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

3 Running title: Zoophytophagy restricts TSWV

4

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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

331 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),^{5,13,14} 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
67 obtained 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
69 predator 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
71 attracts *T. absoluta*. In contrast, the feeding activity of these three mirids results in an
72 attraction of *E. formosa*.^{18,20-24} Furthermore, the feeding behaviour of these zoophytophagous
73 predators has been verified to induce direct defences through the activation of the JA pathway
74 with an increase in protease inhibitor activity.^{20,23,24} Plants previously induced by mirids have
75 been 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:
77 Tetranychidae) in sweet pepper,^{19,26} along with *T. urticae* in tomato.^{18,20,24}

78 Regardless of the above mentioned studies, more investigations are needed to expand our
79 understanding of plant mediated effects on pest and disease management induced by
80 zoophytophagous predators. Interestingly, an important facet of research, previously not
81 addressed but already hypothesized, is the evaluation of plant mediated effects of
82 zoophytophagous predators on viral and microbial infection.^{18,33} Recently, beneficial microbes
83 have been observed to modulate the performance of zoophytophagous predators.^{34,35} The
84 colonization of tomato plants by the endophytic fungi *Fusarium solani* strain K reduces the
85 capability of *N. tenuis* to induce necrotic rings on tomato stems and leaves. The upregulation
86 of ethylene and JA pathways induced by *F. solani* give protection to tomato from *N. tenuis*
87 feeding.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
89 significantly enhanced when tomato plants are infected with PepMV.³⁶ This interaction was
90 attributed to the antagonistic effects of SA-mediated responses on JA-mediated responses,
91 since PepMV infection induces the SA defence pathway³⁷ meanwhile *M. pygmaeus* mainly
92 activates the JA pathway.^{20, 22} Additionally, tomato plants with high expression of methyl
93 jasmonate are less likely to be infected with the *Tomato yellow leaf curl virus* (TYLCV).³⁸
94 Therefore, we hypothesized that possible interaction can occur between induced defences by
95 zoophytophagous predator influence the incidence of plant viruses.

96 In 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
98 sweet pepper. TSWV is one of the most harmful plant viral pathogens, ranking second in the
99 list 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

1072 MATERIAL AND METHODS

1082.1 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.

1212.2 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).

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

144 One 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
146 plant 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
148 symptoms.

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

154 2.3 Quantification of TSWV infection by RT-qPCR

155 Total RNAs from 0.1 g of fresh leaf tissue from TSWV-infected and non-infected sweet pepper
156 plants were extracted using TRIzol (Invitrogen, CA, USA) as described above. RNA
157 concentrations were measured in duplicate with the UV-Vis spectrophotometer nanodrop
158 1000 (Thermo Scientific, Waltham, MA, USA) and adjusted to approximately 10 ng/μl to
159 normalize the different extractions. Aliquots were stored at -80°C until use. RT-qPCR was
160 carried out using the LightCycler® 480 System (Roche Molecular Systems, Inc., Switzerland),
161 using 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
163 Technologies, Rockville, MD, USA), 2 U of RNase inhibitor (Applied Biosystems, Foster City, CA,
164 USA), 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
166 min, incubation at 95°C for 10 min, 45 cycles of 95°C for 15 s and 60°C for 1 min.⁴⁴

167

1682.4 Plant gene expression

169 In a previous work, we showed how sweet pepper plants cv Lipari were activated defensively
170 when exposed to adults of both *N. tenuis* and *M. pygmaeus*.²⁶ In this work, unlike the previous
171 work, the cultivar Salmeron and fourth instar nymphs of both mirid species, were used.
172 Therefore, to confirm that sweet pepper plants used in this experiment were defensively
173 activated, plant gene expression analysis were performed. The relative expression of three
174 marker genes, commonly used as indicators of JA, SA and ABA-related defences, was
175 estimated: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
179 instructions.^{23,26} The RNA was treated with a Turbo DNA-free DNase kit (Applied Biosystems)
180 according to the manufacturer's protocol to eliminate any traces of genomic DNA. cDNA was
181 later synthesized using a prime script™ RT reagent kit (perfect real time) (TAKARA Bio, CA,
182 USA). Real-time PCR amplifications were performed with Maxima SYBR Green qPCR Master
183 Mix (Thermo Fisher Scientific, MA, USA). PCR reactions were run in duplicate, in accordance
184 with manufacturer recommendations. Quantitative PCR was carried out using the LightCycler®
185 480 System (Roche Molecular Systems, Inc., Switzerland), under standard amplification
186 conditions.²⁶ EF1 (elongation factor-1) was used as a standard control gene for normalization.

1872.5 Statistical analysis

188 The 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
190 quantification of TSWV isolates were log (concentration +1) transformed prior to analysis using
191 ANOVA to differentiate between treatments for each of the three post inoculation days (7, 14
192 and 21 dpi), followed by comparison of means (Tukey's test) at $\alpha < 0.05$.

193

1943 RESULTS

1953.1 Plant defence by mirids restrict TSWV infection

196 TSWV titer increased with time in intact sweet pepper plants; it reached a maximum at 21 dpi.
 197 However, it remained low and almost constant with time in both *N. tenuis*- and *M. pygmaeus*-
 198 punctured plants (Fig. 2). No significant differences for TSWV titer were found at day 7 nor day
 199 14 post inoculation ($F_{2-29} = 1.018$; $P = 0.3748$ and $F_{2-29} = 1.788$; $P = 0.1865$, respectively).
 200 However, at 21 dpi TSWV titer was significantly higher in intact sweet pepper plants as
 201 opposed to that in plants punctured with both mirids ($F_{2-29} = 36.25$; $P < 0.0001$). At day 21 intact
 202 sweet pepper plants presented chlorotic flecking on the leaves, while these symptoms were
 203 not observed in either of the two mirid phytophagy exposure treatments (Fig. 3). No virus
 204 contamination was detected in the negative control plants.

2053.2 Phytophagy of mirids alters JA pathway

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

2134 DISCUSSION

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

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

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296REFERENCES

- 2971 Heimpel GE and Mills NJ, *Biological Control: Ecology and Applications*, Cambridge
298 University Press, UK (2017).
- 2992 Barratt BIP, Moran VC, Bigler F, and van Lenteren JC, The status of biological control and
300 recommendations for improving uptake for the future, *BioControl* **63**:155–167 (2018).
- 3013 van Lenteren JC, Bolckmans K, Köhl J, Ravensberg WJ, and Urbaneja A, Biological control
302 using invertebrates and microorganisms: plenty of new opportunities, *BioControl*
303 **63**:39–59 (2018).
- 3044 Alarcón-Roldán R, *Control Biológico, apuesta de futuro*, ed. by Gutiérrez-Escobar J,
305 Horticultura intensiva de Almería, Anuario Agrícola FH, Almería, Spain, p. 88 (2016).
- 3065 Egea FJ, Torrente RG, and Aguilar A, An efficient agro-industrial complex in Almería
307 (Spain): Towards an integrated and sustainable bioeconomy model, *N Biotechnol*
308 **40**:103–112 (2018).
- 3096 Pérez-Hedo M, Suay R, Alonso M, Ruocco M, Giorgini M, Poncet C, *et al.*, Resilience and
310 robustness of IPM in protected horticulture in the face of potential invasive pests, *Crop*
311 *Prot* **97**:119–127 (2017).
- 3127 Blom J, *Pimiento bajo abrigo*, ed. by Jacas JA and Urbaneja A, *Control biológico de*
313 *plagas agrícolas*, Phytoma, Valencia, España, pp. 399–409 (2008).
- 3148 Calvo FJ, Knapp M, van Houten YM, Hoogerbrugge H, and Belda JE, *Amblyseius swirskii*:
315 What made this predatory mite such a successful biocontrol agent?, *Exp Appl Acarol*
316 **65**:419–433 (2015).
- 3179 Calvo FJ, Bolckmans K, and Belda JE, Biological control-based IPM in sweet pepper
318 greenhouses using *Amblyseius swirskii* (Acari: Phytoseiidae), *Biocontrol Sci Technol*
319 **22**:1398–1416 (2012).
- 32010 Pérez-Hedo M and Urbaneja A, Prospects for predatory mirid bugs as biocontrol agents
321 of aphids in sweet peppers, *J Pest Sci* (2004) **88**:65–73 (2015).
- 32211 Messelink GJ, Bloemhard CMJ, Hoogerbrugge H, van Schelt J, Ingegno BL, and Tavella L,
323 Evaluation of mirid predatory bugs and release strategy for aphid control in sweet
324 pepper, *J Appl Entomol* **139**:333–341 (2015).
- 32512 Bouagga S, Urbaneja A, and Pérez-Hedo M, Combined Use of Predatory Mirids With
326 *Amblyseius swirskii* (Acari: Phytoseiidae) to Enhance Pest Management in Sweet
327 Pepper, *J Econ Entomol* **111**:1112–1120 (2018).
- 32813 Urbaneja A, González-Cabrera J, Arnó J, and Gabarra R, Prospects for the biological
329 control of *Tuta absoluta* in tomatoes of the Mediterranean basin, *Pest Manag Sci*
330 **68**:1215–1222 (2012).
- 33114 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,

- 373 Chicago (1997).
- 37429 Walling, The Myriad Plant Responses to Herbivores, *J Plant Growth Regul* **19**:195–216
375 (2000).
- 37630 Heil M and Ton J, Long-distance signalling in plant defence, *Trends Plant Sci* **13**:264–272
377 (2008).
- 37831 Ponzio C, Gols R, Pieterse CMJ, and Dicke M, Ecological and phytohormonal aspects of
379 plant volatile emission in response to single and dual infestations with herbivores and
380 phytopathogens, *Funct Ecol* **27**:587–598 (2013).
- 38132 Bouagga S, Urbaneja A, Rambla JL, Granell A, and Pérez-Hedo M, *Orius laevigatus*
382 strengthens its role as a biological control agent by inducing plant defenses, *J Pest Sci*
383 (2004) **91**:55–64 (2018).
- 38433 Pappas ML, Broekgaarden C, Broufas GD, Kant MR, Messelink GJ, Steppuhn A, *et al.*,
385 Induced plant defences in biological control of arthropod pests: a double-edged sword,
386 *Pest Manag Sci* (2017).
- 38734 Garantonakis N, Pappas ML, Varikou K, Skiada V, Broufas GD, Kavroulakis N, *et al.*,
388 Tomato Inoculation With the Endophytic Strain *Fusarium solani* K Results in Reduced
389 Feeding Damage by the Zoophytophagous Predator *Nesidiocoris tenuis*, *Front Ecol Evol*
390 **6**:126 (2018).
- 39135 Pappas ML, Liapoura M, Papantoniou D, Avramidou M, Kavroulakis N, Weinhold A, *et al.*,
392 The Beneficial Endophytic Fungus *Fusarium solani* Strain K Alters Tomato Responses
393 Against Spider Mites to the Benefit of the Plant, *Front Plant Sci* **9**:1603 (2018).
- 39436 Moerkens R, Berckmoes E, Van Damme V, Ortega-Parra N, Hanssen I, Wuytack M, *et al.*,
395 High population densities of *Macrolophus pygmaeus* on tomato plants can cause
396 economic fruit damage: interaction with Pepino mosaic virus ?, *Pest Manag Sci*
397 **72**:1350–1358 (2016).
- 39837 Hanssen IM, Peter van Esse H, Ballester A-R, Hogewoning SW, Parra NO, Paeleman A, *et al.*,
399 Differential Tomato Transcriptomic Responses Induced by Pepino Mosaic Virus
400 Isolates with Differential Aggressiveness, *Plant Physiol* **156**:301–318 (2011).
- 40138 Escobar-Bravo R, Alba JM, Pons C, Granell A, Kant MR, Moriones E, *et al.*, A Jasmonate-
402 Inducible Defense Trait Transferred from Wild into Cultivated Tomato Establishes
403 Increased Whitefly Resistance and Reduced Viral Disease Incidence, *Front Plant Sci* **7**,
404 **1732** (2016).
- 40539 Scholthof K-BG, Adkins S, Czosnek H, Palukaitis P, Jacquot E, Hohn T, *et al.*, Top 10 plant
406 viruses in molecular plant pathology, *Mol Plant Pathol* **12**:938–954 (2011).
- 40740 Rybicki EP, A Top Ten list for economically important plant viruses, *Arch Virol* **160**:17–20
408 (2015).
- 40941 Margaria P, Ciuffo M, and Turina M, Resistance breaking strain of Tomato spotted wilt
410 virus (Tospovirus; Bunyaviridae) on resistant pepper cultivars in Almeria, Spain, *Plant*
411 *Pathol* **53**:795–795 (2004).
- 41242 Debreczeni DE, López C, Aramburu J, Darós JA, Soler S, Galipienso L, *et al.*, Complete
413 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 Tosspoviruses 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

450Figure captions**451Figure 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).

456Figure 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

464Figure 3.

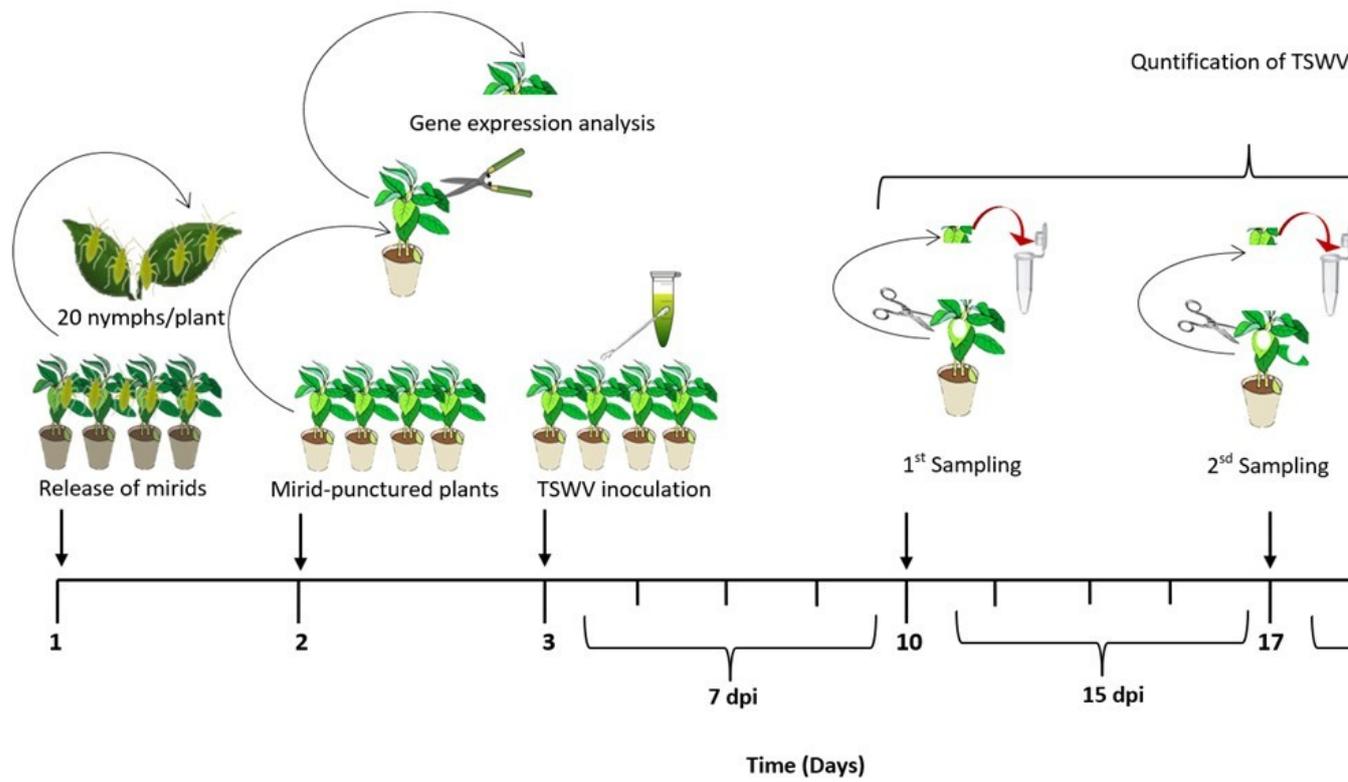
465Symptoms of TSWV in sweet pepper leaves at 21 days post inoculation (dpi), (a) intact plants,
466(b) *N. tenuis*-punctured plants and (c) *M. pygmaeus*-punctured plants.

467

468Figure 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 \pm SE ($n = 6$). Bars with different letters are significantly
474different (ANOVA with Tukey's multiple comparison test; $P < 0.05$).

475**Figure 1.**

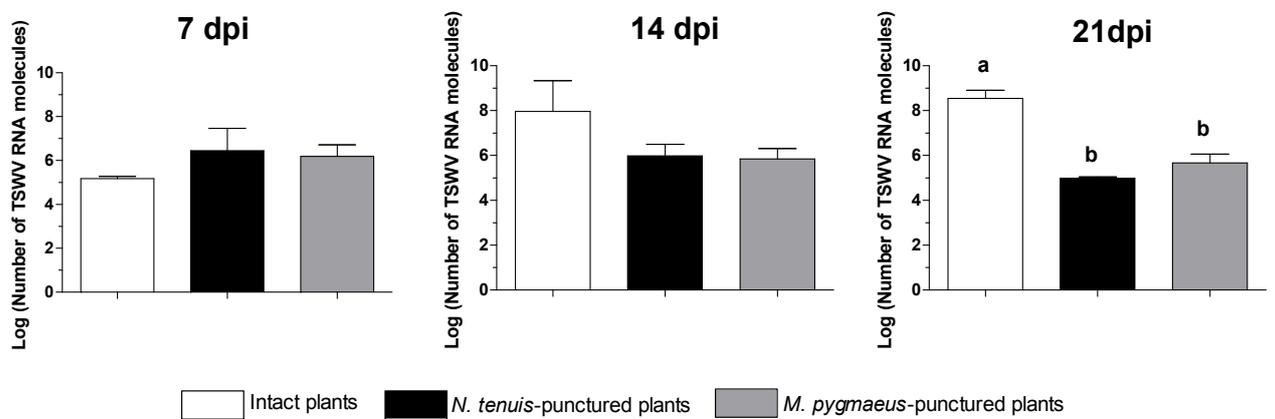


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477 Figure 2.

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483 **Figure 3.**

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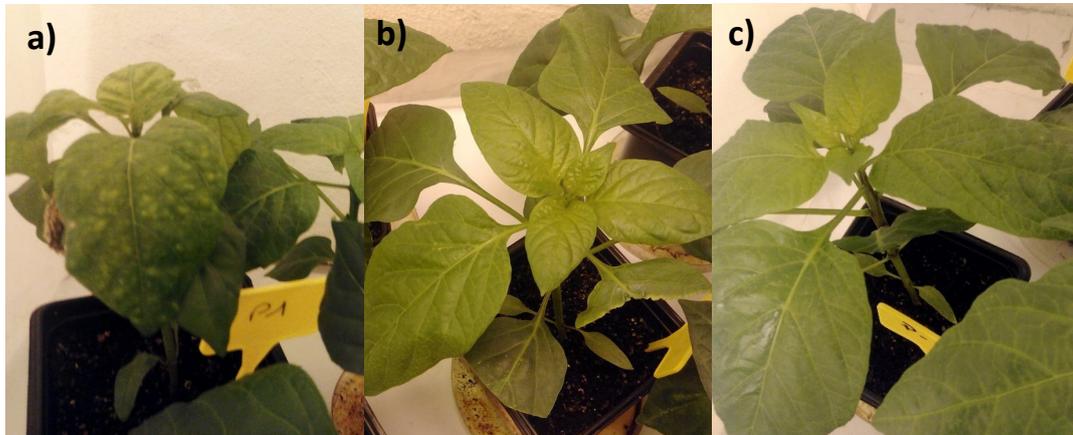
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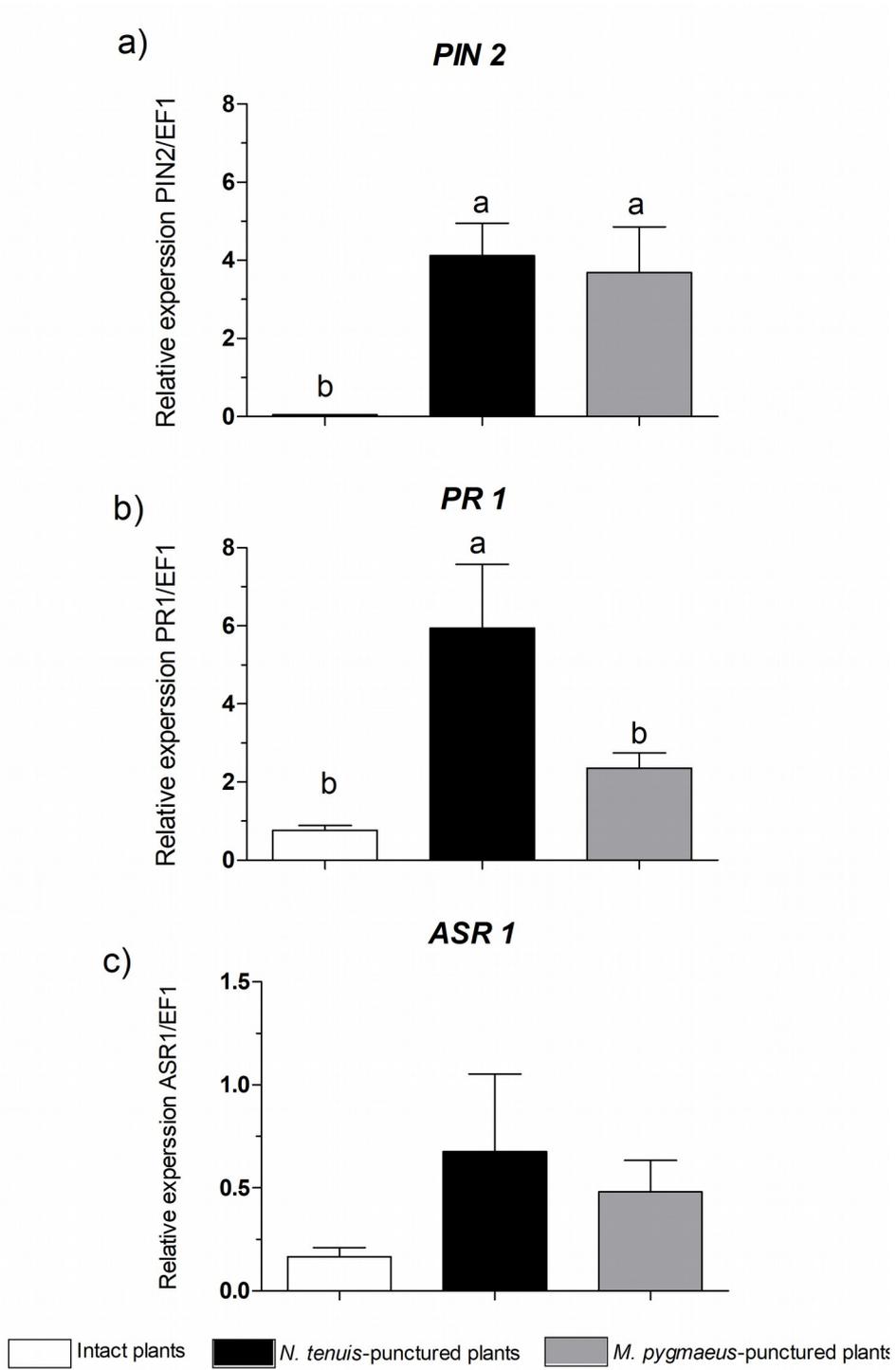
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493 **Figure 4.**



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