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ORIGINAL ARTICLE

Effect of disease prevalence and growth stage on symptom severity in the *Turnip mosaic virus* – *Arabidopsis thaliana* pathosystem

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Abstract

In response to stresses, plants are capable of communicating their physiological status to other individuals in the community using several chemical cues. Nearby receivers then adjust their own homeostasis to increase resilience. The majority of studies to date have concentrated on the communication of abiotic stressors (e.g., salinity or drought) or herbivory. Less attention has been paid to the role of communication during microbial infections and almost nothing has focused on viruses. Here we investigated the effect that the prevalence of a turnip mosaic virus in a community of *Arabidopsis thaliana* has on the severity of symptoms developed in a group of receivers. First, we looked at the influence of two factors on the kinetics of symptom progression in the receivers, namely the prevalence of infection among emitters and the growth stage of the receiver plants at inoculation. We found that young receiver plants developed milder symptoms than older ones, and that high infection prevalence resulted in slower disease progression in receivers. Second, we tested the possibility that jasmonates could act as chemical signaling cues. To do this, we examined the kinetics of symptom progression in jasmonate-insensitive and wild-type plants. The results showed that the protective effect vanished in the mutant plants. Third, we investigated the possibility that root communication could also be relevant. We found that the kinetics of symptom progression across receivers was further slowed down in an age-dependent manner when plants were planted in the same pot. Together, these preliminary findings point to a potential function for disease prevalence in plant communities in regulating the severity of symptoms, this effect being mediated by some volatile organic compounds.

Keywords: disease severity, plant-to-plant communication, plant virus epidemiology, *Potyvirus*, volatile organic compounds

Introduction

To protect themselves from biotic and abiotic stresses, plants create and emit a wide variety of volatile organic compounds (VOCs) (Bitas *et al.* 2013; Brosset and Blande 2022; Loreto and D'Auria 2022). These VOCs may also serve as warning signals to prime defenses in unaffected areas of the same plant and in neighboring plants. Plant-to-plant communication is a phenomenon that was first identified by Baldwin and Schultz (1983). Since this groundbreaking research, a plethora of research projects have provided strong experimental support for the hypothesis that plants release VOCs in response to a variety of stresses, like salinity (Lee and Seo 2014), drought (Falik *et al.* 2022), herbivory (Dolch and Tscharntke 2000), infection by bacterial (Riedlmeier *et al.* 2017), fungal (Moreira *et al.* 2020) or viral (Ghosh *et al.* 2022) pathogens, or even triggering plant-plant allelopathy to minimize intraspecific competition (Santonja *et al.* 2019).

The chemical nature of VOCs is diverse and, indeed, VOCs typically act as complex blends of various volatiles rather than as single molecular species (Ueda *et al.* 2012). Among the most frequently described VOCs

are terpenoids, oxylipins [most notably jasmonic acid (JA) and some of its derivatives, such as methyl-JA and *cis*-JA], olefins (e.g., isoprene and ethylene), and methylsalicylate (MeSA). The phenyl-propanoid pathway, which produces the volatile MeSA, and the methyl erythritol phosphate pathway, which produces volatile isoprenoids like monoterpens and hemiterpens, are examples of biochemical pathways induced by abiotic and biotic stresses and producing VOC emissions that may be used as signals or to prime defenses (Loreto and D'Auria 2022).

VOCs modulate JA signaling involved in the induction of defenses, and it is usually assumed that by enhancing defenses, VOCs would inhibit the growth of receivers if investment in defense trades off with growth and development. Indeed, several studies support the theory that exposure to VOCs affect growth and reproduction to varying extents among plant species (reviewed in Brosset and Blande 2022).

The impact of plant-to-plant communication on virus epidemiology is unknown. Given that infected plants emit VOCs, it stands to reason to assume that as epidemics spread through a population of vulnerable hosts and the incidence of infection rises, the concentration of VOCs will rise as well. This will prime antiviral defenses in receiver non-infected plants more effectively, increasing tolerance to infection and slowing the progression of symptoms in primed plants. Furthermore, it is anticipated that plants at different developmental stages would respond to this priming in different ways, with younger plants being more sensitive to VOCs than older ones that are already investing in reproduction. To test these two hypotheses, we used the turnip mosaic virus (TuMV) – *Arabidopsis thaliana* natural pathosystem. In the first set of experiments, we tested whether or not the kinetics of disease progression (i.e., the severity of symptoms) of receiver plants was affected by the prevalence of infection in the population (i.e., the frequency of infected plants). Then, we observed if the effect was dependent on the developmental stage of the receivers and duration of exposition. In a second set of experiments, we examined the potential role of JA as a chemical primer of increased tolerance to infection. Finally, we showed results of a third experiment to determine if root communication may also contribute to priming a more tolerant state in receiver plants.

Materials and Methods

Plants and growth conditions

Arabidopsis thaliana (L.) Heynh of the accession Col-0 were maintained in a BSL2 climatic chamber under a photoperiod of 16 h light (LED tubes at PAR 90 – 100 μmol m^{-2} s^{-1}) at 24°C and 8 h dark at 20°C and 40% relative humidity.

Mutant *jasmonate insensitive 1* (*jin1*) (AT1G32640) was used to test the potential role of jasmonates as VOCs in signaling viral infection. Mutant *jin1* exhibits decreased induction of JA-responsive genes, including *PR1*. However, mutant plants showed reduced sensitivity to *Pseudomonas syringae* and *Botrytis cinerea* infections (Nickstadt *et al.* 2004; Laurie-Berry *et al.* 2006). Indeed, Nickstadt *et al.* (2004) showed that *jin1* was defective in JA sensing but not in jasmonates biosynthesis, and that infections were associated with two-fold increases in salicylic acid (SA), suggesting a possible contribution of the SA pathway to *jin1* plant resistance to the above-mentioned pathogens. This would suggest that over-expression of one pathway does not necessarily down-regulate the other (Nickstadt *et al.* 2004). Furthermore, these authors concluded that *JIN1* affected basal resistance rather than resistance based on gene-for-gene interactions. In general, enhanced JA accumulation favors plant defense against viral infections (Islam *et al.* 2019) and for example, *jin1* plants showed increased susceptibility to TuMV infection (Navarro *et al.* 2022).

Virus, inoculation procedure and characterization of symptoms

TuMV (species *Turnip mosaic virus*, genus *Potyvirus*, family *Potyviridae*) infectious sap was obtained from TuMV-infected *Nicotiana benthamiana* Domin plants inoculated with the infectious plasmid p35STunos containing a cDNA of the TuMV genome (GeneBank accession AF530055.2) under the control of the cauliflower mosaic virus 35S promoter and the *nos* terminator (Chen *et al.* 2003) as described elsewhere (González *et al.* 2019; Corrêa *et al.* 2020). This TuMV sequence variant corresponds to the YC5 strain from calla lily (*Zantesdeschia* sp.) (Chen *et al.* 2003). After plants showed symptoms of infection they were pooled and frozen with liquid N_2. This frozen plant tissue was homogenized into a fine powder using a Mixer Mill MM400 (Retsch GmbH, Haan, Germany).

For inoculation of *A. thaliana* plants, 0.1 g of powder was diluted in 1 ml of inoculation buffer (50 mM phosphate buffer pH 7.0, 3% PEG6000, 10% Carborundum) and 5 μl of the inoculum was gently rubbed onto each of three leaves per plant (in total 15 μl per plant). Depending on the particular experiment, plants were inoculated at either of the following two growth stages (Boyes *et al.* 2001): (*i*) at stage 3.32, when the rosette is ~25% of its final size; (*ii*) at stage 5.10, the precise moment at which the first flower buds are visible, marking the transition from juvenile vegetative growth to adult flower (reproductive) development. Hereafter we will

refer to plants at growth stage 3.32 as *prebolting* and to plants at growth stage 5.10 as *bolting*.

The severity of symptoms was evaluated in the semiquantitative discrete scale shown in Figure 1 of Butković *et al.* (2021). This scale ranges from zero for non-infected and asymptomatic plants to five for plants showing a generalized necrosis and wilting. Symptom severity was annotated daily for each individual plant from inoculation up to 14 days post-inoculation (dpi).

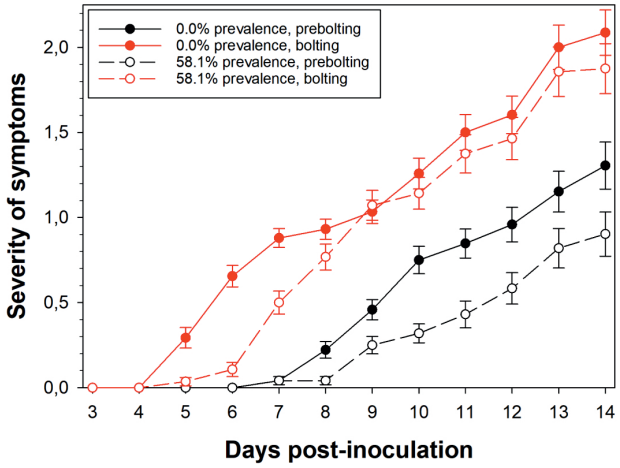

Fig. 1. Symptom progression curves at low and high prevalence of infection and considering the plant growth stage (3.32 as prebolting and 5.10 as bolting) at the time of inoculation. Error bars represent ±1 SEM

Evaluating the role of disease prevalence and growth stage in symptom progression

The interaction between the growth stage of the inoculated plants and the prevalence of disease in the plant community was investigated in the first experiment. There were two distinct prevalence scenarios simulated: the first was zero prevalence, in which no plants in the community were infected during the growing of the 130 wild-type (WT) receiver plants to be infected once they reached the desired growth stage. Second, high prevalence, in which the median prevalence of infection among emitters during the development of an additional set of 128 WT receiver plants was 0.318 ± 0.109 (±1 IQR). After inoculation of the receivers at the predetermined growth stage, the median prevalence increased to 0.581 ± 0.303. For both prevalence values, 72 plants were inoculated when they reached prebolting while the remaining 56 were inoculated at bolting. The zero and high prevalence treatments were done in two separated growth chambers.

In the second experiment, meant to examine the role of JA as VOC in this pathosystem, WT control and *jin1* plants were all inoculated at prebolting under two prevalence conditions: (*i*) zero prevalence, with 86 WT and 86 *jin1* plants inoculated at the same time, (*ii*) A prevalence of 0.662 ± 0.034 among WT emitters during the vegetative growth of the receivers, reaching 0.974 ± 0.107 after inoculation of 80 WT and 72 *jin1* receivers.

Evaluating the role of root-mediated communication

A third experiment was designed to test the possible contribution of root communication. Two treatments were done, all using WT plants. First, two plants were sowed per pot to allow for possible additional root communication. Second, only one plant was sown per pot thus blocking root communication. In both treatments, the distance between plants was kept constant. For the same pot treatment, 130 emitters, one per pot, were inoculated at prebolting. Eighty-two receivers were inoculated the same day and 48 more 12 days later (thus at bolting) sharing a pot with the emitters.

For the different pot treatments, the inoculation design was as follows: 64 emitters were inoculated at prebolting and, the same day, 40 receivers were also inoculated. Twelve days later 24 plants were inoculated at bolting.

Statistical analysis

To examine the kinetics of disease severity progression, two different tests were conducted. To assess overall differences between curves, nonparametric Wilcoxon signed-rank tests (Wilcoxon 1945) were performed. Second, daily severity data were fitted to repeated measures ANOVA models (Girden 1992) in which days-post inoculation was treated as the within-plant factor (repeated measures) whereas other between-plant factors were treated as orthogonal. In all these models, the type III sum of squares (*SS*) was used to partition total variance between factors and the Greenhouse-Geisser assumed sphericity was used for the within-plant tests (Greenhouse and Geisser 1959). The power of each test, $1 - \beta$, was also computed, where β is the type II error. Cohen's η_p^2 statistic, which gauges the percentage of total variability attributable to each model factor, was used to assess the effect of size of the different factors in the ANOVA models (Cohen 1973). Values of $\eta_p^2 \geq 15\%$ are typically regarded as large effects.

All these statistical analyses were done with SPSS version 28.0.1.1 (IBM, Armonk, NY, USA).

Data availability

Three Excel files containing the disease severity raw data from each experiment described above are available at zenodo.org under DOI: https://doi.org/10.5281/zenodo.7663956.

Results

The kinetics of symptom development were determined by disease prevalence among emitters and the receivers' growth stages in an additive manner

Our first set of experiments evaluated the combined contribution of two factors on the progression of disease symptoms: the prevalence of disease among emitter plants and the growth stage of the receiver plants. Figure 1 shows the disease severity progression curves for two prevalence values and two growth stages for inoculated receiver plants. In both cases the dynamics of symptom development were slower if receivers grew in a plant community with a high incidence of infection. When receiver plants were inoculated at prebolting, symptoms started appearing 7 dpi, being on median 19.44% ± 38.20 (±1 IQR) stronger for receiver plants grown in a community with zero prevalence of infection during their development (Fig. 1, black symbols; Wilcoxon signed-rank test: $z = -2.3664$, $p = 0.0180$). However, when receiver plants were infected at bolting, symptoms started being visible 5 dpi, also being on median 14.10% ± 13.60 more severe than receivers grown in a community with zero prevalence (Fig. 1, red symbols; $z = -2.7011$, $p = 0.0069$).

To further understand the source of statistical differences, we fitted the data in Figure 1 to a repeated-measures ANOVA (Table S1 – in supplementary material) with the degree of prevalence and growth stage as random between-plant orthogonal factors. Obviously, the most significant and of larger magnitude effect corresponds to differences among days ($p < 0.0001$, $\eta_p^2 = 57.08\%$). But three other interesting conclusions can be drawn from Table S1: (*i*) Prevalence had significant effects both within- and between-plants ($p = 0.0442$ and $p = 0.0006$, respectively) although of small magnitude ($\eta_p^2 = 1.24\%$ and $\eta_p^2 = 4.57\%$, respectively). This confirms that receiver plants grown at high prevalence of TuMV infection develop weaker symptoms (compare solid *vs* open symbols in Figure 1), (*ii*) More severe symptoms were developed by older than by younger plants. This effect was highly significant both within- and between-plants ($p < 0.0001$ in both cases), although the effect was of moderate magnitude in the within-plant test ($\eta_p^2 = 10.72\%$) but very large in the among-plant test ($\eta_p^2 = 30.54\%$) (compare red *vs* black symbols in Figure 1), (*iii*) Finally, the test of the interaction between prevalence and growth stage was significant in the within-plant test ($p = 0.0050$), although of small effect ($\eta_p^2 = 2.11\%$), but not significant in the between-plant test ($p = 0.8104$). These tests suggest a similar overall effect of prevalence at both growth stages (compare the difference between solid and open red symbols *vs* the difference between solid and open black symbols in Figure 1).

Jasmonate as a candidate for signaling VOCs

After observing a significant effect of disease prevalence among emitters in the kinetics of symptom development of receivers, we sought to explore the potential role of JA as a candidate VOC regulating the response of receptor plants. The choice of this hormone was done based on its well stablished role in plant defense against viral infections (Islam *et al.* 2019). Keeping the growth stage constant at prebolting and varying only disease prevalence, we inoculated WT and *jin1* receiver plants. The results of this experiment are shown in Figure 2. As in the previous experiment, a highly significant effect was observed in WT plants; in median, plants grown at high TuMV prevalence showed 27.76% ± 32.89 weaker symptoms (black symbols in Figure 2: $z = -2.9341$, $p = 0.0033$). However, the effect vanished in *jin1* plants, where symptom progression curves overlapped for both prevalences (red symbols in Figure 2: $z = -0.9780$, $p = 0.3281$), supporting the role of JA as VOC.

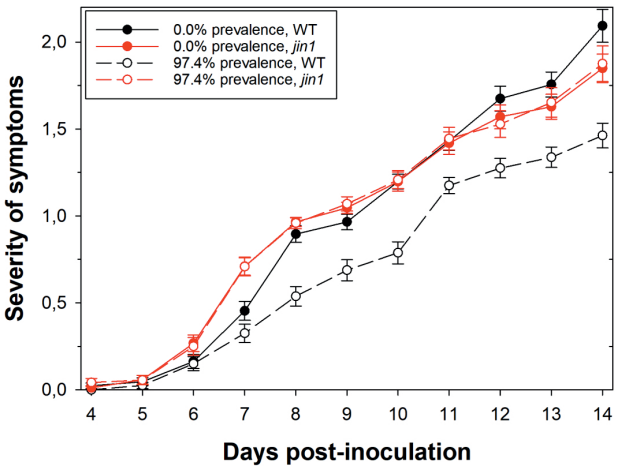

Fig. 2. Jasmonte insensitive (*jin1*) plants did not show reduced susceptibility to infection when grown at high prevalence of infection. Error bars represent ±1 SEM

To gain further statistical insight, we fitted the data in Figure 2 to a repeated-measures ANOVA (Table S2 – in supplementary material) with degree of disease prevalence values and plant genotype as random between-plant orthogonal factors. Again, the largest amount of observed variability was explained by differences among days ($p < 0.0001$, $\eta_p^2 = 70.68\%$). Regarding the three other factors in the model: (*i*)

confirming the previous results, prevalence *per se* had a highly significant effect both within- and between-plants ($p < 0.0001$ in both cases), although it was of very large magnitude within-plants ($\eta_p^2 = 70.68\%$) but small between-plants ($\eta_p^2 = 4.51\%$), (*ii*) Highly significant differences existed between the symptoms developed by WT and *jin1* plants both when testing within- ($p = 0.0020$) and between-plant ($p < 0.0001$) effects, although both were of small magnitude ($\eta_p^2 = 1.42\%$ and $\eta_p^2 = 5.10\%$, respectively), and (*iii*) a highly significant genotype-dependent effect of disease prevalence was observed both within- ($p = 0.0013$) and between-plants ($p < 0.0001$). In both tests, the magnitude of the effects was rather small ($\eta_p^2 = 1.54\%$ and $\eta_p^2 = 4.95\%$, respectively).

Root contact between emitters and receivers slowed the rate at which bolting, but not prebolting, receivers developed symptoms

In our last investigation, we explored the possibility that the TuMV – *A. thaliana* pathosystem may possibly be influenced by root chemical communication between infected and non-infected plants. To verify this idea, seeds were sown singly in pots or in pairs, always keeping the same spacing between them. First, at prebolting, emitters and receivers were inoculated concurrently. In a subsequent experiment, half the plants (emitters) were inoculated at prebolting while the other half were at growth stage 5.12. (receivers). Figure 3 summarizes the results from these experiments. When emitters and receivers were in separate pots, the growth stage effect described above held ($z = -2.2258$, $p = 0.0262$), with receiver older plants developing 15.12% ± 15.61 median stronger symptoms than younger ones. However, if plants were potted in pairs, symptoms were on median 28.27% ± 11.31 weaker in the receiver older plants ($z = -3.3510$, $p = 0.0008$), a result that sharply contrasted with all previous observations and which suggests a possible role of root communication.

Once more, we fitted the data in Figure 3 to a repeated-measures ANOVA with two between-plant orthogonal factors: difference in growth stage among emitters and receivers and if the plants were in the same or different pots (Table S3 – in supplementary material). Focusing first on within-plant effects, the largest amount of variability was explained by the progression of symptom severity with time ($p < 0.0001$, $\eta_p^2 = 72.47\%$). Significant overall effects within- ($p = 0.0129$, $\eta_p^2 = 1.67\%$) and between-plants ($p = 0.0022$, $\eta_p^2 = 4.33\%$) were found for the number of plants per pot, although at a small magnitude. Also, significant overall effects were associated with the age of the

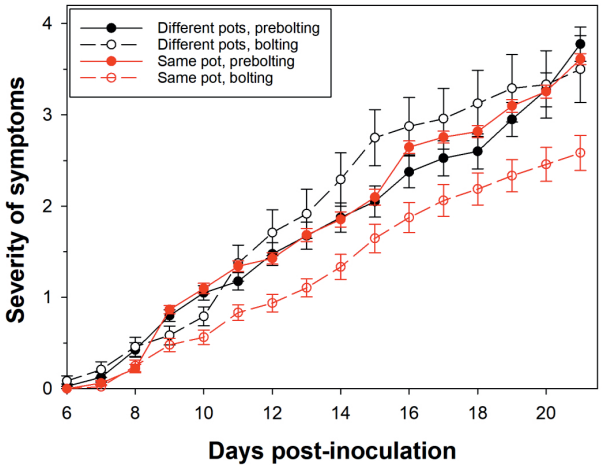

Fig. 3. Effect of emitter and receiver plants sharing or not the same pot in symptom progression curves. Differences between prebolting (3.32) and bolting (5.10) growth stages were tested. Error bars represent ±1 SEM

receivers at the time of their inoculation, both within- ($p = 0.0002$, $\eta_p^2 = 4.09\%$) and between-plants ($p = 0.0053$, $\eta_p^2 = 4.81\%$), although again, the magnitude of these effects was small. Finally, the interaction between these two factors was also highly significant, both within- ($p = 0.0006$, $\eta_p^2 = 2.68\%$) and between-plants ($p = 0.0005$, $\eta_p^2 = 2.68\%$). This significant interaction, although certainly of small magnitude, supports the theory that the growth-stage dependent effect on symptom severity strongly depended on whether plants were capable of root communication or not, being weaker in older plants sharing a pot with an emitter during development.

Discussion

Plants release VOCs in response to biotic and abiotic stimuli, as has been well established. These VOCs represent chemical information on the physiological status of the emitter plants that is sensed by the receiver plants. In response, receiver plants activate defense mechanisms to ensure their homeostasis (Peñuelas and Llusià 2004; Ueda *et al.* 2012; Brosset and Blande 2021; Loreto and D'Auria 2022). In particular, for cultivated systems plant diseases are a significant source of stress. Several studies have documented how infectious diseases affect plant VOCs (Hammerbacher *et al.* 2019; Moreira *et al.* 2020). Although the majority of research on VOCs and infection has been done on bacterial and fungal pathogens, viruses have also been studied (reviewed in Hammerbacher *et al.* 2019). For example, Shulaev *et al.* (1997) showed that increased emissions of MeSA by *Nicotiana tabacum*

plants infected with tobacco mosaic virus (TMV) boosted resistance against this pathogen in near-by healthy plants.

In this preliminary study, we focused on four main questions that might exert an impact in virus epidemiology and evolution: (*i*) in *A. thaliana* communities with varying levels of TuMV prevalence, do VOCs play a role in signaling infection among plants? (*ii*) Does the receiver plants' age affect how they react to VOCs? (*iii*) Do jasmonates function as VOCs to alert *A. thaliana* populations to TuMV infection? and (*iv*) Do root exudates aid in the dissemination of disease information at the community level?

Social communication and disease prevalence

Our findings suggest a negative correlation between the speed of disease progression and the prevalence of TuMV infection in the plant community. VOCs are effective messengers for establishing a communication network among plants. However, the potential epidemiological implications of these communication networks have not been considered yet. In recent years, theoretical epidemiologists have been examining the relationship between the propagation of epidemic awareness and the actual epidemic infection in spatially-structured populations of susceptible hosts (Granell *et al*. 2013; Wang *et al*. 2015; Scatà *et al*. 2016; Li and Xiao 2021; Gao *et al.* 2022). In such models, the spread of epidemics is no longer properly described by classic models (e.g., susceptible – infected – removed, SIR) but more complex and richer dynamics emerge, in which the onset of epidemics has a critical value defined by the awareness dynamics. In the particular case of plant disease epidemics, such awareness dynamics would depend, e.g., on the gradients of spatial spread of VOCs across the community. Theoretically, the efficient activation of defense mechanisms in receivers, with a concomitant reduction in the rate of symptom development and virus accumulation are expected to contain the spread of the disease at long distances. Clearly, this possibility deserves further theoretical and experimental work.

Age-dependent susceptibility to VOCs and the severity of symptoms

Related to our second question, in a recent study, our group has shown that the severity of symptoms associated with TuMV infection of *A. thaliana* actually depends on the growth stage of the plant at the time of inoculation, with older plants developing stronger symptoms (Melero *et al.* 2023). This observation is extensible to viruses from other families and host species (Huang *et al*. 2020; Melero *et al*. 2023). Here, we have verified this earlier finding and shown that the magnitude of the observed effect was unrelated to the prevalence of the disease in the population: older plants systematically show stronger symptoms and are less sensitive to VOCs than younger ones.

Although the defense mechanism known as age-related resistance (ARR) appears to be widespread in plants, it has primarily been studied in the context of plant-bacterial interactions (Kus *et al*. 2002; Hu and Yang 2019). Our findings, which concur with those made by others (Huang *et al*. 2020; Melero *et al*. 2023), appear to be at odds with ARR. There are instances where older plants are more resistant to viral infections than younger plants, but other species exhibit the opposite pattern. In the cucumber mosaic virus (CMV) – *Capsicum annuum* pathosystem, García-Ruiz and Murphy (2001) demonstrated that the severity of symptoms and viral accumulation were lower in older plants. Similarly, Levy and Lapidot (2008) demonstrated that plant age had little bearing on the severity of symptoms in the Tomato yellow leaf curl virus – *Solanum lycopersicum* pathosystem, despite juvenile plants having much reduced fruit output. In stark contrast, Huang *et al.* (2020) discovered that the Tomato spotted wilt virus – *A. thaliana* and CMV – *A. thaliana* pathosystems had a developmentally regulated rise in susceptibility, with older plants being more susceptible and exhibiting more severe symptoms.

In the contemporary context, when anthropogenic activities and climate change are changing plant developmental patterns and timing, the influence of the host growth stage on its vulnerability to viruses is particularly pertinent. Abiotic factors connected to climate change have been shown to have genotype-dependent effects on the induction and retardation of developmental processes, including blooming (Craufurd and Wheeler 2009; Tun *et al*. 2021). Moreover, it has been noted that human activities like urbanization (Neil and Wu 2006) or biodiversity loss (Wolf *et al*. 2017) can influence flowering timing. Viral populations will be exposed to host populations with altered levels of sensitivity as a result of these changes in plant growth. One crucial component to enhancing the management of viral infections in the future is understanding how these developmental changes may affect the evolution of viruses.

Jasmonate as a candidate for VOC in the TuMV – *Arabidopsis thaliana* pathosystem

Regarding our third question, although VOCs are composed of a complex mixture of molecules (Brosset and Blande 2021; Loreto and D'Auria 2022), jasmonates (e.g., methyl-jasmonate) have been recognized as essential airborne signals generated by the emitters and

inducing defense responses in the receivers (Tamogami *et al*. 2008). Furthermore, the role of JA in defense responses against viral infections has been well established (Islam *et al*. 2019). To test such a role of JA in the TuMV – *A. thaliana* pathosystem we used *jin1* mutants, which are insensitive to JA, as receivers. In agreement with the predicted role of jasmonates, *jin1* plants grown in a community with high TuMV prevalence did not show any delay in symptom progression compared to WT plants after inoculation. Other studies have suggested a similar role for MeSA in the TMV – *N. tabacum* pathosystem (Shulaev *et al*. 1997). JA and SA signaling pathways are thought to be engaged in an antagonistic tradeoff (Glazebrook 2005), though this assumption has been jeopardized (Nickstadt *et al*. 2004). Therefore, testing the role of salicylates in our pathosystem would be of special interest.

Root communication also plays a role in the TuMV – *Arabidopsis thaliana* pathosystem

Addressing our fourth and final question, there is abundant data demonstrating the functions of mycorrhizae and root exudates in supplying between-plant cues and signals (Babikova *et al*. 2013; Wang *et al*. 2020), but there is significantly less recorded evidence of belowground volatile-mediated interplant communication. Our exploratory experiment produced somewhat puzzling results. No changes in disease progression were seen in young receiver plants, regardless of whether they were capable of root-communication or not. Adult receivers, on the other hand, experienced milder symptoms and a more gradual onset of symptoms which allowed for belowground communication. This growth stage-dependent effect could simply result from slower soil diffusion of signaling molecules, which do not reach receivers when emitters were inoculated concurrently but do so when emitters were inoculated well in advance. Root exudates, like VOCs, contain a complex mixture of compounds, such as ethylene, strigolactones, JA, and allantoin (Wang *et al*. 2020), as well as microRNAs arranged into extracellular vesicles (Betti *et al*. 2021). Further research is necessary to determine the chemical nature of these underground signals.

Conclusions

Our exploratory work suggests a possible role of VOCs, and in particular of JA, in the attenuation of symptom progression in newly infected plants living alongside already infected ones. In this sense, high disease prevalence will result in more and more plants emitting VOCs and in a reduction of susceptibility of noninfected receiver individuals. It is interesting to note that younger plants, which are more sensitive to VOCs signaling than their more mature neighbors, exhibit higher levels of protection. Finally, although it merits further investigation, another root communication pathway may also have an impact on this protective community effect.

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