (arthropods, nematodes, fungi, etc.). However, many plant viruses are vertically transmitted from parent to offspring through seeds, which requires that hosts produce progeny. Therefore, parasites transmitted through strict vertical transmission would evolve toward very low or no virulence (Lipsitch *et al.*, 1996; Hamelin *et al.*, 2016). Although more than 25% of all known plant viruses (including latent and persistent ones) are seed-transmitted, this hypothesis has seldom been tested (Stewart *et al.*, 2005; Pagán *et al.*, 2014; Pagán, 2022). Interestingly, Verhoeven *et al.*, report very high ArLV1 seed transmission rates in asymptomatic infections. Although it is not currently known whether ArLV1 can be horizontally transmitted, these results are compatible with conditions predicted to favor evolution toward asymptomatic infections.

Altogether, Verhoeven *et al.*, provide at least some evidence that supports each of the above explanations for the evolution of asymptomatic virus infections. Surely, none of them independently, but a combination of all, explains the success of ArLV1. This gives a compelling idea of the great evolutionary potential of this virus and the complex interactions that plants establish with it.

The results of Verhoeven *et al.*, not only have implications for understanding the paths leading to the evolution of asymptomatic infections, but also open interesting questions about the

This article is a Commentary on Verhoeven *et al.* (2023), pp. 1146–1153.

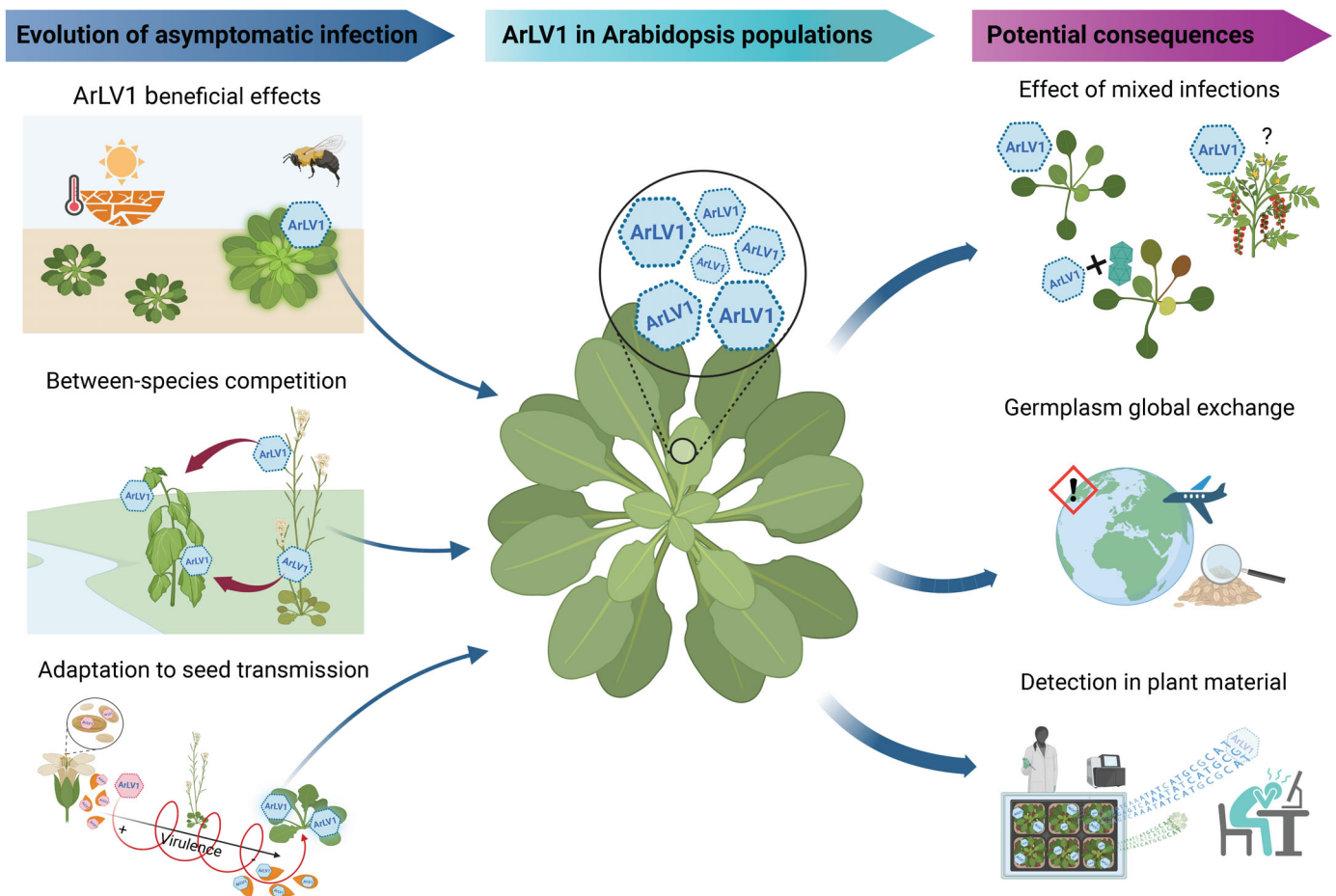


Fig. 1 Schematic representation of the contribution of the article published in this issue of *New Phytologist* by Verhoeven *et al.* (2023; pp. 1146–1153) to the understanding of asymptomatic plant virus infections. Possible scenarios that conduced to Arabidopsis latent virus 1 (ArLV1) asymptomatic infection in Arabidopsis include mutualistic interactions between ArLV1 and Arabidopsis (note the image of the bee represents beneficial insects in general) (top left), differential effects of ArLV1 infection according to the host plant (middle left), and evolution toward ArLV1 lower virulence through adaptation to seed transmission (bottom left). Potential consequences arising from ArLV1 presence in Arabidopsis span from unexpected infection outcomes when ArLV1 is in mixed infection with other viruses (top right), to implications for Arabidopsis germplasm exchange (middle right) and routine detection (for instance, by sequencing) in laboratory plant materials (bottom right).

consequences of ArLV1 presence in Arabidopsis: plant–virus infections are complex processes that do not exclusively involve a plant and a virus. In nature, plants (including Arabidopsis) are commonly infected by more than one virus at the same time (Montes & Pagán, 2022). Verhoeven *et al.*, report that ArLV1 can be detected at high prevalence in Arabidopsis plants collected from wild populations, including some from the Iberian Peninsula where other viruses have been reported to reach a prevalence of up to 80% (Pagán *et al.*, 2010). Merging these observations, it seems likely that ArLV1 co-occurs with other viruses, supporting the increasingly extended view that virus-mixed infections are frequent in wild plants. Such mixed infections may have far-reaching consequences for the fitness of both the plant (by rendering host defenses inefficient and altering virus virulence) and the virus (by establishing synergistic or antagonistic interactions affecting within-host multiplication and between-host transmission) (Alcaide *et al.*, 2020). Hence, ArLV1 may act as a main modulator of the consequences of the infection by other viruses on Arabidopsis. Moreover, because many Arabidopsis-infecting viruses are horizontally transmitted by insects, mixed infections with ArLV1 may

alter the interaction with their vectors. This may deeply impact the extent and severity of virus epidemics and therefore the size and genetic composition of plant populations. Virus-mixed infections are not limited to the interaction between viruses, but also of viruses with phytopathogenic or mutualistic bacteria and fungi. Thus, the characterization of ArLV1 opens the possibility of studying cross-kingdom interactions, which may shed light on currently unknown or poorly understood factors modulating the interactions between plants and microorganisms.

The consequences of ArLV1 infection expand beyond wild plant populations, as Verhoeven *et al.*, detected the virus in mutant lines and in accessions present in the Arabidopsis stocks of laboratories worldwide (including the widely used Col-0). This is one of the most notable contributions of the manuscript, because it opens a new dimension to how plant biologists approach the plant material used in their experiments. Most plant–virus interactions are genotype-per-genotype dependent. Thus, the effect of ArLV1 presence in wild-type and mutant Arabidopsis can be unpredictable, and a safer use of such plants would require testing for the presence of this virus in the stocks maintained in the laboratory. This technically simple

precaution would also contribute to ensuring a virus-free exchange of plant materials. If ArLV1 has a significant prevalence in Arabidopsis laboratory stocks as reported by Verhoeven *et al.*, why was it not detected until now? The authors provide a reasonable answer to this question: ArLV1 accumulation in Arabidopsis can be extremely high, accounting for up to 90% of the reads in RNA-seq data. In these cases, researchers would often consider this result as a technical problem during library preparation or RNA amplification, discarding the sequence data as not useful. Hence, the characterization of ArVL1 highlights that unexpected results may have a biological meaning and provides highly useful information for plant scientists when dealing with troubleshooting of their RNA-seq libraries. This observation also has implications beyond Arabidopsis as to what extent other plants commonly used in laboratory work may also contain asymptomatic viruses. This makes the work by Verhoeven *et al.*, more than just the characterization of a plant virus, but a potential game changer for the management of Arabidopsis in the laboratory.

In summary, Verhoeven *et al.*, characterize a plant–virus interaction that not only supports the idea that plant viruses can establish mutualistic relationships with plants, but also has great potential for studying scientific questions highly relevant to understanding the epidemiology and evolution of plant and virus populations. Moreover, this work makes plant biologists aware of the potential risk of asymptomatic virus infections in their laboratory seed stocks, which is extremely useful information when designing their experiments.



Acknowledgements

This work was supported by Plan Nacional I+D+i, Ministerio de Ciencia e Innovación, Spain (PID2019-109579RB-I00) to IP.

ORCID

Israel Pagán  <https://orcid.org/0000-0003-4061-5758>

Cristina Sáez  <https://orcid.org/0000-0001-8876-1194>

Cristina Sáez  and Israel Pagán* 

Centro de Biotecnología y Genómica de Plantas UPM-INIA and E.T.S. Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid 28223, Spain
(*Author for correspondence: email jesusisrael.pagan@upm.es)

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Key words: Arabidopsis latent virus 1, *Arabidopsis thaliana*, asymptomatic infection, mutualism, plant–virus interaction.