Priming potato with thiamin to control Potato Virus Y

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Keywords potato; virus; thiamin; insects; aphids

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Accepted for publication _____

Abstract

Potato virus Y (PVY) is a major potato pathogen affecting potato yields worldwide. Thiamin, a water-soluble B vitamin (vitamin B_1) has been shown to boost the plant's immunity, thereby increasing resistance against pathogens. In this study, we tested different concentrations of thiamin (1 mM, 10 mM, 50 mM, 100 mM) and multiple thiamin applications (once, biweekly and monthly,) on potato resistance to PVY in Ranger Russet potatoes. Plants were mechanically inoculated with $PVY^{N:O}$. This PVY strain is known for causing well-defined foliar symptoms. We collected leaflets weekly through April and May 2014 and tested them with an enzyme-linked immunosorbent assay specific to PVY as well as by real time quantitative RT-PCR. These assays allowed us to determine the presence and level of PVY in different parts of the plants. We found that the highest thiamin concentration treatment (100 mM) produced the lowest virus level in potatoes across all dates and leaflet samples. Also, it was found that multiple applications of thiamin had a positive effect on reducing virus level, especially when thiamin was applied every four weeks.

Introduction

Potato virus Y (PVY), the type member of the genus *Potyvirus* of the family *Potyviridae*, is an economically important disease of potato with a worldwide distribution, causing significant losses in solanaceous crops. There are many strains of PVY, including the common (ordinary) strain (PVY^{O}), the tobacco veinal necrotic strain (PVY^{N}), the recombinant N:O strain ($PVY^{N:O}$), and the non-recombinant potato tuber necrotic strain (PVY^{NTN}) (Karasev and Gray 2013a, Karasev and Gray 2013b, Nie et al. 2012). Each potato cultivar responds differently to each strain or isolate of the virus (Rowley et al. 2014). Generally, symptom detection and recognition is relied upon to manage and control PVY and its spread in a potato field. However, some isolates may produce mild foliar symptoms but may display severe symptoms in tubers (Nie et al. 2012, Rowley et al. 2014). Symptoms expressed in plants infected with PVY^{O} include mosaic, leaf and stem necrosis, and leaf drop (Nie et al. 2012). With PVY^{N}, the symptoms tend to be milder ranging from no symptoms at all to mosaic, veinal, petiole, and stem necrosis, and possibly premature leaf death (Nie et al. 2012). PVY^{NTN} elicits similar symptoms to PVY^{N} with additional mosaic and chlorotic mottling (Nie et al. 2012). If the plant dies prematurely no tubers are produced and yield is decreased (Nie et al. 2012). The necrotic strains can lead to Potato Tuber Necrotic Ringspot Disease, or PTNRD, resulting in lesions on tuber skins and internal necrosis which makes the tubers commercially unacceptable.

PVY is a virus transmitted by aphids in a non-persistent manner (Nanayakkara et al. 2012). The most efficient aphid vector is *Myzus persicae* Sulzer (Hemiptera: Aphididae), the Green Peach Aphid, though many other species are vectors of PVY. Chemical control is not effective because the aphid can inoculate a plant with PVY faster than any insecticide could control it. The window between inoculation and infection is too short for chemical control to be effective.

One attractive strategy for disease control is to boost the plant's immune system to protect against attempted invasions by pathogens (Conrath 2009, Conrath et al. 2015, Conrath et al. 2002). The enhanced capacity of the plants to express pertinent defense mechanisms is called priming, and can be triggered by application of defense activators. Some of the best known priming-active compounds are the synthetic chemical benzo (1,2,3) thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) and the nonprotein amino acid β-aminobutyric acid (BABA), which were shown to induce tolerance in potato against *Phytophtora infestans*, *Alternaria solani*, and *Fusarium* spp. (Beckers and Conrath 2007).

One of the newly identified defense activators is thiamin. Thiamin, also known as vitamin B_1, has been shown to boost the plant's immunity to diseases in several crops. For instance, thiamin induced resistance to *Plasmopara viticola* in grapevine (Boubakri et al. 2012), to sheath blight disease and bacterial leaf blight in rice (Ahn et al. 2005, Bahuguna et al. 2012), to *Pepper mild mottle virus* in tobacco (Ahn et al. 2005), and to anthracnose in cucumber (Ahn et al. 2005). More recently, thiamin treatments alleviated aphid infestations in barley and pea (Hamada and Jonsson 2013). Priming with thiamin against *Pseudomonas syringae* pv *tomato* in Arabidopsis was shown to be dependent on salicylic acid, hydrogen peroxide accumulation, and *NPR1* (nonexpressor of *Pathogenesis-Related* genes 1) (Ahn et al. 2007). In grapevine, thiamin treatment also triggered hydrogen peroxide accumulation, callose deposition in stomata cells, total phenolics accumulation, phenylalanine ammonia lyase (PAL) and superoxide dismutase (SOD) activities, and hypersensitive response (HR)-like cell death (Boubakri et al. 2013, Boubakri et al. 2012). Thiamin was also shown to activate NADPH oxidase (NOX) and trigger the accumulation of NOX-

generated reactive oxygen species in Arabidopsis plants infected with *Sclerotinia sclerotiorum* (Zhou et al. 2013).

There is currently no report on priming potato with thiamin. In this study, we tested the effect of thiamin application on potato resistance to PVY. We used the strain $PVY^{N:O}$ and the potato variety Ranger Russet. $PVY^{N:O}$ was used because it produces clear foliar symptoms of mosaic (mottling), chlorosis (yellowing), and leaf drop in Ranger Russet, but typically does not cause premature plant death.

Materials and Methods

Plant Material

Virus-free potato plantlets of the variety Ranger Russet were propagated in tissue culture. After 2-3 weeks on synthetic medium, fifty plantlets were transferred to 3-liters pots containing Sunshine Mix 1 supplemented with Osmocote and grown in a greenhouse in Hermiston, OR. Plants were arranged in a randomized complete block design with nine plants per each thiamin treatment and five untreated control plants. For each of the nine plants, three received the specified thiamin dose once at the beginning of the experiment, three received it every four weeks, and three received it every two weeks. With nine plants per thiamin treatment, there were three replicates of thiamin application rate.

Thiamin Treatments

Thiamin was sprayed with a spray bottle on the whole plant 24 h prior to inoculation with PVY one month after transferring plantlets to soil from tissue culture. Thiamin solutions contained Tween 80 at 250 mg/L. Thiamin was applied at 5 different concentrations: 0, 1, 10, 50, and 100 mM. Non-inoculated untreated plants were included as healthy plant controls. Since thiamin-triggered immunity was shown to last about fourteen days in Arabidopsis (Ahn et al. 2005), the effect of multiple thiamin applications was also tested. Thus, one third of the plants from each treatment (=3 plants) received thiamin applications only once (at start of experiment on April 2, 2015), one third received thiamin applications twice (on April 2 and 30, 2015), and one third of the plants received thiamin applications four times (on April 2, 16 and 30, and May 14, 2015).

PVY Inoculation

Plants were mechanically inoculated with $PVY^{N:O}$ (isolate Alt (Szajko et al. 2014)) on April 2, 2015, as described before (Nishimura et al. 1984). Previously inoculated and symptomatic tobacco leaves were ground in cold 30 mM potassium phosphate buffer, pH 8.0 (0.1 g infected leaf material for 10 mL phosphate buffer). Three leaflets on three leaves from the lower canopy were then dusted with carborundum and inoculated by rubbing the inoculum on the whole adaxial surface area.

Leaf Sampling

Leaflets were sampled and tested for PVY by ELISA (see below). Specific leaflets were sampled every week for six weeks starting two weeks after inoculation (Figure 1). Leaflet 1 represents the younger, uninoculated leaf right above the top inoculated leaf on each plant. Leaflets 2+3 were

located in the medium canopy of the plant. At 14 days post-inoculation (dpi) (April 16), leaflets 4+5 were the new emerging leaves. The leaves where leaflets were collected were marked so that leaflets could be collected each week from the same leaves to test for the development and spread of PVY throughout the plant. At 26 dpi (April 28), leaflets 6+7 which were the new emerging leaves were collected. At 35 dpi (May 7), leaflets 8+9 were collected from new emerging leaves as the plant continued to grow. Leaflets 10+11 were collected 43 dpi (May 15) from new emerging leaves. There was no new emergence by five weeks post-inoculation. Leaflet 1 had fallen off of plants 26 dpi (April 28) and leaflets 2+3 had fallen off of most plants by 43 dpi (May 15). The last sampling date was 50 dpi (May 22).

PVY Detection and Quantification by ELISA

PVY was detected by using a triple antibody sandwich ELISA kit (ELISA Reagent Set for Potato Virus Y, Agdia). Leaflet samples were freeze-dried and then ground with a mortar and pestle and placed in 1.5 mL centrifuge tubes. Twenty milligrams of freeze-dried ground leaflet tissue were used for detection. This exact amount of material enabled us to normalize the virus titer across samples. Microreader plates were read at 1 hour and at 3 hours, and the ELISA values from the 3 hour read were transformed and used in all graphs and data analyses.

PVY Detection and Quantification by Real Time Quantitative RT-PCR

RNAs were extracted from freeze-dried leaf samples (10 mg) in biological triplicates using TriZol (Invitrogen) according to the manufacturer's recommendations with the following modifications. Because the RNA absorbance ratio 260/280 and 260/230 were not always satisfactory after TriZol extraction, RNAs were precipitated with one volume of 4 M LiCl overnight at 4°C. After centrifugation at 13000 rpm for 15 min, the pellet was washed with 70% ethanol, air dried, and resuspended in 40 µl DEPC-treated water. RNA extracts were then treated with DNase I (Ambion) to remove any trace of genomic DNA. All RNA extracts had a final absorbance ratio 260/280 and 260/230 greater than 1.8.

RNAs (1 µg) were reverse transcribed to cDNAs by using M-MuLV (New England Biolabs) and an 18-mer oligonucleotide Oligo$(dT)_{18}$ (Invitrogen). After dilution (four times) in RNase-free water, cDNAs (2 µl) were used as template for real-time quantitative PCR using the Brilliant III Ultra-Fast SYBR Green QPCR Master Mix (Agilent Technologies) in 20-µl reaction volume. PVY-specific primers were PVYF 5'-ATACTCGRGCAACTCAATCACA-3' and PVYR 5'-CCATCCATCATAACCCAAACTC-3' (Du et al. 2006). PVY RNAs were quantified relative to the expression of the housekeeping gene EF1α as described before (Goyer et al. 2015). EF1α-specific primers were EF1a-Fwd1 5'-CTGGTATGGTTGTGACCTTTG-3' and EF1a-Rev1 5'-TTGAACCCAACATTGTCACC-3'. Primers were used at a final concentration of 500 nM. Primers efficiencies were determined by amplifying serial dilutions of cDNAs as described before (Schmittgen and Livak 2008). Efficiencies were 2.02 and 2.06 for EF1α and PVY, respectively. For each biological replicate (three replicates per condition), quantitative PCR was run in three technical replicates, so data are from 9 independent determinations. For each measurement, a $2^{-\Delta Ct}$ value was calculated, and data are presented relative to the highest $2^{-\Delta Ct}$ value (i.e. value found for PVY-inoculated plants treated with 0 mM thiamine at 50 dpi).

Statistical Analysis

One-way ANOVA was used. Differences among treatments were determined using the Tukey's least significant means test at a significance level of $\alpha = 0.05$.

Results

Priming of Ranger Russet potatoes against $PVY^{N:O}$ was tested by spraying thiamin at five concentrations (0, 1, 10, 50, and 100 mM) and applied either once, twice, or four times over the course of the experiment (50 days). A control that was not inoculated with PVY was included. Leaflets from different canopy levels (Figure 1) were collected for PVY quantification by ELISA at various time points after PVY inoculation (14, 26, 35, 43, and 50 dpi). Thiamin concentrations had a significant effect on the virus levels within the potato plants (Table 1). The number of thiamin applications also had a significant effect on the relative virus level within the plant (Table 1). There was a significant interaction between thiamin concentrations and the number of applications (Table 1). The relative virus level was significantly different depending on which leaflet was sampled (Table 1). There was a significant interaction between leaflet and concentrations (Table 1).

We further analyzed our data per number of applications (Table 2 and Figures 2-4). In all treatments (one, two, or four applications), thiamin concentrations, sampling date, and leaflet position had a significant effect (Table 2). The lowest relative virus level amongst PVY-inoculated plants was found in plants treated with 100 mM thiamin, regardless of the number of thiamin applications and leaflet position (Figures 2-4). Although the virus level in plants treated with 100 mM thiamin increased over time, it never reached the levels found in plants treated with other thiamin concentrations and remained at levels at least four times lower than the other treatments. We confirmed these ELISA results by real-time quantitative RT-PCR on a subset of samples shown previously in Figure 3 "Leaflets 4+5". As shown in Figure 5, leaflets 4+5 from the 100 mM thiamin-treated plants had PVY RNA levels below 5% of those found in the 0 mM thiamin-treated plants. In addition, PVY could not be detected until 26 dpi in leaflets 1 through 5 of plants treated with 10, 50, and 100 mM thiamin, while it could be detected at 14 dpi in plants treated with 0 or 1 mM thiamin (Figure 2-4). These results show that there was a delay in the increase of the relative virus level in plants treated with thiamin concentrations higher than 1 mM. The relative virus level throughout the plants was then calculated by averaging ELISA values in all sampled leaflets and sampling date and compared between thiamin concentrations (Table 3). Only plants that were treated with 100 mM thiamin consistently showed significantly lower PVY levels than plants treated with a mock solution (0 mM thiamin) regardless of the application frequency. In plants that were treated twice with 100 mM thiamin, the relative PVY level was very low and was not significantly different than that of control plants that were not inoculated with PVY (Table 3). When thiamin was applied twice, the relative PVY level in plants treated with 10 mM thiamin was significantly different than that in plants treated with a mock solution (Table 3). When thiamin was applied four times, the relative virus level was significantly lower in plants treated with 10 and 50 mM thiamin compared to plants treated with a mock solution (Table 3).

Leaflets collected 14 and 26 dpi had lower PVY levels than leaflets sampled 35, 43, and 50 dpi (Figures 2-4), which shows the progressive increase of the relative virus level in the plant over time. This is particularly obvious in leaflets 4+5 that we were able to sample throughout the whole experiment. The emerging leaflets (4+5, 6+7, 8+9, 10+11) had higher PVY levels than mid-canopy leaflets 2+3 (and possibly leaflets 1 but it was not possible to evaluate because leaflets 1 dropped by 26 dpi) (Figures 2-4 and Table 1).

Discussion

Thiamin application has recently been shown to increase tolerance or resistance to various stresses, abiotic or biotic, in plants (Ahn et al. 2005, Bahuguna et al. 2012, Boubakri et al. 2012, Hamada and Jonsson 2013, Tunc-Ozdemir et al. 2009). In this study, we show that application of 100 mM thiamin delayed the detection of PVY and kept the virus level in systemic leaves as low as below 5% of the level found in infected plants treated with 0 mM thiamin. Thiamin applications did not have an effect on foliar symptoms except to delay their appearance in plants where relative virus level was lower. It is noteworthy that plants treated four times during the course of the experiment (or every two weeks) with 100 mM thiamin, and some of the plants treated with 50 mM thiamin, began showing severe scorching symptoms after the last thiamin application 42 dpi. This was not the case for plants treated once or twice (or every four weeks). Therefore, future studies are warranted to determine the most appropriate number of applications to find the balance between lowering the PVY titer and preventing detrimental effect of thiamin application on plant foliage.

Low and mid-canopy leaflets (leaflets 1, 2, and 3) had low, sometimes undetectable virus, while upper-canopy emerging leaflets (leaflets 4 through 11) had high relative virus level by comparison, showing that PVY did not accumulate in mature leaves but was transported to young leaves where it accumulated. These results are in agreement with previous studies that showed virus movement from source (mature) to sink (young) tissues (Rajamaki and Valkonen 2002, Roberts et al. 1997), and that PVY multiplies more rapidly in younger, more metabolically active tissues (Kogovsek et al. 2011).

After initial entry into and infection of epidermal or mesophyll cells, potyviruses move from cell-to-cell through plasmodesmata and then are loaded into sieve elements for long distance transport following photoassimilates partitioning (Kogovsek et al. 2011, Rajamaki and Valkonen 2002). Unloading occurs in sink tissues. The lower PVY level observed in plants treated with 100 mM thiamin indicate that thiamin treatment may have interfered with entry and transport mechanisms. Indeed, thiamin application was shown to trigger callose deposition in stomata guard cells and lignin in leaf tissues (Ahn et al. 2007, Boubakri et al. 2012), thereby possibly limiting initial entry into leaf cells and cell-to-cell movement. Additional studies are necessary to depict the cellular and molecular effects of thiamin application in potato.

In summary, our data show that thiamin can limit the level of PVY in the plant and suggest that thiamin may be an additional tool compatible with efficient, integrated, sustainable management of PVY in potato production systems. Further investigation is warranted to validate our findings in a field environment and determine the impact of thiamin application on yield. Future research should also focus on its potential use to control other strains of PVY and in other potato cultivars.

Acknowledgements

The authors would like to thank the Oregon State University Agricultural Research Foundation for funding part of this trial. We would also like to thank Matthew Warman for his help during PVY inoculation and ELISA testings, and Mark Barnett for his technical help with the real-time quantitative RT-PCR experiments.

References

Ahn, I. P., S. Kim and Y. H. Lee. 2005. Vitamin B1 functions as an activator of plant disease resistance. *Plant Physiology*. 138: 1505-1515.

Ahn, I. P., S. Kim, Y. H. Lee and S. C. Suh. 2007. Vitamin B1-induced priming is dependent on hydrogen peroxide and the NPR1 gene in Arabidopsis. *Plant Physiology*. 143: 838-48.

Bahuguna, R. N., R. Joshi, A. Shukla, M. Pandey and J. Kumar. 2012. Thiamine primed defense provides reliable alternative to systemic fungicide carbendazim against sheath blight disease in rice (*Oryza sativa* L.). *Plant Physiology and Biochemistry*. 57: 159-167.

Beckers, G. J. and U. Conrath. 2007. Priming for stress resistance: from the lab to the field. *Current Opinion in Plant Biology*. 10: 425-431.

Boubakri, H., A. Poutaraud, M. A. Wahab, C. Clayeux, R. Baltenweck-Guyot, D. Steyer, C. Marcic, A. Mliki and I. Soustre-Gacougnolle. 2013. Thiamine modulates metabolism of the phenylpropanoid pathway leading to enhanced resistance to Plasmopara viticola in grapevine. *Bmc Plant Biology*. 13.

Boubakri, H., M. A. Wahab, J. L. Chong, C. Bertsch, A. Mliki and I. Soustre-Gacougnolle. 2012. Thiamine induced resistance to *Plasmopara viticola* in grapevine and elicited host-defense responses, including HR like-cell death. *Plant Physiology and Biochemistry*. 57: 120-133.

Conrath, U. 2009. Priming of Induced Plant Defense Responses. *In* Plant Innate Immunity. Vol. 51. L. C. VanLoon, editor. Academic Press Ltd-Elsevier Science Ltd, London. 361-395.

Conrath, U., G. J. M. Beckers, C. J. G. Langenbach and M. R. Jaskiewicz. 2015. Priming for Enhanced Defense. *In* Annual Review of Phytopathology, Vol 53. Vol. 53. N. K. VanAlfen, editor. Annual Reviews, Palo Alto. 97-119.

Conrath, U., C. M. Pieterse and B. Mauch-Mani. 2002. Priming in plant-pathogen interactions. *Trends Plant Sci.* 7: 210-6.

Du, Z. Y., J. S. Chen and C. Hiruki. 2006. Optimization and application of a multiplex RT-PCR system for simultaneous detection of five potato viruses using 18S rRNA as an internal control. *Plant Disease*. 90: 185-189.

Goyer, A., L. Hamlin, J. M. Crosslin, A. Buchanan and J. H. Chang. 2015. RNA-Seq analysis of resistant and susceptible potato varieties during the early stages of Potato Virus Y infection *BMC Genomics*: Under Review.

Hamada, A. M. and L. M. V. Jonsson. 2013. Thiamine treatments alleviate aphid infestations in barley and pea. *Phytochemistry*. 94: 135-141.

Karasev, A. V. and S. M. Gray. 2013a. Continuous and Emerging Challenges of Potato virus Y in Potato. *Annual Review of Phytopathology, Vol 51*. 51: 571-586.

Karasev, A. V. and S. M. Gray. 2013b. Genetic Diversity of Potato virus Y Complex. *American Journal of Potato Research*. 90: 7-13.

Kogovsek, P., A. Kladnik, J. Mlakar, M. T. Znidaric, M. Dermastia, M. Ravnikar and M. Pompe-Novak. 2011. Distribution of Potato virus Y in Potato Plant Organs, Tissues, and Cells. *Phytopathology*. 101: 1292-1300.

Nanayakkara, U. N., X. Nie, M. Giguere, J. Zhang, S. Boquel and Y. Pelletier. 2012. Aphid Feeding Behavior in Relation to Potato Virus Y (PVY) Acquisition. *Journal of Economic Entomology*. 105: 1903-1908.

Nie, B. H., M. Singh, A. Murphy, A. Sullivan, C. H. Xie and X. Z. Nie. 2012. Response of Potato Cultivars to Five Isolates Belonging to Four Strains of Potato virus Y. *Plant Disease*. 96: 1422-1429.

Nishimura, H., Y. Uehara, K. Sempuku and A. Iwashima. 1984. Purification and some properties of thiamine-binding protein from rice bran. *Journal of Nutritional Science and Vitaminology*. 30: 1-10.

Rajamaki, M. L. and J. P. T. Valkonen. 2002. Viral genome-linked protein (VPg) controls accumulation and phloem-loading of a potyvirus in inoculated potato leaves. *Molecular Plant-Microbe Interactions*. 15: 138-149.

Roberts, A. G., S. S. Cruz, I. M. Roberts, D. A. M. Prior, R. Turgeon and K. J. Oparka. 1997. Phloem unloading in sink leaves of Nicotiana benthamiana: Comparison of a fluorescent solute with a fluorescent virus. *Plant Cell*. 9: 1381-1396.

Rowley, J. S., S. M. Gray and A. V. Karasev. 2014. Screening potato cultivars for new sources of resistance to Potato virus Y. *American Journal of Potato Research*.

Schmittgen, T. D. and K. J. Livak. 2008. Analyzing real-time PCR data by the comparative C-T method. *Nature Protocols*. 3: 1101-1108.

Szajko, K., D. Strzelczyk-Zyta and W. Marczewski. 2014. Ny-1 and Ny-2 genes conferring hypersensitive response to potato virus Y (PVY) in cultivated potatoes: mapping and marker-assisted selection validation for PVY resistance in potato breeding. *Molecular Breeding*. 34: 267-271.

Tunc-Ozdemir, M., G. Miller, L. Song, J. Kim, A. Sodek, S. Koussevitzky, A. N. Misra, R. Mittler and D. Shintani. 2009. Thiamin confers enhanced tolerance to oxidative stress in Arabidopsis. *Plant Physiol*. 151: 421-32.

Zhou, J., A. Z. Sun and D. Xing. 2013. Modulation of cellular redox status by thiamine-activated NADPH oxidase confers Arabidopsis resistance to *Sclerotinia sclerotiorum*. *Journal of Experimental Botany*. 64: 3261-3272.

Figure Captions

Fig. 1. Diagram of leaflet sampling. Leaflets were sampled for testing for the presence of potato virus Y (PVY) by ELISA. Specific leaflets were sampled every week for six weeks starting two weeks after PVY inoculation. Leaflet 0 represents the top inoculated leaflet. Leaflet 1 represents the leaflet above leaflet 0 on each plant. Leaflets 2+3 were located in the medium canopy of the plant. At 14 days post-inoculation (dpi) (April 16), leaflets 4+5 were the new emerging leaves. The leaves where leaflets were collected were marked so that leaflets could be collected each week from the same leaves to test for the development and spread of PVY throughout the plant. At 26 dpi (April 28), leaflets 6+7, which were the new emerging leaves, were collected. At 35 dpi (May 7), leaflets 8+9 were collected from new emerging leaves as the plant continued to grow. Leaflets 10+11 were collected 43 dpi (May 15) from new emerging leaves. There was no new emergence by five weeks post-inoculation. Leaflet 0 had fallen off of all plants by 18 dpi (April 20). Leaflet 1 had fallen off of plants 26 dpi (April 28) and leaflets 2+3 had fallen off of most plants by 43 dpi (May 15). The last sampling date was 50 dpi (May 22).

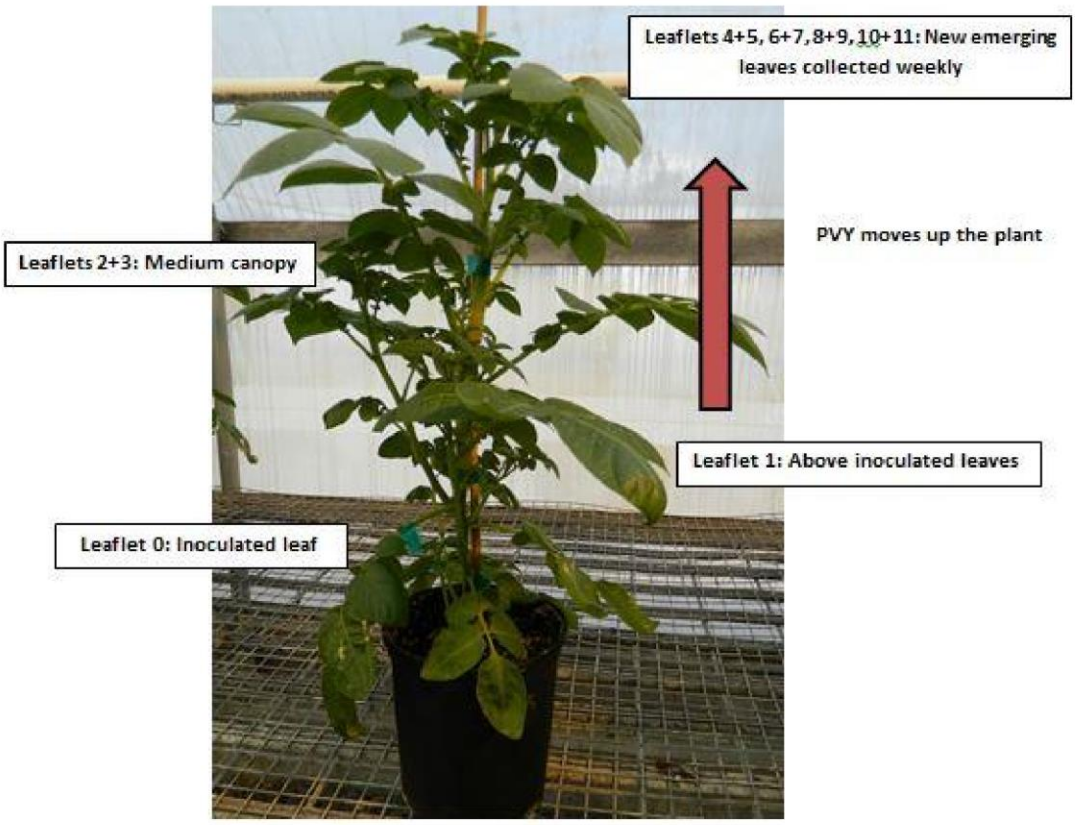
Fig. 2. Relative potato virus Y (PVY) level in leaflets sampled (see Fig. 1) at various days post-inoculation (dpi) and treated with thiamin concentrations (0 mM, 1 mM, 10 mM, 50 mM, 100 mM) once 24 h prior to inoculation with $PVY^{N:O}$. PVY level was determined by ELISA. All plants were treated with thiamin and inoculated with $PVY^{N:O}$ except the control which was not treated with thiamin and not inoculated with PVY. Data are mean ELISA values per leaflet at each sampling date for all plants where thiamin was applied once, 24 hours prior to inoculation with $PVY^{N:O}$.

Fig. 3. Relative potato virus Y (PVY) level in leaflets sampled (see Fig. 1) at various days post-inoculation (dpi) and treated with thiamin concentrations (0 mM, 1 mM, 10 mM, 50 mM, 100 mM) twice, once at the start of the experiment 24 h prior to inoculation with $PVY^{N:O}$, and once 28 dpi. PVY level was determined by ELISA. All plants were treated with thiamin and inoculated with $PVY^{N:O}$ except the control which was not treated with thiamin and not inoculated with PVY. Data are mean ELISA values per leaflet at each sampling date for all plants where thiamin was applied twice, once at the start of the experiment 24 h prior to inoculation with $PVY^{N:O}$, and once 28 dpi.

Fig. 4. Relative potato virus Y (PVY) level in leaflets sampled (see Fig. 1) at various days post-inoculation (dpi) and treated with thiamin concentrations (0 mM, 1 mM, 10 mM, 50 mM, 100 mM) biweekly, once at the start of the experiment 24 h prior to inoculation with $PVY^{N:O}$, at 15 dpi, 28 dpi, and 43 dpi. PVY level was determined by ELISA. All plants were treated with thiamin and inoculated with $PVY^{N:O}$ except the control which was not treated with thiamin and not inoculated with PVY. Data are mean ELISA values per leaflet at each sampling date for all plants where thiamin was applied biweekly, once at the start of the experiment 24 h prior to inoculation with $PVY^{N:O}$, and 15 dpi, 28 dpi, and 43 dpi.

Fig.5. Potato virus Y RNA quantification by real-time quantitative RT-PCR. All plants were treated with thiamin and inoculated with $PVY^{N:O}$ except the control which was not treated with thiamin and not inoculated with PVY. Samples are identical to those in Figure 3 “Leaflets 4+5”. Data are based on three biological replicates and three technical replicates, thus a total of 9 determinations per sample/condition. Data represent the amount of PVY RNA relative to the

expression of the housekeeping gene eF1α. Data are presented in percentage of PVY RNA relative to the sample with the highest PVY RNA detected (i.e. 0 mM thiamin, 50 dpi).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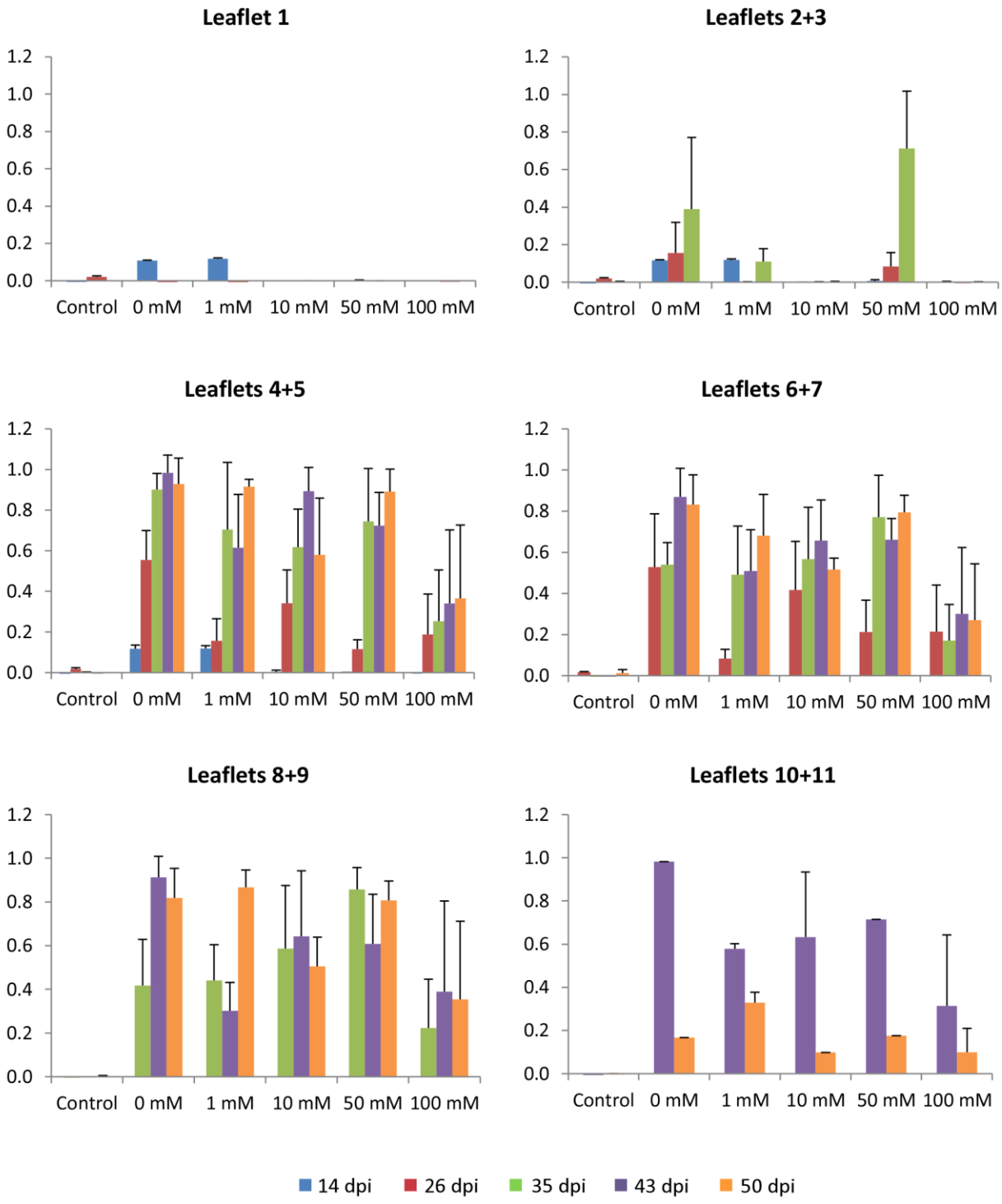
PVY moves up the plant
Leaflets 2+3: Medium canopy
Leaflet 1: Above inoculated leaves
Leaflet 0: Inoculated leaf

432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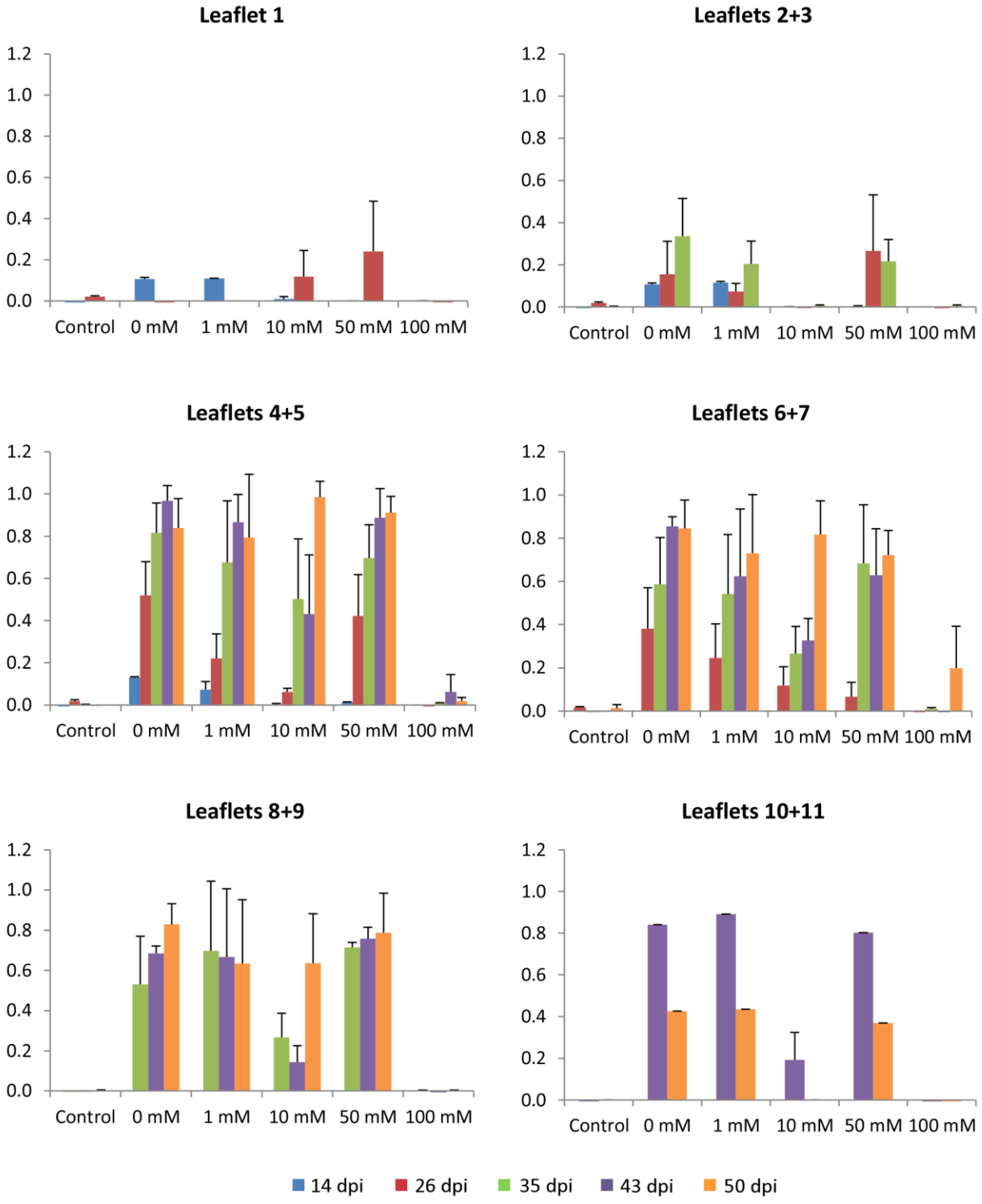
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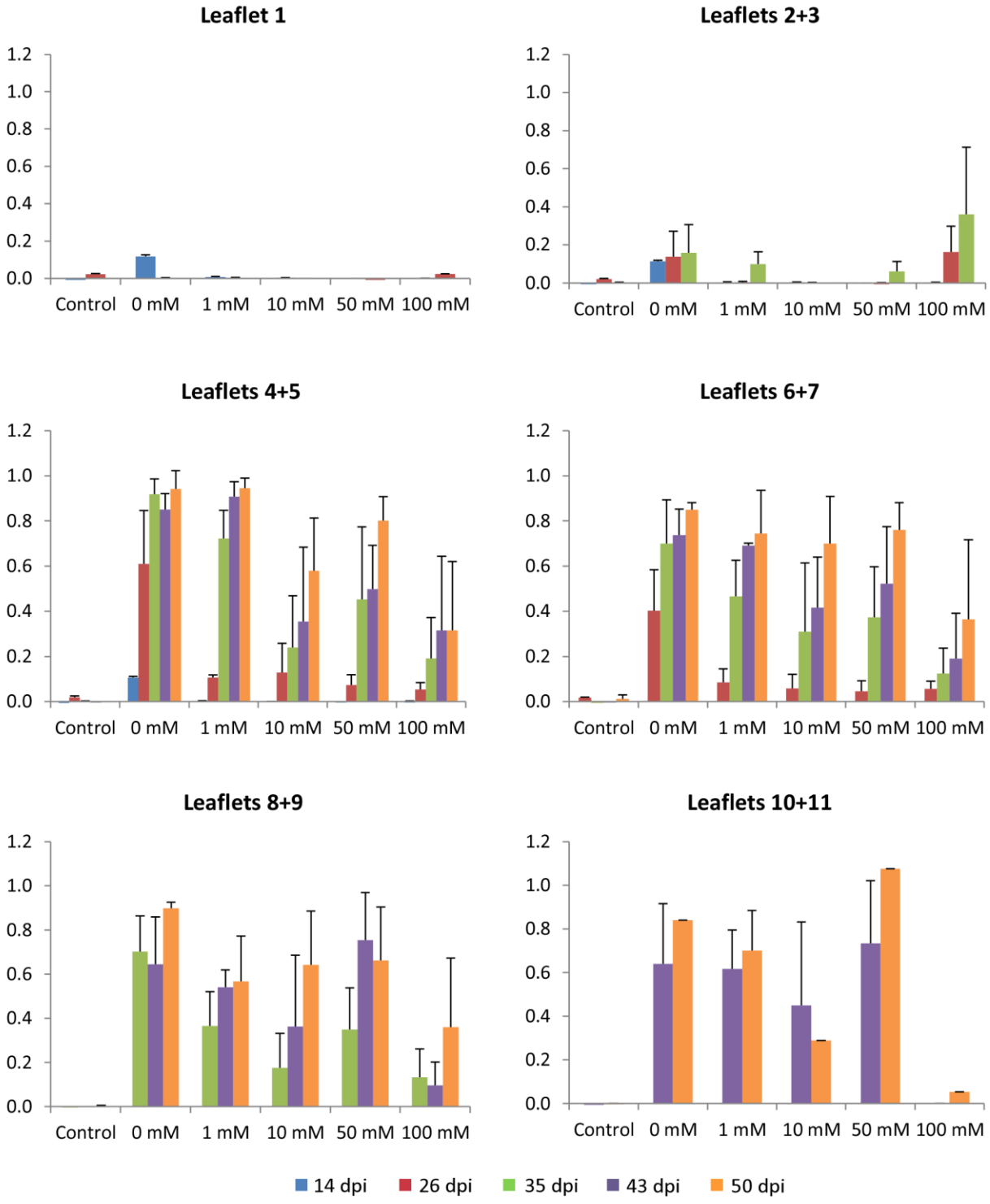
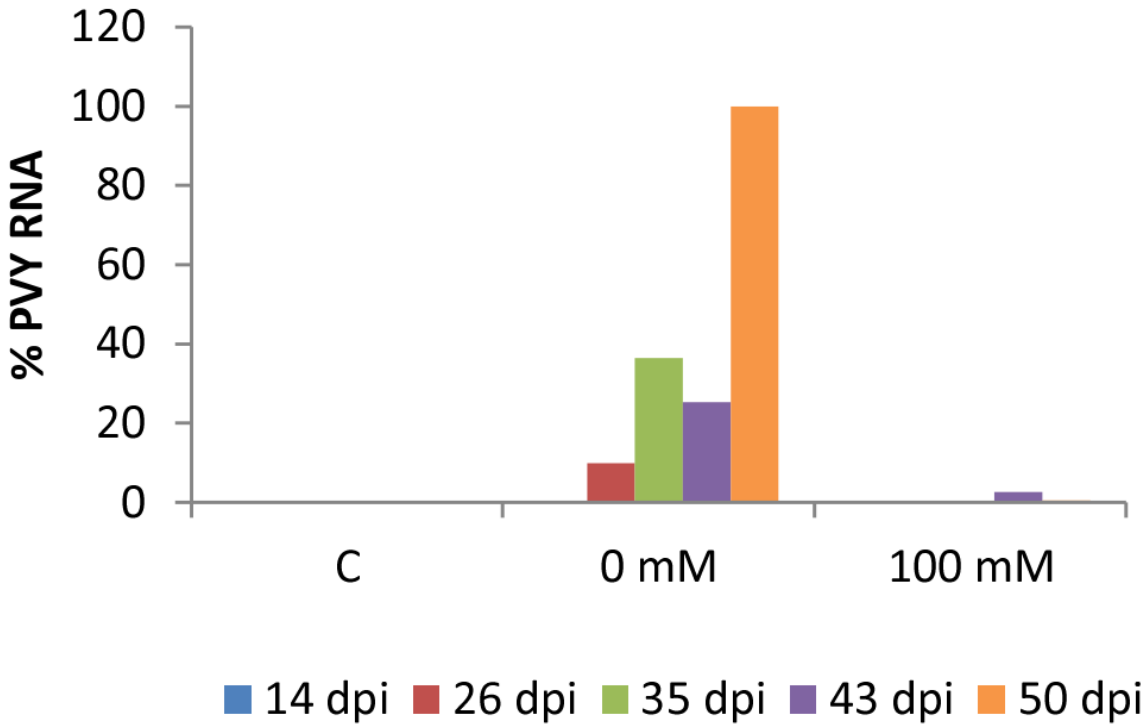

Leaflet 1
Leaflets 2+3
Leaflets 4+5
Leaflets 6+7
Leaflets 8+9
Leaflets 10+11
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Control
0 mM
1 mM
10 mM
50 mM
100 mM
14 dpi
26 dpi
35 dpi
43 dpi
50 dpi

435


120
100
80
60
40
20
0
% PVY RNA
C
0 mM
100 mM
14 dpi
26 dpi
35 dpi
43 dpi
50 dpi

436