

1 **ExoRNAi exposes contrasting roles for sugar exudation in host-finding by** 2 **plant pathogens**

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11

12 **Abstract**

13 Plant parasitic nematodes (PPN) locate host plants by following concentration gradients of
14 root exudate chemicals in the soil. We present a simple method for RNAi-induced
15 knockdown of genes in tomato seedling roots, facilitating the study of root exudate
16 composition, and PPN responses. Knockdown of sugar transporter genes, *stp1* and *stp2* in
17 tomato seedlings triggers corresponding reductions of glucose and fructose, but not xylose,
18 in collected root exudate. This corresponds directly with reduced infectivity and stylet
19 thrusting of the promiscuous PPN *Meloidogyne incognita*, however we observe no impact
20 on the infectivity or stylet thrusting of the selective Solanaceae PPN *Globodera pallida*. This
21 approach can underpin future efforts to understand the early stages of plant-pathogen
22 interactions in tomato, and potentially other crop plants.

23

24 **Keywords:** exoRNAi, *Meloidogyne incognita*, *Globodera pallida*, Sugar, Root exudate

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26 RNA interference (RNAi) is widely used for the analysis of plant gene function, primarily
27 through the transgenic production of dsRNA constructs *in planta*, and secondarily through
28 Virus-Induced Gene Silencing (VIGS) (Watson *et al.*, 2005). Publications from the Wolniak
29 lab have shown that exogenous dsRNA can silence genes of the water fern *Marsilea vestita*
30 (Klink and Wolniak, 2001), and crude lysate from *Escherichia coli* expressing virus-specific
31 dsRNA have also been used to protect plants from viral pathology (Tenllado *et al.*, 2003).
32 Here we present a similar approach to triggering RNAi in tomato seedlings, which we term
33 exogenous (exo)RNAi. In this approach, aqueous dsRNA is delivered exogenously to tomato
34 seedlings.

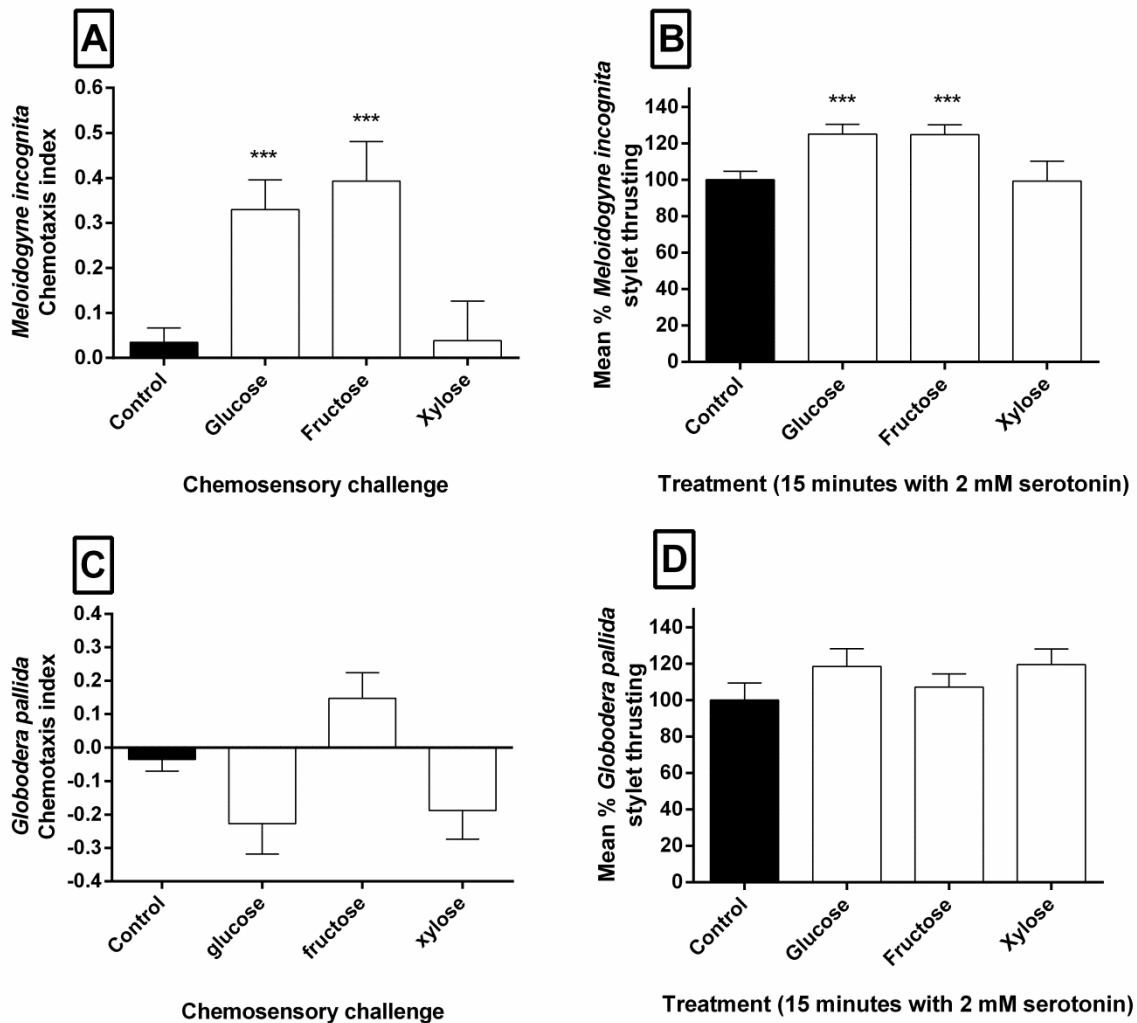
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36 Plant root exudate comprises a complex mixture of compounds including volatile
37 and soluble chemicals which may derive from intact or damaged root cells, or sloughed-off
38 root border cells (Dakora and Phillips, 2002). It has been estimated that 11% of
39 photosynthetically-assimilated carbon is released as root exudate (Jones *et al.*, 2009). The
40 monosaccharides glucose, fructose and xylose represent the major sugar component of
41 tomato root exudates (Kamilova *et al.*, 2006). Plant parasitic nematodes (PPNs) are
responsible for an estimated 12.3% loss in crop production globally each year (Sasser and

42 Freckman, 1987), and are attracted to host plants by components of plant root exudate.
43 Here we assess the chemosensory response of the root knot nematode, *Meloidogyne*
44 *incognita* (a promiscuous pathogen of flowering plants), and the potato cyst nematode,
45 *Globodera pallida* (a selective pathogen of Solanaceae plants) to each of the three major
46 monosaccharide sugars of tomato plant root exudate, and the efficacy of exoRNAi against
47 *stp1* and *stp2*, known transporters of monosaccharide sugars in tomato (Gear *et al.*, 2000).

48 *Meloidogyne incognita* infective stage juveniles were attracted to glucose (CI: 0.33
49 ± 0.07 ; $P < 0.001$) and fructose (CI: 0.39 ± 0.09 ; $P < 0.001$), but not xylose (CI: 0.04 ± 0.09 ;
50 $P > 0.05$) as compared to control treated worms (Fig 1A). Glucose (125.1% ± 5.5 ; $P < 0.001$)
51 and fructose (124.8% ± 5.4 ; $P < 0.001$) also triggered an elevated level of serotonin-triggered
52 stylet thrusting in treated juveniles; xylose failed to trigger any significant response (99.36%
53 ± 10.87 ; $P > 0.05$) when compared to control treatments (Fig 1B). *Globodera pallida* infective
54 stage juveniles were mildly repelled by glucose (CI: -0.23 ± 0.09 ; $P > 0.05$), and did not
55 respond to fructose (CI: 0.15 ± 0.08 ; $P > 0.05$), or xylose (CI: -0.19 ± 0.09 ; $P > 0.05$) as compared
56 to control treated worms (Fig 1C). Glucose (118.6% ± 9.7 ; $P > 0.05$), fructose (107.2% ± 7.3 ;
57 $P > 0.05$), or xylose (119.6% ± 8.6 ; $P > 0.05$) had no significant impact on the frequency of
58 serotonin-triggered stylet thrusting in *G. pallida* infective juveniles when compared to
59 control treatments (Fig 1D).

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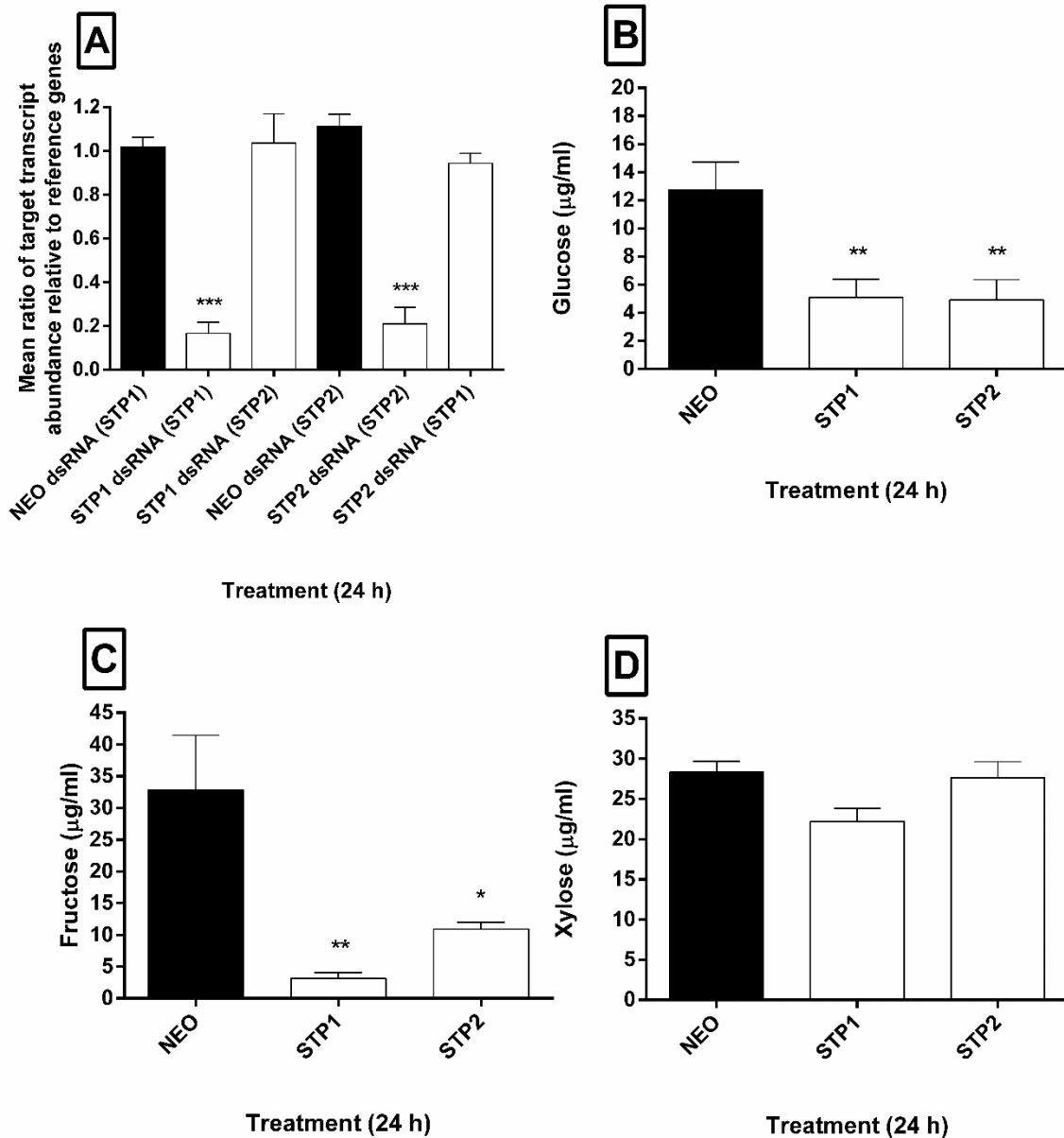
61
 62 **Figure 1. Glucose and fructose stimulate different chemotaxis and stylet thrusting responses in *M. incognita***
 63 **and *G. pallida*.** (A) Chemosensory response (chemotaxis index) of *M. incognita* infective juveniles to
 64 glucose, fructose, xylose and control (water) assay challenge. Each data point represents the mean (\pm SEM) of
 65 10 assays of 100 infective juveniles each. (B) Mean percentage (\pm SEM) stylet thrusting of glucose, fructose and
 66 xylose treated *M. incognita* infective stage juveniles ($n=100$) relative to control (2 mM serotonin in water). (C)
 67 Chemosensory response of *G. pallida* infective juveniles to glucose, fructose, xylose and control (water) assay
 68 challenge. (D) Mean percentage (\pm SEM) stylet thrusting of glucose, fructose and xylose treated *G. pallida*
 69 infective stage juveniles ($n=100$) relative to control (2 mM serotonin in water). An agar slurry (0.25% agar, pH
 70 7) was used to flood Petri dishes for chemosensory assays. Specifically, 3 ml of agar slurry was poured to
 71 provide the medium through which the infective stage juveniles could move. Sugar plugs were prepared by
 72 dissolving 50 mM of the relevant sugar (glucose / fructose / xylose) in 0.25% agar and allowed to set. Plugs
 73 were picked with a Pasteur pipette which had been cut half way down the pipette barrel, and placed onto one
 74 side of a Petri dish, with a negative plug (water instead of 50 mM sugar) on the other. 100 *M. incognita* or *G.*
 75 *pallida* infective stage juveniles were suspended in 5 μ l of water, and spotted onto the centre point of each
 76 dish. A Petri dish lid was marked with two parallel vertical lines 0.5 cm either side of the centre point forming
 77 a 1 cm 'dead zone' that ran vertically along the lid. Assay plates were set onto the lid for scoring of nematode
 78 positions following a two hour assay period. Only nematodes outside the dead zone were counted. The
 79 distribution of *M. incognita* infective stage juveniles was used to generate the chemotaxis index (Hart, 2006)
 80 for each assay plate which formed one replicate. For the stylet thrusting assay, 100 *M. incognita* or *G. pallida*
 81 infective stage juveniles were suspended in 20 μ l of water (autoclaved and adjusted to pH 7) containing 2 mM

82 serotonin and 50 mM of glucose, fructose or xylose (Sigma-Aldrich). Worms were incubated in this solution for
83 15 minutes, pipetted onto a glass slide with a coverslip, and stylet thrusts were counted in randomly selected
84 infective stage juveniles for 1 minute each. Control treatments were expressed as a percentage, including
85 technical variation, and experimental treatments were normalised to control percentages across individual
86 experiments and days. Chemosensory and stylet thrusting results were analysed by One-way ANOVA and
87 Tukey's Honestly Significant Difference test using Graphpad Prism 6. Probabilities of less than 5% ($P < 0.05$)
88 were deemed statistically significant *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

89

90 Treatment of tomato seedlings with *stp1* dsRNA triggered a significant reduction in *stp1*
91 transcript abundance (0.17 ± 0.05 ; $P < 0.001$), yet had no impact on *stp2* abundance (1.037
92 ± 0.13 ; $P > 0.05$) relative to neomycin transferase (*neo*) dsRNA treatment. Likewise, *stp2*
93 dsRNA induced significant reductions in *stp2* transcript abundance (0.21 ± 0.06 ; $P < 0.001$),
94 but not *stp1* (0.94 ± 0.05 ; $P > 0.05$) relative to *neo* dsRNA treatments (Fig 2A). Corresponding
95 reductions in glucose and fructose exudate concentration were observed for both *stp1* (5.10
96 $\mu\text{g/ml} \pm 1.31$; $P < 0.01$ and $3.14 \mu\text{g/ml} \pm 0.92$; $P < 0.01$, respectively) and *stp2* ($4.90 \mu\text{g/ml} \pm 1.45$;
97 $P < 0.01$ and $10.90 \mu\text{g/ml} \pm 1.07$; $P < 0.05$, respectively) dsRNA treated seedlings. No significant
98 changes in xylose exudate concentration were observed across treatment groups (Fig 2B-D).

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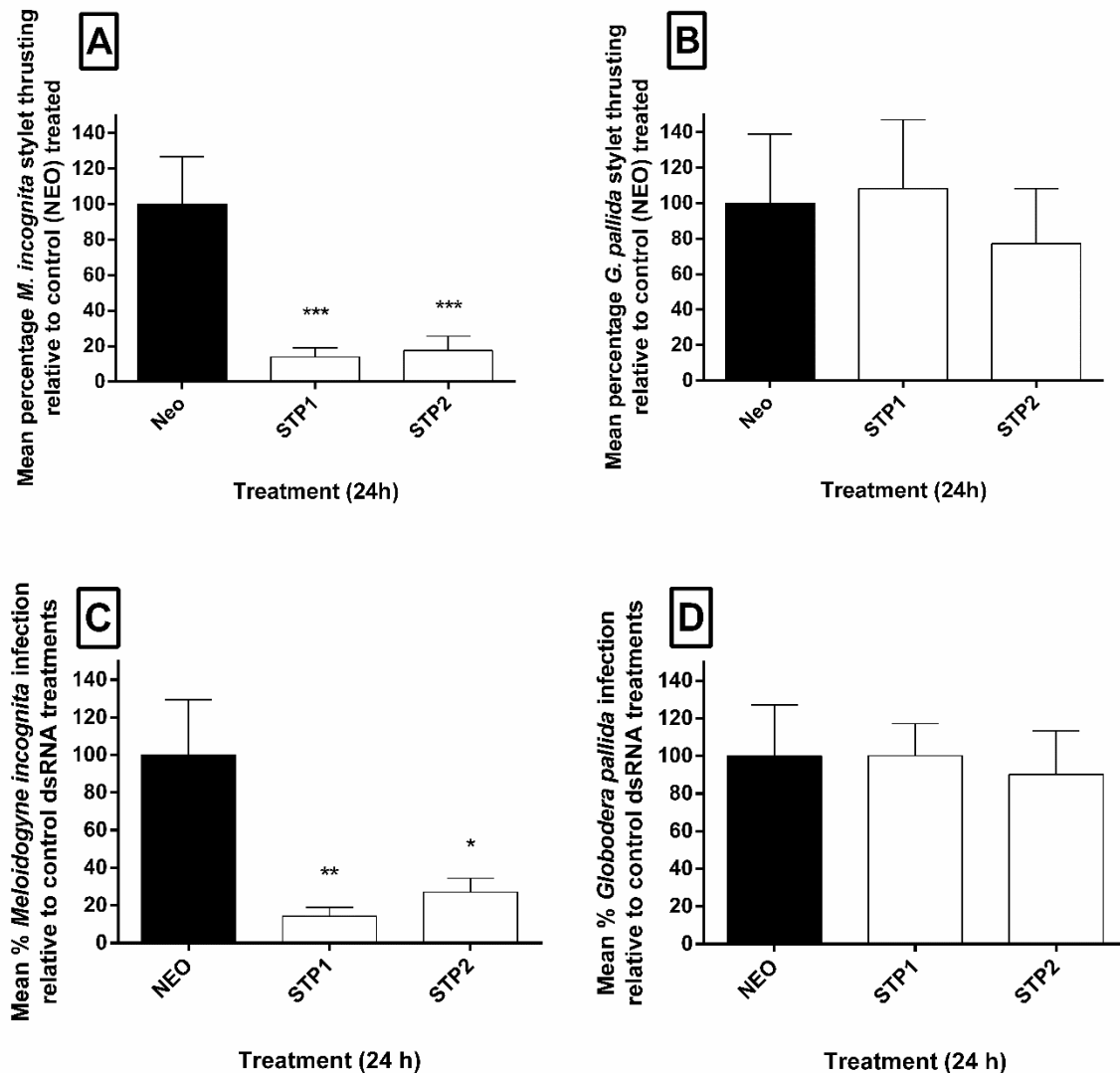
100
 101 **Figure 2. ExoRNAi induces target-specific knockdown of tomato sugar transporter genes; alters root exudate**
 102 **composition. (A)** Mean ratio of target transcript (in parentheses) abundance relative to three endogenous
 103 reference genes. Each data point represents the mean (\pm SEM) of three replicates of five seedlings each.
 104 Forward and reverse primers including 5' T7-recognition sites were used to generate specific amplicons for
 105 dsRNA synthesis to EST-supported fragments of *stp1* (Solyc02g079220.2), and *stp2* (Solyc09g075820.2)
 106 (Reuscher *et al.*, 2014). Primers for dsRNA synthesis were as follows: (Neomycin Phosphotransferase, *neoF* 5'
 107 -GGTGGAGAGGCTATTCGGCT-3', *neoFT7* 5'-TAATACGACTCACTATAGGGGTGGAGAGGCTATTCGGCT -3', *neoR* 5'-
 108 CCTCCCCTTCAGTGACAA-3', *neoRT7* 5'-TAATACGACTCACTATAGGCCTCCCCTTCAGTGACAA -3'); (Sugar
 109 Transporter 1, *stp1F* 5'- CTGCTGTGATCACTGGTGA-3', *stp1FT7* 5'-
 110 TAATACGACTCACTATAGGCTGCTGTGATCACTGGTGA -3', *stp1R* 5'-ATCCCCTGGAGTTCCATTT-3', *stp1RT7* 5'-
 111 TAATACGACTCACTATAGGATCCCCTGGAGTTCCATTT -3'); (Sugar Transporter 2, *stp2F* 5'-
 112 ACGTTCTCTCCACCGTTGTC -3', *stp2FT7* 5'-TAATACGACTCACTATAGGACGTTCTCTCCACCGTTGTC -3', *stp2R* 5'-
 113 CTACGAAGATTCCCCAACCA-3', *stp2RT7* 5'-TAATACGACTCACTATAGGCTACGAAGATTCCCCAACCA-3');
 114 PCR products were assessed by gel electrophoresis, and cleaned using the Chargeswitch PCR clean-up kit (Life
 115 Technologies). dsRNA was synthesised using the T7 RiboMAX™ Express Large Scale RNA Production System

116 (Promega), and quantified by Nanodrop 1000 spectrophotometer. Tomato cv. Moneymaker seeds (Suttons)
117 were sterilised by 30 minute treatment in dilute bleach, followed by five, 15 minute washes in 1 ml deionised
118 water. Seeds were germinated on 0.5X MS salts, 0.6% agar plates at 23°C, and taken for exoRNAi treatment on
119 the first day post radicle emergence. Ten seedlings were used per well of a 24-well plate (SPL Lifesciences),
120 and incubated with 300 µl of 10 ng/µl dsRNA solution for 24h at 23°C, in darkness. Five seedlings were snap
121 frozen in liquid nitrogen per biological replicate, and total RNA isolated using Trizol reagent. Total RNA was
122 treated with the Turbo DNase free kit (Life Technologies), and cDNA was synthesised using the High-capacity
123 RNA-to-cDNA kit (Applied Biosciences) according to manufacturer's instructions using the maximum input
124 concentration of RNA. Three biological replicates were performed for each treatment. Quantitative RT-PCR
125 primers were as follows: (Sugar Transporter 1, *qstp1F* 5'-ATGTTGCTGGATTCGCTTGGTC-3', *qstp1R* 5'-
126 TGTGCAGCTGATCGAATTTCCAG-3'); (Sugar Transporter 2, *qstp2F* 5'-ATTATGGCTGCTACCGGAGGTC-3', *qstp2R*
127 5'-TGTAACACCACCAGAACTCCAAC-3'); (Elongation Factor, *qefaF* 5'-TACTGGTGGTTTTGAAGCTG-3', *qefaR* 5'-
128 AACTTCTTCACGATTTTCATCATA-3'); (SAND protein family, *qsandF* 5'-TTGCTTGGAGGAACAGACG-3', *qsandR* 5'-
129 GCAAACAGAACCCTGAATC-3'); (Sugar Transporter 41, *qstp41F* 5'-ATGGAGTTTTGAGTCTTCTGC-3', *qstp41R*
130 5'-GCTGCGTTTCTGGCTTAGG-3') (Dekkers *et al.*, 2012). Primer sets to be used for qPCR were optimised for
131 working concentration, annealing temperature and analysed by dissociation curve for contamination or non-
132 specific amplification by primer-dimer as standard. Each individual reaction comprised 5 µl Faststart SYBR
133 Green mastermix (Roche Applied Science), 1 µl each of the forward and reverse primers (10 mM), 1 µl water, 2
134 µl cDNA. PCR reactions were conducted in triplicate for each individual cDNA using a Rotorgene Q thermal
135 cycler under the following conditions: [95°C x 10 min, 40 x (95°C x 20s, 60°C x 20s, 72°C x 20s) 72°C x 10 min].
136 The PCR efficiency of each specific amplicon was calculated using the Rotorgene Q software, and quantification
137 of each target amplicon calculated by an augmented comparative Ct method (Pfaffl, 2001), relative to the
138 geometric mean of three endogenous reference genes (Vandesompele *et al.*, 2002). Ratio-changes in
139 transcript abundance were calculated relative to control dsRNA treated seedlings in each case Exudate
140 concentration of **(B)** glucose, **(C)** fructose and **(D)** xylose across *neo* (double stranded [ds]RNA control), *stp1*
141 and *stp2* dsRNA treated tomato seedlings. The exudate solution was collected by pipette and transferred to a
142 hydrophobically-lined microcentrifuge tube (Anachem) prior to quantification. The sugars were quantified
143 colorimetrically at 340 nm using Glucose (HK), and Fructose assay kits from Sigma-Aldrich, and the Xylose assay
144 kit from Megazyme as per manufacturer's instructions. Each data point represents the mean (±SEM) of three
145 replicates of ten seedlings each. Data were analysed by ANOVA and Tukey's Honestly Significant Difference
146 test using Graphpad Prism 6. Probabilities of less than 5% (P < 0.05) were deemed statistically significant *,
147 P<0.05; **, P<0.01; ***, P<0.001.

148
149 Root exudates collected from tomato seedlings which had been treated with either *stp1* or
150 *stp2* dsRNA were less capable of stimulating stylet thrusting in *M. incognita* relative to
151 exudates collected from control dsRNA treated seedlings (13.92 ±5.10%, P<0.001; and 17.53
152 ±8.12%, P<0.001, respectively. Fig 3A). No significant difference in stylet thrusting
153 frequency was observed for *G. pallida* juveniles when exposed to root exudates from *stp1* or
154 *stp2* dsRNA-treated seedlings, relative to control treated seedlings (108.2 ±38.87%, P>0.05;
155 and 77.34 ±30.84%, P>0.05, respectively) (Fig 3B).

156 When exoRNAi-treated seedlings were challenged by *M. incognita* infection assay,
157 significant reductions in percentage infection levels relative to control (*neo*) dsRNA
158 treatment were observed for both *stp1* (14.15% ±4.77; P<0.01) and *stp2* (27.08% ±7.32;
159 P<0.05) dsRNA treatments (Fig 3C). Knockdown of *stp1* (14.15% ±4.77; P>0.05) or *stp2*
160 (14.15% ±4.77; P>0.05) did not significantly reduce the percentage infection levels of *G.*
161 *pallida* relative to *neo* dsRNA treatment (Fig 3D).

162



163

164 **Figure 3. ExoRNAi of tomato seedling sugar transporters differentially alters plant nematode infection and**
 165 **activation.**

166 (A) Mean percentage (\pm SEM) stylet thrusting of *M. incognita* and (B) *G. pallida* infective stage juveniles in
 167 response to collected seedling exudates (n=100), relative to control (*neo* dsRNA). Root exudate was collected
 168 and quantified as described in Fig. 2. Nematodes were exposed to 50 μ l of dsRNA treated seedling exudate for
 169 30 minutes, thrusts were counted in randomly selected infective stage juveniles for 1 minute each. (C) Mean
 170 percentage *M. incognita* infection levels of *stp1* and *stp2* dsRNA treated tomato seedlings normalised to
 171 control (*neo*) dsRNA treated seedlings. (D) Mean percentage *G. pallida* infection levels of *stp1* and *stp2* dsRNA
 172 treated tomato seedlings normalised to control (*neo*) dsRNA treated seedlings. Agar slurry was prepared by
 173 autoclaving a 0.55% agar solution which had been autoclaved and adjusted to pH 7. The agar was agitated for
 174 six hours at room temperature, until it had a smooth consistency. 500 *M. incognita* or *G. pallida* infective
 175 stage juveniles were added to each well of a 6 well plate (SPL Lifesciences) with one exoRNAi treated seedling
 176 embedded within 3 ml of agar slurry. Plates were sealed with parafilm, covered above and below with a sheet
 177 of tin foil and incubated for 24 hours at 23°C. Seedlings were subsequently removed from the slurry, gently
 178 washed several times by immersion in deionised water, and stained using acid fuchsin (Bybd *et al.*, 1983). The
 179 number of invading PPN juveniles was counted for each seedling using a light microscope. Control treatments
 180 were expressed as a percentage, including technical variation, and experimental treatments were normalised

181 to control percentages. Each data point represents the mean (\pm SEM) of ten seedlings challenged with 500
182 infective stage juveniles each. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

183

184 These data demonstrate that the exogenous application of aqueous dsRNA onto tomato
185 seedlings is sufficient to trigger specific gene knockdown. However, we found that different
186 experimental populations of tomato seedlings could display wide variation in the expression
187 of both sugar transporter genes, and reference genes which resulted in high standard error
188 of mean (SEM) values. This made it difficult to resolve gene knockdown levels for an
189 isolated number of experiments. This may be due to variation in the susceptibility of
190 tomato seedlings to exoRNAi, as has been observed for Tobacco Rattle Virus (TRV) VIGS
191 approaches in tomato (Liu *et al.*, 2002), or it could indicate that larger replicates of seedlings
192 are required to consistently resolve gene expression data post exoRNAi. It should also be
193 noted that attempts to silence phytoene desaturase in order to observe a bleaching
194 phenotype in the cotyledons were unsuccessful (data not shown). This may indicate that
195 only genes expressed in the tomato root are susceptible to this approach.

196 It is well established that plant root exudates mediate both positive and negative
197 interactions with commensal and pathogenic microbes (Badri *et al.*, 2009), insects (Walker
198 *et al.*, 2003), and other plants (Bais *et al.*, 2006). Plant parasitic nematodes also respond to
199 plant root exudates (Teillet *et al.*, 2013). The present study aimed to probe the involvement
200 of monosaccharide sugars of tomato root exudate for involvement in the attraction and
201 activation of parasitic behaviours in the promiscuous root knot nematode *M. incognita*, and
202 the host-selective potato cyst nematode *G. pallida*. STP1 and STP2 are known transporters
203 of monosaccharide sugars (Gear *et al.*, 2000), and our data demonstrate that both play a
204 role in regulating the level of glucose and fructose (but not xylose) exudation from tomato
205 seedling roots. exoRNAi knockdown of each transporter significantly reduced the amount of
206 glucose and fructose secreted from plant roots, which corresponded with a decrease in *M.*
207 *incognita* infectivity, but not *G. pallida* infectivity. These results suggest that glucose and
208 fructose are important chemical cues which infective stage *M. incognita* use to find host
209 plants. These data indicate that glucose and fructose trigger host-finding and stylet
210 thrusting in promiscuous PPNs, as opposed to host-specific PPNs, an observation which is
211 consistent with the ubiquitous nature of monosaccharide sugars in plant root exudates
212 (Kamilova *et al.*, 2006). The demonstration that STP1 and STP2 are specifically involved in
213 the exudation of both monosaccharides from tomato roots is an important finding which
214 can underpin future efforts to study the link between plant root transporters, and chemical
215 constituents of root exudates.

216

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223

224 **References**

225 **Badri DV, and Vivanco JM.** (2009) Regulation and function of root exudates. *Plant, Cell and*
226 *Environment* 32: 666-681.

227 **Badri DV, Quintana N, El Kassis EG, Kim HK, Choi YH, Sugiyama A, Verpoorte R, Martinoia**
228 **E, Manter DK, and Vivanco JM.** (2009) An ABC transporter mutation alters root exudation of
229 phytochemicals that provoke an overhaul of natural soil microbiota. *Plant Physiology* 151:
230 2006-2017.

231 **Bais HP, Weir TL, Perry LG, Gilroy S, and Vivanco JM.** (2006) The role of root exudates in
232 rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*
233 57: 233-266.

234 **Bybd DW JR, Kirkpatrick TJR, and Barker KR.** (1983). An Improved Technique for Clearing
235 and Staining Plant Tissues for Detection of Nematodes. *Journal of Nematology* 15(1):142-
236 143.

237 **Dakora FD and Phillips DA.** (2002) Root exudates as mediators of mineral acquisition in low-
238 nutrient environments. *Plant and Soil* 245: 35–47.

239 **Dekkers BJ, Willems L, Bassel GW, van Bolderen-Veldkamp RP, Ligterink W, Hilhorst HW,**
240 **Bentsink L.** (2012). Identification of reference genes for RT-qPCR expression analysis in
241 *Arabidopsis* and tomato seeds. *Plant Cell Physiology* 53(1):28-37.

242 **Gear ML, McPhillips ML, Patrick JW, McCurdy DW.** (2000) Hexose transporters of tomato:
243 molecular cloning, expression analysis and functional characterization. *Plant Molecular*
244 *Biology* 44: 687-697.

245 **Hart AC** (2006) Behavior. WormBook, doi/10.1895/wormbook.1.87.1,
246 <http://www.wormbook.org>.

247 **Jones DL, Nguyen C, Finlay RD.** (2009) Carbon flow in the rhizosphere: carbon trading at the
248 soil-root interface. *Plant Soil* 321, 5–33.

249 **Kamilova F, Kravchenko LV, Shaposhnikov AI, Azarova T, Makarova N, Lugtenberg B.**
250 (2006) Organic acids, sugars, and L-tryptophane in exudates of vegetables growing on
251 stonewool and their effects on activities of rhizosphere bacteria. *Molecular Plant-Microbe*
252 *Interactions* 19(3): 250-256.

253 **Liu Y, Schiff M, Dinesh-Kumar SP.** (2002) Virus-induced gene silencing in tomato. *The Plant*
254 *Journal* 31: 777–786.

255 **Pfaffl MW.** (2001) A new mathematical model for relative quantification in real-time RT-
256 PCR. *Nucleic Acid Research* 2001 29(9):e45.

257 **Klink VP, and Wolniak SM.** (2001) Centrin is necessary for the formation of the motile
258 apparatus in spermatids of *Marsilea*. *Mol. Biol. Cell* 12: 761-776.

259 **Reuscher S, Akiyama M, Yasuda T, Makino H, Aoki K, Shibata D, Shiratake K.** (2014) The
260 sugar transporter inventory of tomato: genome wide identification and expression analysis.
261 *Plant and Cell Physiology* 55(6): 1123-1141.

- 262 **Sasser JN, and Freckman DW.** (1987) A world perspective on nematology: the role of the
263 society pp 7-14. *Vistas on Nematology* Ed. Veech, J. A.
- 264 **Teillet A, Dybal K, Kerry BR, Miller AJ, Curtis RHC, Hedden P.** (2013) Transcriptional changes
265 of the root-knot nematode *Meloidogyne incognita* in response to *Arabidopsis thaliana* root
266 signals. *PLoS One* 8(4): e61259. doi:10.1371/journal.pone.0061259.
- 267 **Tenllado F, Martínez-García B, Vargas M, Díaz-Ruíz JR.** (2003) Crude extracts of bacterially
268 expressed dsRNA can be used to protect plants against virus infections. *BMC Biotechnology*
269 March 20;3:3.
- 270 **Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F.**
271 (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging
272 of multiple internal control genes. *Genome Biology* June 18; 3(7): RESEARCH0034.
- 273 **Walker TS, Bais HP, Grotewold E, and Vivanco JM.** (2003) Root exudation and rhizosphere
274 biology. *Plant Physiology* 132: 44-51.
- 275 **Watson JM, Fusaro AF, Wang M, Waterhouse PM.** (2005) RNA Silencing Platforms in Plants.
276 *FEBS Letters* 579(26): 5982-5987.
- 277
- 278