17 June 2024

ARCHIVIO ISTITUZIONALE
DELLA RICERCA

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Zoophytophagous predator-induced defences restrict accumulation of the tomato spotted wilt virus

This is the final peer-reviewed author’s accepted manuscript (postprint) of the following publication:

Published Version:
Zoophytophagous predator-induced defences restrict accumulation of the tomato spotted wilt virus / Bouagga S.; Urbaneja A.; Depalo L.; Rubio L.; Perez-Hedo M.. - In: PEST MANAGEMENT SCIENCE. - ISSN 1526-4998. - ELETTRONICO. - 76:2(2020), pp. 561-567. [10.1002/ps.5547]

This version is available at: https://hdl.handle.net/11585/713506 since: 2020-01-15

Published:

DOI: http://doi.org/10.1002/ps.5547

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

(Article begins on next page)

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/).
When citing, please refer to the published version.

Document donwnloaded from:

[http://redivia.gva.es/handle/20.500.11939/6278]

This paper must be cited as:

[Bouagga, S. , Urbaneja, A. , Depalo, L. , Rubio, L. and Pérez-Hedo, M. (2019), Zoophytophagous predator-induced defences restrict accumulation of the tomato spotted wilt virus. Pest. Manag. Sci.. doi:10.1002/ps.5547]

ivia
Institut Valencià
d'Investigacions Agràries

The final publication is available at

[http://dx.doi.org/10.1002/ps.5547]

Copyright [Wiley]

1 # Zoophytophagous predator-induced defences restrict accumulation of
2 the tomato spotted wilt virus

3 Running title: Zoophytophagy restricts TSWV

4

5 **Sarra Bouagga[1], Alberto Urbaneja[1], Laura Depalo[2], Luís Rubio[1] and Meritxell Pérez-Hedo[1]***

6 [1]Instituto Valenciano de Investigaciones Agrarias (IVIA). Centro de Protección Vegetal y
7 Biotecnología, (IVIA), CV-315, Km 10.7, 46113 Moncada, Valencia, Spain
8 [2]Università di Bologna. DISTAL Department of Agricultural and Food Sciences. Viale G. Fanin 44,
9 40127 Bologna.

10
11

12 *Author for correspondence: mperezh@ivia.es
13

14

15 **Abstract**

16 BACKGROUND: The use of zoophytophagous predators in protected crops has been widely
17 adopted to manage pests in Southern Europe. We hypothesized plant defence responses
18 would be induced by zoophytophagous predators and this induction could affect plant virus
19 occurrence; the phytophagy of these predators induces plant defences similarly to that of viral
20 infection. Therefore, we evaluated whether or not mirid predator activated plant defences
21 limited the accumulation of *Tomato Spotted Wilt Virus* (TSWV) in mechanically infected sweet
22 pepper.

23 RESULTS: Our results revealed TSWV accumulation in mirid-punctured plants to be significantly
24 lower than in intact plants; this is most likely associated with the upregulation of the JA
25 pathway triggered by mirid phytophagy.

26 CONCLUSION: Activation of induced defences by mirid predators has been demonstrated for
27 the first time to limit the accumulation of TSWV in sweet pepper. This novel approach can offer
28 new control strategies for the management of plant diseases.

29

30 **Keywords**: *Nesidiocoris tenuis*, *Macrolophus pygmaeus*, *Tomato spotted wilt virus*, plant
31 defences, biological control

32

33 1 INTRODUCTION

34 In Europe, throughout the last ten years, biological control in protected crops has been widely 35 adopted for pest management.[1–3] The case of sweet pepper and tomato in South-eastern Spain 36 could be a paradigmatic example of how biological control based on the use of omnivorous 37 predators has environmentally, socially and economically transformed an entire region of 38 more than 30,000 ha of protected crops.[4,5] In this short period of time the agricultural 39 paradigm in this zone has evolved from chemical dependency to the implementation of an 40 integrated pest management program based on the release and conservation of natural 41 enemies; where preventive and sustainable control methods are now prioritized.[3,6]
42 In sweet pepper, (*Capsicum annuum*), the release of two generalist predators native to the 43 Mediterranean region, the predatory mite *Amblyseius swirskii* (Athias-Henriot) (Acari: 44 Phytoseiidae) together with the minute pirate bug *Orius laevigatus* (Fieber) (Hemiptera: 45 Anthocoridae) results in highly efficient management of the two key sweet pepper pests; the 46 western flower thrip, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and the 47 whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae).[7–9] Moreover, recent studies 48 with the mirid predators, *Nesidiocoris tenuis* (Reuter) and *Macrolophus pygmaeus* (Rambur) 49 (Hemiptera: Miridae), sustained even better biological control results in this crop since these 50 two are also able to control aphid species.[10–12] Similarly in tomatoes, the cosmopolitan 51 predatory mirid *N. tenuis* enables effective control of *B. tabaci* and the tomato borer *Tuta* 52 *absoluta* (Meyrick) (Lepidoptera: Gelechiidae),[6,13,14] an important invasive tomato pest 53 detected for the first time in Spain in 2007.[15]

54 Zoophytophagy is a special case of omnivory; predators belonging to this group use a mixture 55 of both prey and plant resources to complete development and reproduction.[16] 56 Zoophytophagous predators can affect herbivore populations directly by preying upon them as 57 well as indirectly through plant-mediated effects.[17–27] Plant responses to herbivory feeding are 58 known to result in a stunning array of structural, chemical, and protein-based defences 59 designed to detect invading organisms and stop them before they are able to cause extensive 60 damage.[28–31] Zoophytophagous predators have been observed to induce both direct and 61 indirect plant defences in sweet pepper and tomato. In sweet pepper, the phytophagy of the 62 anthocorid *O. laevigatus* and the mirids *N. tenuis* and *M. pygmaeus* activated the jasmonate 63 acid (JA) and salicylic acid (SA) signalling pathways and triggered the release of an altered 64 blend of volatiles (green leaf volatiles, terpenoids and methyl salicylate). Those volatiles 65 repelled *B. tabaci* and *F. occidentalis* and at the same time attracted the whitefly parasitoid,

66*Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae). [26,27,32] Similar results have been 67obtained in tomato with the mirid predators, *N. tenuis*, *M. pygmaeus* and *Dicyphus bolivari* 68(Lindberg) [= *D. maroccanus* (Wagner)], yet the specific responses were attributed to each 69predator species in these cases. Thus, while plants punctured by *N. tenuis* repel *B. tabaci* and 70*T. absoluta*, the phytophagy of *M. pygmaeus* and *D. bolivari* did not repel *B. tabaci* and even 71attracts *T. absoluta*. In contrast, the feeding activity of these three mirids results in an 72attraction of *E. formosa*.[18,20–24] Furthermore, the feeding behaviour of these zoophytophagous 73predators has been verified to induce direct defences through the activation of the JA pathway 74with an increase in protease inhibitor activity.[20,23,24] Plants previously induced by mirids have 75been found to reduce the establishment and performance of important pests such as *B. tabaci*, 76*F. occidentalis* and the two-spotted spider mite, *Tetranychus urticae* (Koch) (Acari: 77Tetranychidae) in sweet pepper,[19,26] along with *T. urticae* in tomato.[18,20,24]

78Regardless of the above mentioned studies, more investigations are needed to expand our 79understanding of plant mediated effects on pest and disease management induced by 80zoophytophagous predators. Interestingly, an important facet of research, previously not 81addressed but already hypothesized, is the evaluation of plant mediated effects of 82zoophytophagous predators on viral and microbial infection.[18,33] Recently, beneficial microbes 83have been observed to modulate the performance of zoophytophagous predators.[34,35] The 84colonization of tomato plants by the endophytic fungi *Fusarium solani* strain K reduces the 85capability of *N. tenuis* to induce necrotic rings on tomato stems and leaves. The upregulation 86of ethylene and JA pathways induced by *F. solani* give protection to tomato from *N. tenuis* 87feeding.[34] An interaction between the pepino mosaic virus (PepMV) and the mirid *M.* 88*pygmaeus* has been also found. The severity of crop damage caused by *M. pygmaeus* is 89significantly enhanced when tomato plants are infected with PepMV.[36] This interaction was 90attributed to the antagonistic effects of SA-mediated responses on JA-mediated responses, 91since PepMV infection induces the SA defence pathway[37] meanwhile *M. pygmaeus* mainly 92activates the JA pathway.[20, 22] Additionally, tomato plants with high expression of methyl 93jasmonate are less likely to be infected with the *Tomato yellow leaf curl virus* (TYLCV).[38] 94Therefore, we hypothesized that possible interaction can occur between induced defences by 95zoophytophagous predator influence the incidence of plant viruses.

96In this research, we focused on evaluating whether plant defences triggered separately by *N.* 97*tenuis* or *M. pygmaeus* affect the multiplication of the *Tomato Spotted Wilt Virus* (TSWV) in 98sweet pepper. TSWV is one of the most harmful plant viral pathogens, ranking second in the 99list of the most important plant viruses worldwide.[39,40] It is transmitted in a persistent manner

100by several thrips species; with *F. occidentalis* being its main vector. Eradication or control of 101TSWV has become even more difficult by the emergence of resistant TSWV isolates in 102pepper.[41] Herein, we evaluated the effect of plant defence activation on TSWV multiplication 103by quantifying TSWV RNA accumulation. Plant defence activation was confirmed by analyzing 104gene expression of defence pathways. The implications of these results to improve TSWV 105disease management in pepper are discussed.

106

107**2 MATERIAL AND METHODS**

108**2.1 Plants, insects, and virus isolate**

109Sweet pepper plants [*Capsicum annuum* (Solanaceae)] *cv* ('Salmerón') (California rojo, 110Mascarell semillas S.L, Valencia, Spain) were used in the experiments herein described. Two 111weeks after germination the seedlings were transplanted to plastic pots (8 × 8 × 8 cm) 112containing a mixture of soil with peat moss and were maintained undisturbed at 25 ± 2°C, with 113constant relative humidity of 65% ± 5%, and a photoperiod of 14:10 h (light: dark). Plants were 114irrigated twice a week. Pesticide-free sweet pepper plants were used for the experiments at 6 115weeks of age (approximately 20 cm high). Fourth instar nymphs of *N. tenuis* and *M. pygmaeus* 116were provided directly by Koppert Biological Systems, S.L. (Águilas, Spain). *Tomato spotted wilt* 117*virus*, TSWV PVR isolate (TSWV-PVR), from the IVIA plant virus collection was used.[42] The virus 118was maintained in *Nicotiana benthamiana* Domin (Solanales: Solanaceae). Preliminary 119research showed that the sweet pepper cultivar used in our experiments can be successfully 120infected with TSWV-PVR when mechanically inoculated.

121**2.2 Biological assays**

122Three treatments were assayed: i) *N. tenuis*-punctured plants, ii) *M. pygmaeus*-punctured 123plants and iii) intact plants (control plants free of arthropod contact). Mirid-punctured plants 124were obtained by individually exposing sweet pepper plants to either 20 *N. tenuis* or 20 *M.* 125*pygmaeus* fourth instar nymphs in a 30 x 30 x 30 cm plastic cage (BugDorm-1 insect tents; 126MegaView Science Co., Ltd, Taichung, Taiwan). Nymphs were selected instead of adults to 127avoid defence induction by adult oviposition.[21] All nymphs were removed twenty-four hours 128after placing them on the plants. Ten replicates per treatment were considered. Each replicate 129consisted of a plastic cage 60 x 60 x 60 cm (BugDorm-2; MegaView Science Co., Ltd, Taichung, 130Taiwan), inside which 4 pepper plants of the corresponding treatment were introduced. A total 131of 40 plants were used per treatment. Cages were maintained in a climate chamber at the 132same environmental conditions as described above (Fig. 1).

133Once the experimental design was assembled, six pepper plants per treatment were removed 134to quantify the transcriptional response of the genes involved in defence responses. The apical 135region of the sweet pepper plants (the first 5 cm of the plant formed by the apical stem and 136young leaves) were cut and then ground in liquid nitrogen for RNA extraction. Next, the leaves 137of all remaining pepper plants for all three treatments (34 plants in each treatment) were 138mechanically inoculated with TSWV-PVR (Fig. 1). Inoculation was performed by rubbing a 139dilution of the following leaf extract inoculation solution (1:20, w:v) onto pepper leaves with a 140cotton bud and celite (diatomaceous earth).[43] The inoculation solution was obtained by 141grinding 250 mg of TSWV infected *N. benthamiana* leaves in a mortar in a mixture containing 5 142ml 0.05 M phosphate buffer, pH 7.2; 0.2% 2-mercaptoethanol; 1% polyvinylpyrrolidone 143(average molecular weight 10.000).[43]

144One plant per replicate and treatment was removed at 7, 14 and 21 days after inoculation 145(dpi), respectively, to quantify virus accumulation (n=10). As above, the apical region of each 146plant was excised and immediately immersed in liquid nitrogen for subsequent RNA extraction 147(Fig. 1). The remaining four plants per treatment were used to visually detect the virus 148symptoms.

149In addition, a negative control treatment for the virus inoculation was also performed (mock 150inoculation). For this, ten plastic cages were also arranged with the same conditions as 151described above. Four intact pepper plants were placed inside each cage. Samples were 152collected at 7, 14, and 21 days post inoculation (dpi) to check and verify the absence of any 153contamination.

154**2.3 Quantification of TSWV infection by RT-qPCR**

155Total RNAs from 0.1 g of fresh leaf tissue from TSWV-infected and non-infected sweet pepper 156plants were extracted using TRIzol (Invitrogen, CA, USA) as described above. RNA 157concentrations were measured in duplicate with the UV-Vis spectrophotometer nanodrop 1581000 (Thermo Scientific, Waltham, MA, USA) and adjusted to approximately 10 ng/µl to 159normalize the different extractions. Aliquots were stored at -80°C until use. RT-qPCR was 160carried out using the LightCycler® 480 System (Roche Molecular Systems, Inc., Switzerland), 161using 25 µL of a reaction mix that contained 12.5 µL LightCycler®480 Probe Master Mix 162(ROCHE), 4.38 µL of RNase-free water, 15 units (U) RT Multiscribe Reverse Transcriptase (Life 163Technologies, Rockville, MD, USA), 2 U of RNase inhibitor (Applied Biosystems, Foster City, CA, 164USA), 5 µM of primers 1M-F and 1M-R, 0.25 µM TaqMan®MGB probe and 5 µL of total RNA

165(∼10 ng µL−1). The Thermo cycling conditions consisted of reverse transcription at 48°C for 30
166min, incubation at 95°C for 10 min, 45 cycles of 95°C for 15 s and 60°C for 1 min.[44]

167

168**2.4 Plant gene expression**

169In a previous work, we showed how sweet pepper plants cv Lipari were activated defensively
170when exposed to adults of both *N. tenuis* and *M. pygmaeus*.[26] In this work, unlike the previous
171work, the cultivar Salmeron and fourth instar nymphs of both mirid species, were used.
172Therefore, to confirm that sweet pepper plants used in this experiment were defensively
173activated, plant gene expression analysis were performed. The relative expression of three
174marker genes, commonly used as indicators of JA, SA and ABA-related defences, was
175estimated:[26] (i) *PIN2* (wound-induced proteinase inhibitor II precursor) a marker gene for JA,
176(ii) *PR1* (basic PR-1 protein precursor) a marker gene for salicylic acid (SA), and (iii) *ASR1*
177(abscisic acid stress ripening protein 1) a marker gene for ABA signalling pathway. Total RNA
178(1.5 µg) was extracted using TRIzol (Invitrogen, CA, USA) according to the manufacturer's
179instructions.[23,26] The RNA was treated with a Turbo DNA-free DNase kit (Applied Biosystems)
180according to the manufacturer's protocol to eliminate any traces of genomic DNA. cDNA was
181later synthesized using a prime script™ RT reagent kit (perfect real time) (TAKARA Bio, CA,
182USA). Real-time PCR amplifications were performed with Maxima SYBR Green qPCR Master
183Mix (Thermo Fisher Scientific, MA, USA). PCR reactions were run in duplicate, in accordance
184with manufacturer recommendations. Quantitative PCR was carried out using the LightCycler®
185480 System (Roche Molecular Systems, Inc., Switzerland), under standard amplification
186conditions.[26] EF1 (elongation factor-1) was used as a standard control gene for normalization.

187**2.5 Statistical analysis**

188The relative expression of defence genes was analysed using one-way analysis of variance
189(ANOVA), followed by a comparison of means (Tukey's test) at $\alpha < 0.05$. Data from RNA
190quantification of TSWV isolates were log (concentration +1) transformed prior to analysis using
191ANOVA to differentiate between treatments for each of the three post inoculation days (7, 14
192and 21 dpi), followed by comparison of means (Tukey's test) at $\alpha < 0.05$.
193

194**3 RESULTS**

195**3.1 Plant defence by mirids restrict TSWV infection**

196TSWV titer increased with time in intact sweet pepper plants; it reached a maximum at 21 dpi. 197However, it remained low and almost constant with time in both *N. tenuis*- and *M. pygmaeus*- 198punctured plants (Fig. 2). No significant differences for TSWV titer were found at day 7 nor day 19914 post inoculation ($F_{2\text{-}29}$ = 1.018; P = 0.3748 and $F_{2\text{-}29}$ = 1.788; P = 0.1865, respectively). 200However, at 21 dpi TSWV titer was significantly higher in intact sweet pepper plants as 201opposed to that in plants punctured with both mirids ($F_{2\text{-}29}$ =36.25; $P < 0.0001$). At day 21 intact 202sweet pepper plants presented chlorotic flecking on the leaves, while these symptoms were 203not observed in either of the two mirid phytophagy exposure treatments (Fig. 3). No virus 204contamination was detected in the negative control plants.

205**3.2 Phytophagy of mirids alters JA pathway**

206Both *N. tenuis* and *M. pygmaeus* were found to influence the upregulation of JA pathways in 207the apical part of exposed sweet pepper plants when compared to intact plants. The relative 208expression of the corresponding defence genes, *PIN2* (JA pathway), significantly increased in 209mirid-punctured plants ($F_{2\text{-}17}$ = 7.251; P = 0.0063; Fig. 4a) compared to intact plants. Only *N.* 210*tenuis* was able to upregulate the gene *PR1* (SA pathway) ($F_{2\text{-}17}$ = 7.440; P = 0.0057; Fig. 4b). In 211contrast, the *ASR1* gene (ABA pathway) was not significantly upregulated in mirid-punctured 212plants when compared to intact sweet pepper plants ($F_{2\text{-}17}$ = 1.190; P = 0.3313; Fig. 4c).

213**4 DISCUSSION**

214Two predators used extensively in biological control programs have been found, for the first 215time, to limit the accumulation of one of the most important widespread plant viruses. The RT- 216qPCR revealed that three weeks after the mechanical inoculation of TSWV, the number of RNA 217copies in mirids-punctured plants were significantly lower in comparison to intact plants.

218The production of a number of plant hormones are directly related to the process of virus 219infection; especially the JA and SA pathways.[45] Some components of these pathways function 220as necessary signalling molecules that modulate responses to different stimuli.[31,47,48] Exogenous 221treatments with methyl jasmonate (MeJA) or JA have been shown to reduce incidence of viral 222infection. For example, tomato plants treated with MeJA were less infected with TYLCV.[37] The 223accumulation of *Cucumber mosaic virus* (CMV) in *Momordica charantia* L. (Cucurbitales: 224Cucurbitacea) was significantly suppressed when plants received an exogenous application of 225JA.[49] On the other hand, the infection process of CMV in *M. charantia* was almost unaffected 226by the exogenous application of SA, hence revealing how JA, not SA, inhibited virus infection.[49] 227The activation of the JA pathway is precisely what the phytophagy of the mirids in sweet

228pepper plants stimulates, which could be the explanation for the minor infection by TSWV 229shown in our experiments. Nevertheless, SA also plays an important role in plant defence 230against certain plant viruses. SA exogenous treatments have been reported to reduce the coat 231protein levels of *Tobacco Mosaic Virus* (TMV) and *Potato Virus X* (PVX) during their interactions 232with *N. benthamiana* plants.[46] Both MeJA and Methyl salicylate (MeSA) are required for the 233systemic resistance response of *N. benthamiana* plants against TMV.[50] The foliar application of 234MeJA at early stages of TMV infection followed by a later application of SA activated the 235strongest systemic defence response and upregulated the expression of defence related genes 236against TMV.[50] This is also consistent with another study which showed plant resistance to a 237broad spectrum of RNA viruses could be improved with the application of JA and SA.[47] Future 238identification of the roles of hormones in plant-virus interactions, how these hormones may 239interact with other biotic stressors, and cross talk among hormone pathways is still needed to 240fully understand the mechanisms by which plants resist infection.

241Sweet pepper plants defensively activated by mirids became less attractive to *F. occidentalis*;[26] 242the TSWV vector. Interestingly, TSWV infected plants are more attractive to the vector, *F.* 243*occidentalis*, than healthy plants; indeed thrips themselves develop faster on TSWV infected 244plants.[48] How mirid induced plant responses influence these TSWV-thrips interactions is not 245known, hence further research is needed to evaluate how mirid plant puncturing can limit viral 246infection of TSWV transmitted by thrips. However, not only the mutualistic interactions 247occurring between mirids and plants but also the interactions between vectors and viruses can 248affect the final response of the plant.[48] Additionally, environmental conditions, the presence of 249alternative food on the plant (pollen and nectar) and the presence of prey are crucial factors to 250be considered for further evaluation of plant mediated effects by mirids and its impact on the 251accumulation of TSWV in sweet pepper plants.

252Current control strategies for TSWV include elimination of infected plants, use of clean stock 253material, exclusion of thrips with greenhouse screens or air locks, and introduction of natural 254enemies.[49–51] As these control strategies are only partially successful, additional measures are 255needed to limit virus spread. Until recently, resistance to TSWV was obtained through the 256introgression of the two main resistance genes, *Sw5* and *Tsw*, in tomato and pepper, 257respectively. However, the emergence of resistant TSWV isolates (as the one used in our 258experiment)[43] has limited the durability of this strategy.[52,53] Therefore, breeding for durable 259TSWV resistance in plants is still a challenge upon which our results could provide new insight 260into plant viruses resistance. Probably, the activation of JA signalling pathway through genetic 261and chemical manipulation might improve plant defence against plant viruses.

262The possible implementation of strategies based on the above mentioned hypothesis has been 263verified in young plants; the size which is similar to those habitually transplanted from the 264nursery. Previously, nursery inoculation with mirids was proposed since the activation of 265defence responses reduces the infestation of important pests such as the whitefly, *B. tabaci* in 266sweet pepper and tomato plants[23,26] along with the two-spotted spider mite, *T. urticae* in 267tomato plants.[18] Our results support this strategy since the plants would also be protected 268from diseases such as the TSWV. In this sense, sweet pepper plants can be kept defensively 269activated (upregulated JA pathway) up to 14 days after a single 24 h exposure to mirids.[26] The 270same time period of defence activation was obtained also in *M. pygmaeus*-infested tomato 271plants.[20] In zones where transplanting occurs at the end of summer there is great insect vector 272pressure, thus protecting young plants from viral infection is crucial. Therefore, these results 273promote the use of biological control which could limit viral incidence at the beginning of the 274cultivation period. Further research must clarify the duration of defence activation under field 275conditions when a part of high vector pressure, the plant is subjected to multiple infestations 276which could work synergistically or antagonistically with each other to activate or block the 277metabolic pathways responsible for defences.[54]

278Herein included is a new perspective which had not been previously considered in the use of 279biological control programs with zoophytophagous predators; the ability of *N. tenuis* and *M.* 280*pygmaeus* to influence the reduction of TSWV infection incidence. New research lines should 281explore defence response activation against other diseases such as those caused by fungi and 282bacteria along with how pathogenic microbes may modulate mirid performance.[34,35] In 283conclusion, our results provide insights for future studies that can further strengthen pest and 284disease management programs based on these plant-predator-virus interactions.

285

286

287ACKNOWLEDGMENTS

288The research leading to these results was partially funded by the Spanish Ministry of Economy 289and Competitiveness MINECO (RTA2017-00073-00-00) and the Conselleria d'Agricultura, Pesca 290i Alimentació de la Generalitat Valenciana. MP-H was the recipient of a research fellowship 291from the INIA Spain (Subprogram DOC-INIACCAA). The authors profoundly thank Andrés 292Puchades (IVIA) for his technical assistance and Dr. Javier Calvo (KOPPERT BS, Spain) for

supplying the insects and the two anonymous reviewers for their valuable comments on a previous version of this manuscript.

REFERENCES

1. Heimpel GE and Mills NJ, Biological Control: Ecology and Applications, Cambridge University Press, UK (2017).

2. Barratt BIP, Moran VC, Bigler F, and van Lenteren JC, The status of biological control and recommendations for improving uptake for the future, BioControl **63**:155–167 (2018).

3. van Lenteren JC, Bolckmans K, Köhl J, Ravensberg WJ, and Urbaneja A, Biological control using invertebrates and microorganisms: plenty of new opportunities, BioControl **63**:39–59 (2018).

4. Alarcón-Roldán R, Control Biológico, apuesta de futuro, ed. by Gutiérrez-Escobar J, Horticultura intensiva de Almería, Anuario Agrícola FH, Almería, Spain, p. 88 (2016).

5. Egea FJ, Torrente RG, and Aguilar A, An efficient agro-industrial complex in Almería (Spain): Towards an integrated and sustainable bioeconomy model, N Biotechnol **40**:103–112 (2018).

6. Pérez-Hedo M, Suay R, Alonso M, Ruocco M, Giorgini M, Poncet C, *et al.*, Resilience and robustness of IPM in protected horticulture in the face of potential invasive pests, Crop Prot **97**:119–127 (2017).

7. Blom J, Pimiento bajo abrigo, ed. by Jacas JA and Urbaneja A, Control biológico de plagas agrícolas, Phytoma, Valencia, España, pp. 399–409 (2008).

8. Calvo FJ, Knapp M, van Houten YM, Hoogerbrugge H, and Belda JE, *Amblyseius swirskii*: What made this predatory mite such a successful biocontrol agent?, Exp Appl Acarol **65**:419–433 (2015).

9. Calvo FJ, Bolckmans K, and Belda JE, Biological control-based IPM in sweet pepper greenhouses using *Amblyseius swirskii* (Acari: Phytoseiidae), Biocontrol Sci Technol **22**:1398–1416 (2012).

10. Pérez-Hedo M and Urbaneja A, Prospects for predatory mirid bugs as biocontrol agents of aphids in sweet peppers, J Pest Sci (2004) **88**:65–73 (2015).

11. Messelink GJ, Bloemhard CMJ, Hoogerbrugge H, van Schelt J, Ingegno BL, and Tavella L, Evaluation of mirid predatory bugs and release strategy for aphid control in sweet pepper, J Appl Entomol **139**:333–341 (2015).

12. Bouagga S, Urbaneja A, and Pérez-Hedo M, Combined Use of Predatory Mirids With *Amblyseius swirskii* (Acari: Phytoseiidae) to Enhance Pest Management in Sweet Pepper, J Econ Entomol **111**:1112–1120 (2018).

13. Urbaneja A, González-Cabrera J, Arnó J, and Gabarra R, Prospects for the biological control of *Tuta absoluta* in tomatoes of the Mediterranean basin, Pest Manag Sci **68**:1215–1222 (2012).

14. Pérez-Hedo M and Urbaneja A, The zoophytophagous predator *Nesidiocoris tenuis*: a

332 successful but controversial biocontrol agent in tomato crops, ed. by Horowitz AR and
333 Ishaaya I, Advances in Insect Control and Resistance Management, Springer
334 International Publishing, Dordrecht, Netherlands, pp. 121–138 (2016).

33515 Desneux N, Wajnberg E, Wyckhuys KAG, Burgio G, Arpaia S, Narv?ez-Vasquez CA, *et al.*,
336 Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, geographic
337 expansion and prospects for biological control, J Pest Sci (2004) **83** (2010).

33816 Coll M and Guershon M, Omnivory in terrestrial arthropods: Mixing plant and prey
339 diets, Annu Rev Entomol Vol 57 **47**:267–297 (2002).

34017 Halitschke R, Hamilton JG, and Kessler A, Herbivore-specific elicitation of
341 photosynthesis by mirid bug salivary secretions in the wild tobacco *Nicotiana attenuata*,
342 New Phytol **191**:528–535 (2011).

34318 Pappas ML, Steppuhn A, and Broufas GD, The role of phytophagy by predators in
344 shaping plant interactions with their pests, Commun Integr Biol **9**:e1145320 (2016).

34519 Zhang NX, Messelink GJ, Alba JM, Schuurink RC, Kant MR, and Janssen A, Phytophagy of
346 omnivorous predator *Macrolophus pygmaeus* affects performance of herbivores
347 through induced plant defences, Oecologia **186**:101–113 (2018).

34820 Pappas ML, Steppuhn A, Geuss D, Topalidou N, Zografou A, Sabelis MW, *et al.*, Beyond
349 Predation: The Zoophytophagous Predator *Macrolophus pygmaeus* Induces Tomato
350 Resistance against Spider Mites, PLoS One **10** (2015).

35121 Naselli M, Urbaneja A, Siscaro G, Jaques JA, Zappalà L, Flors V, *et al.*, Stage-related
352 defense response induction in tomato plants by *Nesidiocoris tenuis*, Int J Mol Sci **17**
353 (2016).

35422 Pérez-Hedo M, Bouagga S, Jaques JA, Flors V, and Urbaneja A, Tomato plant responses
355 to feeding behavior of three zoophytophagous predators (Hemiptera: Miridae), Biol
356 Control **86**:46–51 (2015).

35723 Pérez-Hedo M, Urbaneja-Bernat P, Jaques JA, Flors V, and Urbaneja A, Defensive plant
358 responses induced by *Nesidiocoris tenuis* (Hemiptera: Miridae) on tomato plants, J Pest
359 Sci **88**:543–554 (2015).

36024 Pérez-Hedo M, Arias-Sanguino ÁM, and Urbaneja A, Induced Tomato Plant Resistance
361 Against *Tetranychus urticae* Triggered by the Phytophagy of *Nesidiocoris tenuis*, Front
362 Plant Sci **9,1419** (2018).

36325 Pérez-Hedo M, Rambla JL, Granell A, and Urbaneja A, Biological activity and specificity
364 of Miridae-induced plant volatiles, BioControl **63**:203–213 (2018).

36526 Bouagga S, Urbaneja A, Rambla JL, Flors V, Granell A, Jaques JA, *et al.*,
366 Zoophytophagous mirids provide pest control by inducing direct defences, antixenosis
367 and attraction to parasitoids in sweet pepper plants, Pest Manag Sci **74**:1286–1296
368 (2018).

36927 Zhang NX, van Wieringen D, Messelink GJ, and Janssen A, Herbivores avoid host plants
370 previously exposed to their omnivorous predator *Macrolophus pygmaeus*, J Pest Sci
371 (2004) (2018).

37228 Karban R and Baldwin IT, Induced Responses to Herbivory, Univ. of Chicago Press,

Chicago (1997).

29 Walling, The Myriad Plant Responses to Herbivores, J Plant Growth Regul **19**:195–216 (2000).

30 Heil M and Ton J, Long-distance signalling in plant defence, Trends Plant Sci **13**:264–272 (2008).

31 Ponzio C, Gols R, Pieterse CMJ, and Dicke M, Ecological and phytohormonal aspects of plant volatile emission in response to single and dual infestations with herbivores and phytopathogens, Funct Ecol **27**:587–598 (2013).

32 Bouagga S, Urbaneja A, Rambla JL, Granell A, and Pérez-Hedo M, *Orius laevigatus* strengthens its role as a biological control agent by inducing plant defenses, J Pest Sci (2004) **91**:55–64 (2018).

33 Pappas ML, Broekgaarden C, Broufas GD, Kant MR, Messelink GJ, Steppuhn A, *et al.*, Induced plant defences in biological control of arthropod pests: a double-edged sword, Pest Manag Sci (2017).

34 Garantonakis N, Pappas ML, Varikou K, Skiada V, Broufas GD, Kavroulakis N, *et al.*, Tomato Inoculation With the Endophytic Strain *Fusarium solani* K Results in Reduced Feeding Damage by the Zoophytophagous Predator *Nesidiocoris tenuis*, Front Ecol Evol **6**:126 (2018).

35 Pappas ML, Liapoura M, Papantoniou D, Avramidou M, Kavroulakis N, Weinhold A, *et al.*, The Beneficial Endophytic Fungus *Fusarium solani* Strain K Alters Tomato Responses Against Spider Mites to the Benefit of the Plant, Front Plant Sci **9:**1603 (2018).

36 Moerkens R, Berckmoes E, Van Damme V, Ortega-Parra N, Hanssen I, Wuytack M, *et al.*, High population densities of *Macrolophus pygmaeus* on tomato plants can cause economic fruit damage: interaction with Pepino mosaic virus ?, Pest Manag Sci **72**:1350–1358 (2016).

37 Hanssen IM, Peter van Esse H, Ballester A-R, Hogewoning SW, Parra NO, Paeleman A, *et al.*, Differential Tomato Transcriptomic Responses Induced by Pepino Mosaic Virus Isolates with Differential Aggressiveness, Plant Physiol **156**:301–318 (2011).

38 Escobar-Bravo R, Alba JM, Pons C, Granell A, Kant MR, Moriones E, *et al.*, A Jasmonate-Inducible Defense Trait Transferred from Wild into Cultivated Tomato Establishes Increased Whitefly Resistance and Reduced Viral Disease Incidence, Front Plant Sci **7**, **1732** (2016).

39 Scholthof K-BG, Adkins S, Czosnek H, Palukaitis P, Jacquot E, Hohn T, *et al.*, Top 10 plant viruses in molecular plant pathology, Mol Plant Pathol **12**:938–954 (2011).

40 Rybicki EP, A Top Ten list for economically important plant viruses, Arch Virol **160**:17–20 (2015).

41 Margaria P, Ciuffo M, and Turina M, Resistance breaking strain of Tomato spotted wilt virus (Tospovirus; Bunyaviridae) on resistant pepper cultivars in Almeria, Spain, Plant Pathol **53**:795–795 (2004).

42 Debreczeni DE, López C, Aramburu J, Darós JA, Soler S, Galipienso L, *et al.*, Complete sequence of three different biotypes of tomato spotted wilt virus (wild type, tomato

414 Sw-5 resistance-breaking and pepper Tsw resistance-breaking) from Spain, Arch Virol
415 **160**:2117–2123 (2015).

41643 Aramburu J, Galipienso L, Soler S, Rubio L, and López C, A severe symptom phenotype in
417 pepper cultivars carrying the Tsw resistance gene is caused by a mixed infection
418 between resistance-breaking and non-resistance-breaking isolates of Tomato spotted
419 wilt virus, Phytoparasitica **43**:597–605 (2015).

42044 Debreczeni DE, Ruiz-Ruiz S, Aramburu J, López C, Belliure B, Galipienso L, *et al.*,
421 Detection, discrimination and absolute quantitation of Tomato spotted wilt virus
422 isolates using real time RT-PCR with TaqMan®MGB probes, J Virol Methods **176**:32–37
423 (2011).

42445 Thaler JS, Humphrey PT, and Whiteman NK, Evolution of jasmonate and salicylate signal
425 crosstalk, Trends Plant Sci **17**:260–270, Elsevier Current Trends (2012).

42646 Lee WS, Fu SF, Verchot-Lubicz J, and Carr JP, Genetic modification of alternative
427 respiration in *Nicotiana benthamiana* affects basal and salicylic acid-induced resistance
428 to potato virus X, BMC Plant Biol (2011).

42947 Shang J, Xi D-H, Xu F, Wang S-D, Cao S, Xu M-Y, *et al.*, A broad-spectrum, efficient and
430 nontransgenic approach to control plant viruses by application of salicylic acid and
431 jasmonic acid, Planta **233**:299–308 (2011).

43248 Belliure B, Janssen A, Maris PC, Peters D, and Sabelis MW, Herbivore arthropods benefit
433 from vectoring plant viruses, Ecol Lett **8**:70–79 (2004).

43449 Maris PC, Joosten NN, Goldbach RW, and Peters D, Decreased preference and
435 reproduction, and increased mortality of *Frankliniella occidentalis* on thrips-resistant
436 pepper plants, Entomol Exp Appl **113**:149–155 (2004).

43750 Momol MT, Olson SM, Funderburk JE, Stavisky J, and Marois JJ, Integrated Management
438 of Tomato Spotted Wilt on Field-Grown Tomatoes, Plant Dis **88**:882–890 (2004).

43951 Awondo SN, Fonsah EG, Riley D, and Abney M, Effectiveness of Tomato-Spotted Wilt
440 Virus Management Tactics, J Econ Entomol **105**:943–948 (2012).

44152 Lopez C, Aramburu J, Galipienso L, Soler S, Nuez F, and Rubio L, Evolutionary analysis of
442 tomato Sw-5 resistance-breaking isolates of Tomato spotted wilt virus, J Gen Virol
443 **92**:210–215 (2011).

44453 Turina M, Kormelink R, and Resende RO, Resistance to Tospoviruses in Vegetable Crops:
445 Epidemiological and Molecular Aspects, Annu Rev Phytopathol **54**:347–371 (2016).

44654 Pappas ML, Broekgaarden C, Broufas GD, Kant MR, Messelink GJ, Steppuhn A, *et al.*,
447 Induced plant defences in biological control of arthropod pests: a double-edged sword,
448 Pest Manag Sci **73**:1780–1788 (2017).

449

450**Figure captions**

451**Figure 1.**

452Time line presenting pepper defence activation by either *N. tenuis* or *M. pygmaeus* fourth 453instar nymphs, gene expression analysis, TSWV inoculation on mirid-punctured plants and 454intact plants, and TSWV quantification using RT-qPCR at 7, 14 and 21 days post inoculation 455(dpi).

456**Figure 2.**

457Quantification of *Tomato spotted wilt virus* by real time quantitative RT-PCR at 7, 14 and 21 458days post inoculation (dpi) in sweet pepper plants with three treatments: I) intact plants, II) 459punctured by *N. tenuis*, and III) punctured by *M. pygmaeus*. Bars correspond to the mean 460TSWV RNA titer (Log of the number of TSWV RNA molecules) from ten plants (n = 10). 461Standard errors are represented by vertical segments. Bars with different letters are 462significantly different (ANOVA with Tukey's multiple comparison test; $P < 0.05$).
463

464**Figure 3.**

465Symptoms of TSWV in sweet pepper leaves at 21 days post inoculation (dpi), (a) intact plants, 466(b) *N. tenuis*-punctured plants and (c) *M. pygmaeus*-punctured plants.

467

468**Figure 4.**

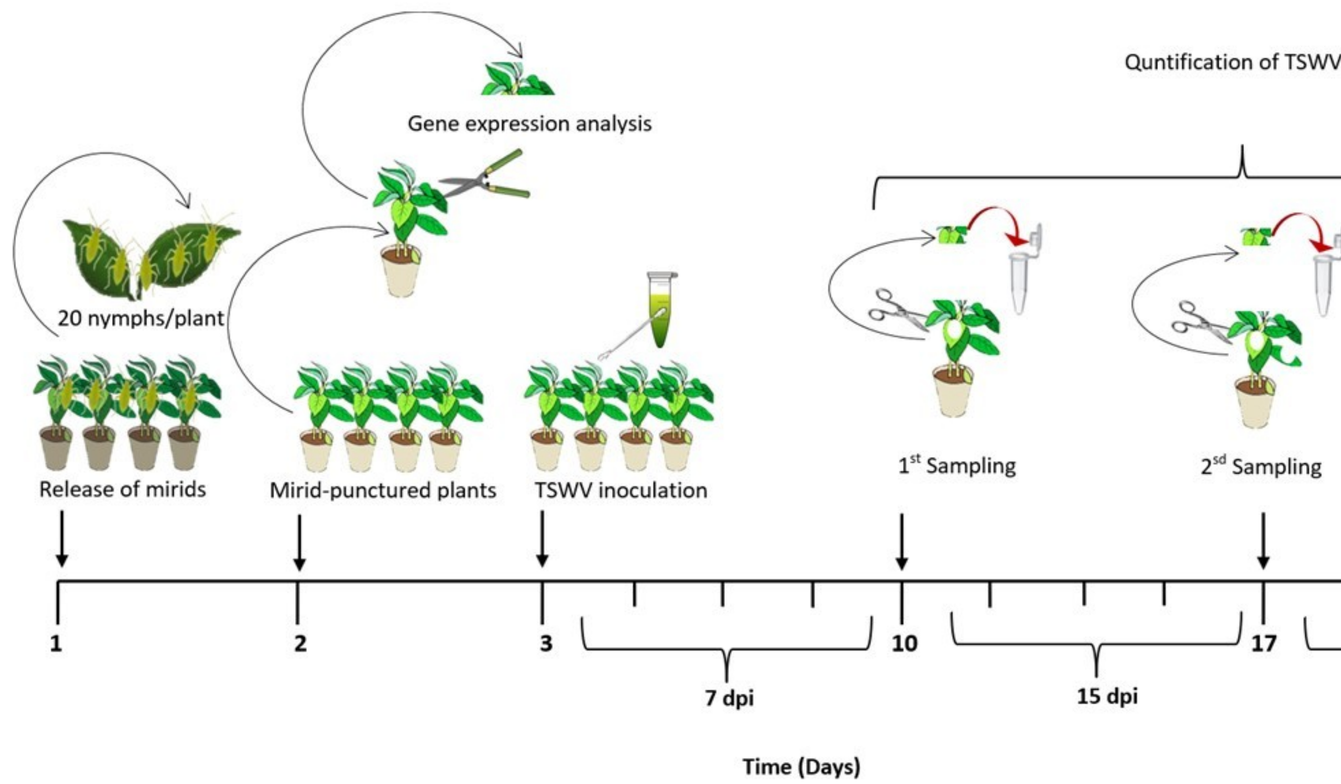
469Relative expression of defensive genes *PIN1* (Jasmonic acid pathway) (a), *PR1* (Salicylic acid 470pathway) (b) and *ASR1* (Abscisic acid pathway) (c), in the apical part of sweet pepper plants 471previously punctured by either *N. tenuis* or *M. pygmaeus* fourth instar nymphs, and in intact 472plants. Data are presented as the mean of six independent analyses of transcript expression 473relative to a housekeeping gene ± SE (n = 6). Bars with different letters are significantly 474different (ANOVA with Tukey's multiple comparison test; $P < 0.05$).

43

475**Figure 1.**

44

45

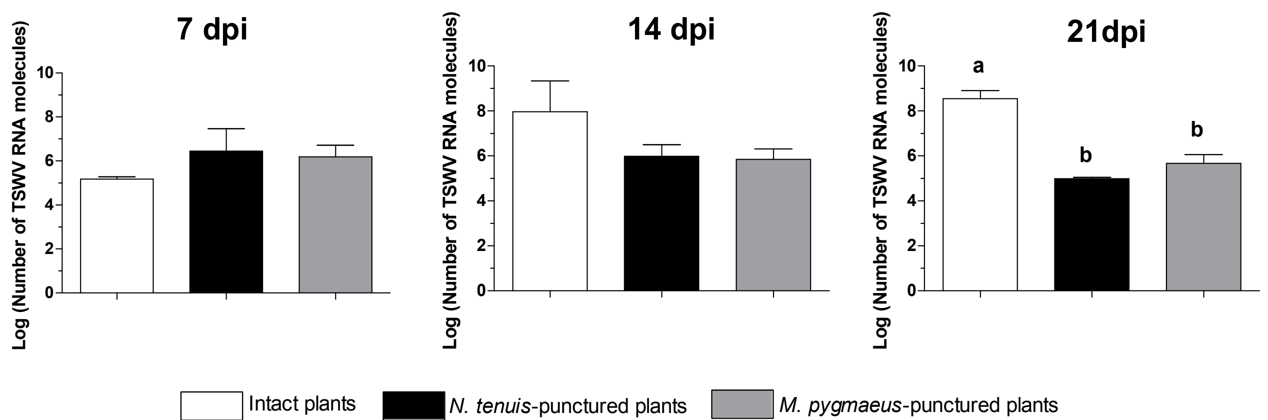


476

477**Figure 2.**

478

479



480

481

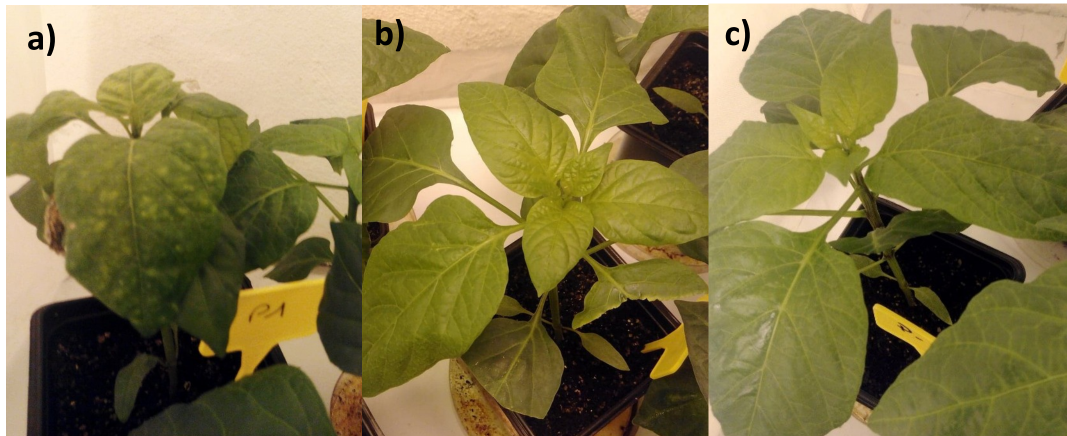
49

482

483 **Figure 3.**

484

485



486

487

488

489

490

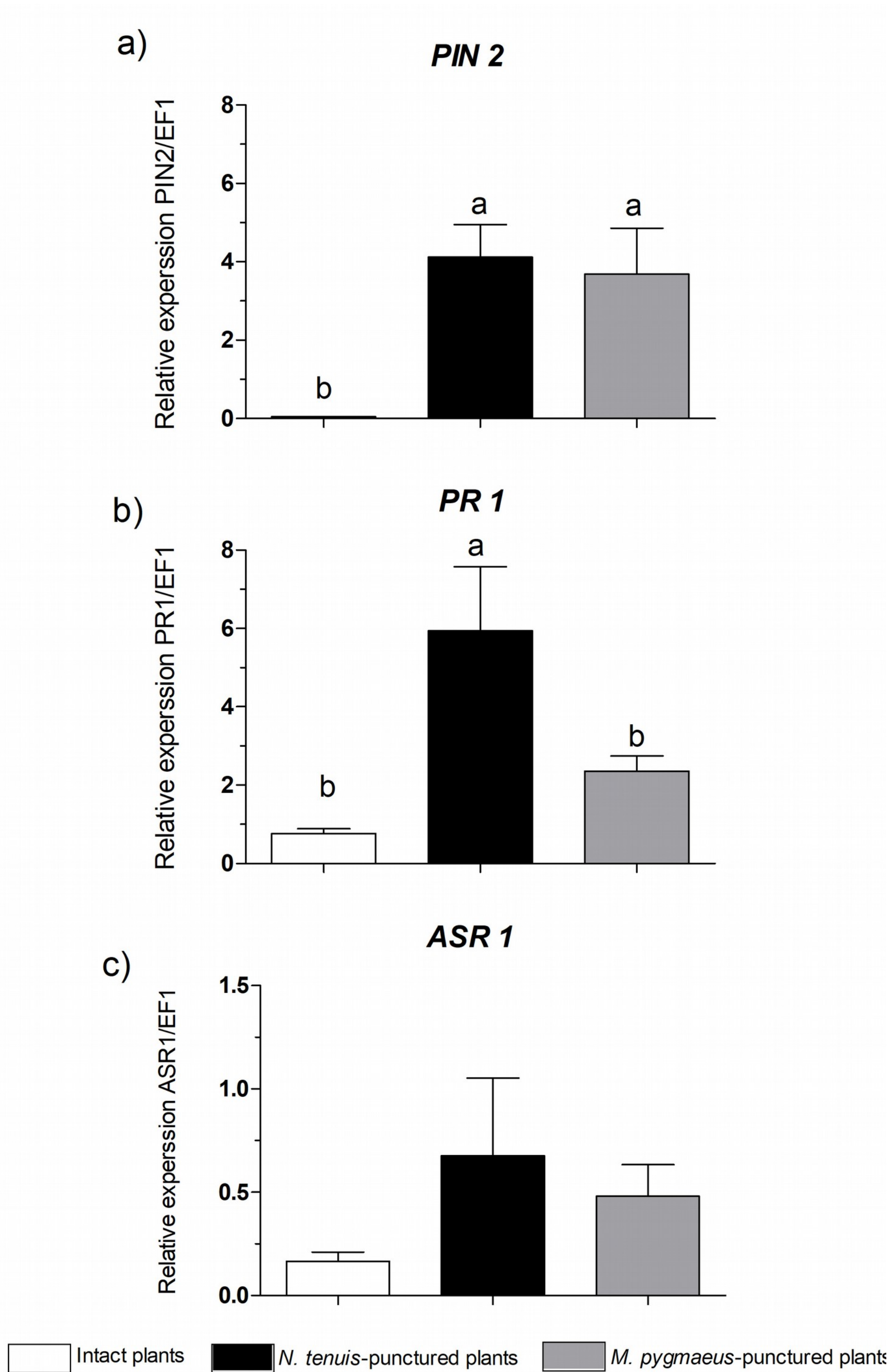
491

492

50

51

493 **Figure 4.**



494

495