The fitness of plant viruses is critically influenced by interactions with the physiology of the host plant, which not only mediate successful propagation within the host but—for insect-vectored viruses—may also influence plant traits that affect vector attraction, pathogen acquisition and dispersal to new hosts. Direct suppression by viruses of plant anti-pathogen defenses (e.g., through RNA silencing) is well documented, and specific viral proteins involved in subverting defenses that would otherwise inhibit propagation have been identified in many systems. But viruses—may also influence plant traits that affect vector attraction, pathogen acquisition and dispersal to new hosts. Direct suppression by viruses of plant anti-pathogen defenses (e.g., through RNA silencing) is well documented, and specific viral proteins involved in subverting defenses that would otherwise inhibit propagation have been identified in many systems.

The well-documented association between Tomato yellow leaf curl China virus (TYLCCNV) and the whitefly vector has spread along with the invasive B. tabaci B Biotype in south China, causing major losses in tomato and tobacco crops. Recent studies demonstrated that B Biotype whiteflies have higher fecundity on TYLCCNV-infected tobacco plants than on healthy hosts. This suggests a mutualistic relationship between the pathogen and vector, in which the presence of the virus enhances vector reproduction and spread, via a potentially adaptive manipulation of the host plant phenotype. The specific mechanisms underlying the effects of TYLCCNV on host plants are not yet well understood, but the obligatory satellite DNA-β of the virus and its single gene, βC1, appear to play important roles. Previous studies have shown that this satellite is essential for pathogenicity, including the onset of visible symptoms and the accumulation of high levels of virus particles. βC1 has also been shown to influence many plant regulation systems including the inhibition of RNA silencing, the disruption of leaf developmental regulation (causing the visual symptoms) and the inhibition of the jasmonic acid signaling pathway.

In the current study, we explored how βC1 impacts host plant phenotype likely involved in herbivores attraction and defense by using transgenic lines of two model plant species, Arabidopsis thaliana and Nicotiana benthamiana, transformed to express this virus gene. Although not usual hosts of the virus the insertion of the βC1 gene in these two biological systems produces morphological symptoms similar to those of TYLCCNV, notably

**Effects of the virus satellite gene βC1 on host plant defense signaling and volatile emission**

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**Keywords:** Tomato yellow leaf curl China virus, Arabidopsis thaliana, Nicotiana benthamiana, phytohormone signaling, olfactory cues

Tomato Yellow Leaf Curl China virus spreads together with its invasive vector, the silverleaf whitefly B biotype, which exhibits higher growth rates on infected plants. Previous studies indicate that the virus satellite gene βC1 accounts for the visible symptoms of infection and inhibits the constitutive expression of jasmonic acid (JA)—a phytohormone involved in plant defense against whiteflies—and of some JA-regulated genes. Here we present new details of the effects of on plant signaling and defense, obtained with (non-host) transgenic Arabidopsis thaliana and Nicotiana benthamiana plants. We found that JA induction in response to wounding was reduced in plants expressing βC1. This result implies that βC1 acts on conserved plant regulation mechanisms and might impair the entire JA defense pathway. Furthermore, transformed N. benthamiana plants exhibited elevated emissions of the volatile compound linalool, suggesting that βC1 also influences plant-derived olfactory cues available to vector and non-vector insects.

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leaf curliness and outgrowth tissues, with a level of phenotype severity correlated with the expression of the gene in the transformed line.\textsuperscript{13,15} These transgenic lines are thus useful tools to investigate the action of this gene on conserved plant regulation mechanisms, in isolation from other physiological perturbations that an actual viral infection could create.

We specifically explored how phytohormone-mediated defense signaling pathways are affected by the presence of this gene. Two key phytohormones involved in plant responses to antagonists are jasmonic acid (JA) and salicylic acid (SA). The former is typically activated in response to physical damage and/or feeding by chewing herbivores and the latter in response to biotrophic pathogens or phloem-feeding insects, though these roles frequently vary.\textsuperscript{16,17} There is furthermore considerable evidence for crosstalk— including mutual inhibition— between these and other defense signaling pathways,\textsuperscript{18} which presumably allows plants to fine-tune defense responses to specific antagonists, but also creates opportunities for manipulation by insect herbivores and pathogens.\textsuperscript{19-21} Despite being phloem feeders, whiteflies are susceptible to JA-mediated defenses, and are furthermore known to inhibit this hormone in Arabidopsis and by so doing increase population growth rates.\textsuperscript{22} In tobacco hosts, Zhang et al.\textsuperscript{12} showed that constitutive JA production was reduced when both the virus and its satellite are present and that JA-responsive genes were inhibited specifically by $\beta$C1, which results in a reduction of defenses against whiteflies. In Arabidopsis, Yang et al.\textsuperscript{15} also reported reduced constitutive expression of some JA-responsive genes in $\beta$C1-transformed Arabidopsis despite no changes in the expression of JA-synthesis genes. Both these studies, however, focused on constitutive states of the plants. Building on these observations, we directly investigated the induced levels of JA and SA as well as their precursors, after mechanical wounding in $\beta$C1-transformed Arabidopsis and $N$. benthamiana plants, in order to explore whether their ability to mount a JA pathway defense in response to external stress was also impaired in the presence of this gene—for example, through a “decoy strategy” exploiting the mutual inhibition of SA and JA\textsuperscript{20}— as a manipulation by TYLCCNV to favor the growth and dissemination of whitefly vectors.

We also explored the impacts of this gene on the olfactory phenotype of host plants. Plant volatile emissions are known to be influenced by the induction of phytohormone signaling pathways, and several aphid-transmitted viruses (from different genera) have been reported to induce qualitative and quantitative alterations in the volatile emissions of host plants that enhance vector attraction.\textsuperscript{6,7,23} The manipulation of such cues has significant potential to influence rates of pathogen transmission through effects on the frequency of opportunities for virus particles to be acquired from infected plants and introduced to healthy ones.\textsuperscript{24}

### Results

**Phytohormones levels in $A$. thaliana and $N$. benthamiana $\beta$C1-transformed plants.** The parental wild type of $A$. thaliana ($\text{Col-0}$) and four $\beta$C1-transformed lines representing two class levels of symptoms severity (Class I: mild phenotype, lines $\beta$C1-#2 and $\beta$C1-#42; class II: severe phenotype, lines $\beta$C1-#5 and $\beta$C1-#30) were compared for their production of JA ($\text{cis}$ and trans isomers), SA, cinnamic acid (CA), Indole-3-acetic acid (i.e. auxin), IAA; linoleic acid, LA; linolenic acid, LN (Table 1).

| Variable       | Block $F_{7,24}$ | Phenotype class $P$ value | Line (class) $F_{3,24}$ | $P$ value |
|----------------|------------------|----------------------------|--------------------------|-----------|
| Total JA       | 2.02             | 0.094                      | 7.16                     | 0.0036    |
| SA             | 2.32             | 0.058                      | 2.95                     | 0.072     |
| CA             | 1.89             | 0.12                       | 1.31                     | 0.29      |
| IAA            | 1.59             | 0.19                       | 0.55                     | 0.59      |
| LA             | 5.75             | 0.0006                     | 2.85                     | 0.078     |
| LN             | 4.51             | 0.0027                     | 2.97                     | 0.071     |

For each variable tested, the $F$-ratios and associated $P$ values of the following explanatory variables are indicated: block, phenotype class and $A$. thaliana line nested within phenotype class. Abbreviations: Total jasmonic acid, Total JA (i.e. sum of $\text{cis}$ and $\text{trans}$ isomers); salicylic acid, SA; cinnamic acid, CA; indole-3-acetic acid (i.e. auxin), IAA; linoleic acid, LA; linolenic acid, LN.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
Variable & Block $F_{7,24}$ & Phenotype class $P$ value & Line (class) $F_{3,24}$ & $P$ value \\
\hline
Total JA & 2.02 & 0.094 & 7.16 & 0.0036 & 3.53 & 0.045 \\
SA & 2.32 & 0.058 & 2.95 & 0.072 & 1.71 & 0.20 \\
CA & 1.89 & 0.12 & 1.31 & 0.29 & 1.66 & 0.21 \\
IAA & 1.59 & 0.19 & 0.55 & 0.59 & 1.33 & 0.28 \\
LA & 5.75 & 0.0006 & 2.85 & 0.078 & 0.96 & 0.91 \\
LN & 4.51 & 0.0027 & 2.97 & 0.071 & 0.97 & 0.39 \\
\hline
\end{tabular}
\caption{ANOVA results on phytohormones production in $A$. thaliana wild type and transgenic lines}
\end{table}

We also explored the impacts of this gene on the olfactory phenotype of host plants. Plant volatile emissions are known to be influenced by the induction of phytohormone signaling pathways, and several aphid-transmitted viruses (from different genera) have been reported to induce qualitative and quantitative alterations in the volatile emissions of host plants that enhance vector attraction.\textsuperscript{6,7,23} The manipulation of such cues has significant potential to influence rates of pathogen transmission through effects on the frequency of opportunities for virus particles to be acquired from infected plants and introduced to healthy ones.\textsuperscript{24}
Discussion

As noted above, the coincident spread of TYLCCNV and its whitefly vector in China appears to be favored by the synergistic effects of virus and vector on transmission. Yang et al. found that *Arabidopsis* transformed with the βC1 gene—carried by the virus’s β satellite—reduced the constitutive expression genes PDF1.2, PR4 and COR13 acting in downstream pathways of the JA defense response, which is effective against whiteflies, but no effect on constitutive expression of the JA biosynthesis genes emission in *N. benthamiana* βC1-transformed plants. The collection of volatile organic compounds was focused on the *N. benthamiana* lines, as the in-vitro cultured *A. thaliana* plants used in this study did not produce any quantifiable volatiles. The volatile organic compounds emitted by the two tobacco lines (wild type and βC1-transformed) were collected during separate day and night collections on HaysepQ filters and analyzed by gas chromatography. All plants consistently emitted linalool during the day collection, but exhibited little or no volatile emissions during the night; thus, we quantified only the linalool emitted during the 14h daytime collections. Relative to plant fresh mass, this emission was higher in the transformed βC1 line than in the wild type *N. benthamiana* (ANOVA on Log-transformed values of Linalool: $F_{1,24} = 5.59$, $P$ value = 0.0265) (Fig. 3).

Volatile organic compounds emission in *N. benthamiana* βC1-transformed plants. The collection of volatile organic compounds was focused on the *N. benthamiana* lines, as the in-vitro cultured *A. thaliana* plants used in this study did not produce any quantifiable volatiles. The volatile organic compounds emitted by the two tobacco lines (wild type and βC1-transformed) were collected during separate day and night collections on HaysepQ filters and analyzed by gas chromatography. All plants consistently emitted linalool during the day collection, but exhibited little or no volatile emissions during the night; thus, we quantified only the linalool emitted during the 14h daytime collections. Relative to plant fresh mass, this emission was higher in the transformed βC1 line than in the wild type *N. benthamiana* (ANOVA on Log-transformed values of Linalool: $F_{1,24} = 5.59$, $P$ value = 0.0265) (Fig. 3).
SA levels were consistently, though not significantly higher in transformed *Arabidopsis* lines. This suggests that the observed suppression of JA induction may not be accomplished via a “decoy strategy” exploiting cross talk between the JA and SA signaling pathways, but by another mechanism, potentially involving direct inhibition of JA synthesis genes with shared homologs between brassicaceae and solanaceae species.

While JA-mediated responses are not known to have any direct effect on TYLCCNV, this hormone appears to be key to plants’ defense against whiteflies. Zarate et al. demonstrated that B biotype silverleaf whiteflies (the vector for TYLCCNV) exhibit a higher developmental rate on JA deficient hosts, and are themselves able to downregulate JA production to their advantage. The disruption of the entire JA pathway in plants expressing βC1 is thus very likely to further benefit the vector and might explain the increase in *B. tabaci* B biotype growth rate on virus infected plants observed in Jiu et al. For a persistent virus such as TYLCCNV, the successful acquisition of transmissible virions by their whiteflies vectors requires prolonged feeding (16–24 h) vector on an infected plant. In contrast to non-persistent viruses that benefit from rapid vector dispersal, TYLCCNV should thus be more likely to benefit from effects on host plants that improve plant quality for vectors.

In addition to changes in phytohormonal response, our analyses of plant volatile emission revealed elevated constitutive emission of volatile linalool by *N. benthamiana* plants. Organic volatile compounds emitted by plants are a key cue for their location by phytophagous insects and pathogen-induced elevation of plant volatile emissions has previously been implicated in vector attraction to infected plants. Whitefly adults are believed to locate their host plants mostly through visual cues but olfactory cues also play a significant role in host plant choice. Moreover,
while tests of whitefly responses to specific olfactory cues are rare, owing to the significant challenges entailed in conducting behavioral assays with these insects, linalool was one of several individual plant-derived compounds reported to elicit positive behavioral assays with these insects, linalool was one of several rare, owing to the significant challenges entailed in conducting while tests of whitefly responses to specific olfactory cues are rare, owing to the significant challenges entailed in conducting behavioral assays with these insects, linalool was one of several individual plant-derived compounds reported to elicit positive behavioral responses from adult B biotype B. tabaci females, individual plant-derived compounds reported to elicit positive behavioral assays with these insects, linalool was one of several rare, owing to the significant challenges entailed in conducting while tests of whitefly responses to specific olfactory cues are rare, owing to the significant challenges entailed in conducting behavior assay...
Volatile collections from *N. benthamiana*. Plants used in volatile collection were grown in vitro for three weeks then transplanted into compost soil 10 d prior to the collection, which was performed in two consecutive blocks (for a total of 12 replicates of βC1 and 15 of the wild type). The potted plants were placed under four liters glass domes with a Teflon® base covering the pot and cotton around the plant stem to prevent air contamination. Total volatiles were collected consecutively for 8 h of night and 14 h of day in a climate-controlled growth chamber (16 h:8 h light:dark, 22°C:20°C) on two separate HaysepQ filters using a clean-air system that pushed 1.5 L/min of air into the domes and pulled 1L/min through the filters. The trapped volatiles were then eluded in 150 μL of dichloromethane with 5 μL of an internal standard added after eluting (80 ng/μL nonyl acetate, 40 ng/μL n-octane). Samples were injected in 1-μL aliquots into an Agilent model 6890 gas chromatograph fitted with a flame ionization detector (column: Agilent 19091Z-331, 0.25 mm internal diameter, 0.1 μm film thickness). The column was held at 35°C for 0.5 min then increased by 7°C per min to 150°C and further increased by 20°C per min to a maximum temperature of 220°C. Linalool was the only compound systematically emitted by all plants during the day and was produced in sufficient quantities to be identified and quantified. This compound was quantified using MSD Chemstation (Agilent Technologies 2003) by measuring volatile output in nanograms relative to the internal standard and corrected by fresh plant mass, rather than surface area as is usually done, in order to avoid bias due to the curliness of βC1-transformed leaves (Fig. 4C and D).

Statistical analyses were performed with JMP (SAS institute). Data were analyzed by ANOVA with treatment (line type) and block (when needed) as explanatory variables. In the *A. thaliana* phytohormones experiment, treatments were divided into three classes of severity (Wild type controls, mild transgene phenotype and severe transgene phenotype), with the individual lines nested within, since the different severity classes have different levels of βC1 expression. *N. benthamiana* JA values as well as volatile emissions of linalool were log-transformed in order to ensure equal variances and a normal distribution of the ANOVA residuals.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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