Soybean *Rhg1* resistance to *Heterodera glycines* impedes the parasitism of *Pratylenchus penetrans*

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Abstract

Rhg1 has been the most effective QTL deployed in soybeans to control soybean cyst nematode (*Heterodera glycines*; SCN). However, the resistance mechanisms and specificity of *Rhg1* towards SCN as opposed to other plant parasitic nematodes are not well known. In this study we report that *Rhg1* can impede the parasitism of the root lesion nematode *Pratylenchus penetrans* (Pp), which is commonly found along with SCN in northern U.S. soybean fields. Two elite public soybean varieties, carrying either *rhg1-a* + *Rhg4* or *rhg1-b*, negatively impacted Pp populations measured at 30 dpi. This contribution of *Rhg1* was more rigorously demonstrated using near isogenic lines for *rhg1-b*. Additionally, in three of the four soybean SCN-susceptible genotypes tested we observed significantly more Pp when Pp were co-inoculated with SCN, consistent with a previous study. No significant changes in SCN cyst numbers upon co-inoculation with Pp were observed for any of the *rhg1* resistant lines. No nematode-induced changes in *Rhg1* transcript abundances were observed when sampling infected root sections in any of the plant genotypes, and no changes in a jasmonate response indicator were observed. However, the 3 dpi salicylate-mediated response to SCN or Pp became more robust during SCN + Pp co-infection. Multiple hypotheses for further dissection and manipulation of soybean resistance to *Pratylenchus* root lesion nematodes are suggested based on the present findings.

Introduction

Root lesion nematodes (RLN; *Pratylenchus* spp.) are important agricultural pests (Jones et al. 2013; Rosa de Araújo et al. 2025). They are migratory nematodes with a wide host range including soybean. *Pratylenchus brachyurus* is often considered to be the most damaging nematode pest of soybeans in South America (Castanheira et al. 2020; Favoreto et al. 2019; Nomura et al. 2024). *Pratylenchus* populations survive cold winters in dead roots through anhydrobiosis or slow desiccation (Townshend 1984). *Pratylenchus* are some of the most common plant parasitic nematodes found in the soybean producing midwestern U.S. states like Wisconsin, Minnesota, Iowa, and Illinois, with *Pratylenchus penetrans* (Pp) being among the most common species, and a species for which soybean yield losses have been documented (Chen et al. 2012; MacGuidwin and Bender 2012; MacGuidwin et al. 2023; Saikai and MacGuidwin 2022; Schumacher and Bird 2025) (Schmitt and Barker 1981). Unlike sedentary endoparasitic nematodes such as the soybean cyst nematode (SCN, *Heterodera glycines*), Pp feed on soybean roots at multiple sites as ectoparasites or endoparasites (Endo 1967; Saikai and MacGuidwin 2020). SCN spend limited time exposed to environmental disturbances as they shelter inside host roots or in cyst-encased eggs for much of their life cycle. In contrast, the migratory lifestyle of Pp makes them more directly exposed to, and to some extent adapted to, changes in soil pH and nutrients, soil moisture, soil type, own population density, microbial communities, and competition from other nematodes.

SCN is the most economically important pathogen or pest on soybeans in the United States (Bradley et al. 2021). Across all of the North Central U.S. and Ontario for ten recent years, SCN is estimated to have caused approximately 4% soybean yield loss (about 1/4

of the total for all soybean pathogens) (Allen et al. 2017; Bradley et al. 2021). Understandably, most efforts in U.S. soybean systems have been directed at managing SCN and not *Pratylenchus* spp. Estimates of the impact of RLN on soybean production are rare, but RLN and SCN frequently co-occur (MacGuidwin et al. 2023; Saikai and MacGuidwin 2022; Schumacher and Bird 2025). Northern U.S. soybean fields are typically rotated with maize, an excellent host for RLN. In one deeply studied set of Wisconsin soybean fields, RLN was found to cause up to 4.5% yield loss by itself (Saikai and MacGuidwin 2022), and about 3.79% yield loss on corn (MacGuidwin and Bender 2016). The farm gate values of corn and soybean in Wisconsin alone was over USD 2.4 billion and USD 1.3 billion respectively (USDA), which illustrates the economic impact of even a 1% yield loss to RLN.

Along with crop rotation, the most important tool for managing SCN over the past three decades has been host resistance provided by the major-effect quantitative trait locus (QTL) *Rhg1* (Resistance to *Heterodera glycines* 1) (Niblack et al. 2006) (Bent 2022; Niblack et al. 2006). There are two widely known resistance-conferring haplotype variants of *Rhg1* in soybean. The haplotype *rhg1-a* is commonly derived from “Peking” while *rhg1-b* is often derived from “PI 88788”, with the latter being used in over 95% of all commercial nematode resistant varieties (McCarville et al. 2017; Niblack et al. 2006). The Peking-type *rhg1-a* haplotype is less effective if it is not coupled with resistance-associated alleles at the unlinked *Rhg4* or *rhg2* loci (Basnet et al. 2022; Brucker et al. 2005; Yu et al. 2016). SCN populations that at least partially overcome three reference sources of *rhg1-b* resistance are designated as HG type 2.5.7. Throughout soybean-producing areas of the U.S., HG type 2.5.7 and other *rhg1-b*-virulent SCN populations are increasingly common

85 and are gradually increasing their virulence on *rhg1-b* (Kleczewski et al. 2023; McCarville
86 et al. 2017). In response, expanded use of Peking-type resistance and introduction of
87 varieties that carry PI 88788-type *rhg1-b* resistance stacked with additional minor-effect
88 resistance QTLs is anticipated (Bent 2022; Brzostowski and Diers 2017; Tylka et al. 2012)

89 The *rhg1-a* and *rhg1-b* haplotypes both carry three tightly linked genes that
90 contribute to SCN resistance (Cook et al. 2014; Cook et al. 2012a). *Glyma.18g022400*,
91 *Glyma.18g022500* and *Glyma.18g022700* respectively encode AAT_{Rhg1} (putative amino
92 acid transporter), α-$SNAP_{Rhg1}$ (vesicle trafficking mediator, also called GmSNAP18) and
93 $WI12_{Rhg1}$ (unknown function, carries a WI12 wound inducible protein domain). While the
94 causal *Rhg1* genes were identified a decade ago and their mechanism(s) of action are
95 being investigated, the resistance has been deployed for multiple decades. The α-
96 $SNAP_{Rhg1}$ proteins differ between the *rhg1-a* and *rhg1-b* haplotypes, as does the copy
97 number of tandem repeats of the entire locus (Cook et al. 2014). The abundance of
98 resistance-associated α-$SNAP_{Rhg1}$ protein is known to increase 10-15-fold in the
99 syncytium, the SCN feeding site (Bayless et al. 2016; Bayless et al. 2019). AAT_{Rhg1} protein
100 increases an order of magnitude along the SCN root penetration path, where a substantial
101 pool of AAT_{Rhg1} is associated with macrovesicles (Han et al. 2023). Less is known about
102 how the $WI12_{Rhg1}$ interacts with SCN, although impacts on root gibberellin levels have
103 been discovered (Dong and Hudson 2022). Silencing or knocking out any one of the three
104 genes increases susceptibility to SCN (Cook et al. 2012b; Dong and Hudson 2022; Liu et
105 al. 2017; Schumacher and Bird 2025). Soybean *Rhg1* genes also conferred resistance to
106 other cyst nematodes when moved into Arabidopsis and potato (Butler et al. 2019; Butler
107 et al. 2021).

We hypothesized that *Rhg1* could also provide some resistance to Pp and that the commercially popular *rhg1-b* may have been beneficial against Pp. Melakeberhan and Dey previously reported that the infection rate of *H. glycines* in some instances decreased with increasing *P. penetrans* proportions, and that the rate of *P. penetrans* infection increased when co-inoculated at a 1:1 ratio with *H. glycines* but did not increase at higher or lower co-inoculation ratios (2003). That work was done using an SCN-susceptible soybean cultivar. Schumacher and Bird recently reported some instances where the presence of Pp increased SCN population densities on *rhg1-a* resistance sources, but with no overall consistent trend (Schumacher and Bird 2025). Interactions between *Rhg1* and other pathogens are only beginning to receive study, although most soybeans grown in recent years across the U.S. carry this genetic resistance (Lopez-Nicora et al. 2023). On soybean, Niblack and colleagues previously found that resistance to SCN was not reduced during co-infections with the root knot nematode *M. incongnita* (Niblack et al. 1986). Significantly, the *rhg1-a* + *rhg2 (GmSNAP11)* combination has recently been shown to enhance quantitative resistance against reniform nematodes (Usovsky et al. 2021; Usovsky et al. 2022)

In this study we assessed (i) the success of Pp and an SCN HG 2.5.7 population on the soybean lines IL3025N (*rhg1-b*), IL3849N (*rhg1-a/Rhg4*) and Williams 82 (SCN-susceptible *rhg1-c*), in terms of total nematodes recovered per plant at the end of 30 days, (ii) the impact on nematode numbers when SCN and Pp were co-inoculated at 2:1 or 1:1 SCN:Pp ratios, and, using a set of near isogenic lines (NILs) for *rhg1-b*, (iii) how *rhg1-b* impacts Pp and SCN nematode numbers and the transcript abundance response of the

130 three *rhg1-b* genes as well as salicylic acid (SA) and jasmonic acid (JA) response indicator
131 genes.

132

133 Materials and Methods

134 Sand cup preparation

135 Torpedo sand was sieved to remove residual gravel and the resulting sand was
136 moistened, placed in heat-tolerant bins at a maximum of 7.5 cm depth, and heated in a 65
137 °C oven for 48 h. The sand was cooled to room temperature and filled in 5 cm x 5 cm cups
138 (plastic pots with drainage holes) and saturated with 50 mL of tap water.

139 Plant genotypes

140 Seeds of Williams 82 (*rhg1-c*), IL3025N (*rhg1-b*) and IL3849N (*rhg1-a,* and *Rhg4*)
141 were used in the first set of cup assays. Seeds of Williams 82 and near-isogenic lines
142 Resistant NIL (*rhg1-b*; LD09-15604) and Susceptible NIL (*rhg1-c*; LD09-15630) were used
143 in the second set of cup assays and differential gene expression studies. The NILs were
144 developed by Brian Diers, University of Illinois at Urbana-Champaign, through four
145 backcrosses using LD00-3309 (*rhg1-b*) as the recurrent parent and IA3023 as donor of the
146 susceptible *rhg1-c* allele (resulting NIL accessions predicted to be >96% LD00-3309
147 genotype). All seeds were surface sterilized using chlorine vapor as described by Clough
148 and Bent (1998). Seeds were germinated in paper towels at 27°C for 4-5 days in the dark
149 until a 5 cm radicle had formed, and were then planted in sand cups. For nematode
150 infection cup assays, five replicates (individual plants in individual cups) for each plant
151 genotype and each nematode treatment were transplanted. Replicate blocks were

152 composed of one replicate of all treatments arranged in a tray. Plants within each replicate
153 block were randomized and covered with a humidity dome for the first 2 days of growth.
154 For expression analyses, three replicate blocks each were set up for 3 dpi and 7 dpi
155 sampling points. Each experiment was repeated twice (two separate dates).

156 **Nematode inoculum**

157 Eggs of an HG 2.5.7 Illinois field population of SCN maintained on IL3025N (FI
158 ~70), termed "SCN HG 2.5.7 IL", were hatched in 4 mM ZnCl2 solution at 27°C and dark
159 conditions. Freshly hatched J2 stage SCN were rinsed and re-suspended in de-ionised
160 water at 1000 J2/mL or 500 J2/mL. The *Pratylenchus penetrans* population was collected
161 in Wisconsin and maintained on 'IO Chief' sweet corn root explants. All life stages of *P.*
162 *penetrans* were extracted from corn root explants grown on Gamborg B5 medium using
163 the Baermann funnel method (Baermann 1917). The nematodes were rinsed and
164 resuspended in de-ionised water. *P. penetrans* inoculum batches contained approximately
165 35% adult females, 15% adult males, 40% third and fourth stage juveniles and 10% first
166 and second stage juveniles. The nematodes were inoculated into the sand cups 24 h after
167 transplanting the germinated soybean seedlings (VE) for most experiments, or for
168 experiments that used V1 plants, when the first set of trifoliate leaves had emerged.
169 Nematode suspensions were inoculated into the sand via a small hole made using a 200
170 uL pipette tip, parallel to and 1 cm away from the soybean seedling, which was then re-
171 covered with sand. The plants were covered with humidity domes for 12 h post inoculation.

172 **Nematode count cup assays**

173 Inoculated plants were maintained in a growth room with 14h daylight, day
174 temperature 27°C, night temperature 22°C, for 30 days. The plants were watered with 10

175 mL of tap water each day for the first week, 15 mL a day the second week, alternating
176 between 15 mL and 30 mL a day the third week and 30 mL a day thereafter. The cups
177 were fertilized once during week 3 and again a week later using MiracleGrow as per
178 manufacturer recommendations instead of tap water.

179 Nematodes were extracted from the sand using water flotation and decanted into a
180 nested sieve system consisting of a 2380 µm, 177 µm and 38 µm sieve (US standard
181 sieves 7, 80, and 400 respectively). Plant materials, coarse sand particles and other debris
182 retained on the 2380 µm sieve after vigorous rinsing with tap water were collected, then
183 the roots of all samples were chopped into ~1 cm pieces and incubated on Baermann
184 funnels with de-ionised water for 48 h at room temperature (25°C). 50 mL of water was
185 eluted from the bottom of the funnels and evaluated for Pp (all life stages) and SCN J2s
186 and males. Contents retained by the 177 µm sieve were retained for SCN cyst
187 enumeration under a stereoscope while contents from the 38 µm sieve were enumerated
188 for *Pratylenchus penetrans* of all life stages and SCN males and juveniles. Nematode
189 samples were further clarified using 70% (70 g into 100ml H_2O) sucrose solution prior to
190 microscopic enumeration. In NIL samples co-inoculated with both nematodes, contents
191 from 38 µm sieve were also evaluated for SCN J2 and male numbers.

192 **NIL *rhg1* differential gene expression assay**

193 For RNA extraction, roots from plants (Williams 82, Resistant NIL and Susceptible
194 NIL) inoculated with either SCN (500 J2) or Pp (500 worms – all life stages) or both (500
195 J2 + 500 Pp worms) as well as non-inoculated controls were rinsed in water and observed
196 under a stereoscope at 3 dpi or 7 dpi. For each sample, about 100 mg of root tissue from
197 regions with visible lesions from a single plant was placed in ice-cold cryovials with 5

198 ceramic beads and immediately frozen in liquid nitrogen. These samples were stored at -
199 80°C. Frozen tissue was processed at 2500 rpm for 30 s in a powerlyzer and returned to
200 liquid nitrogen for a minute. This process was repeated 2-3 times until tissue was
201 pulverized. RNA was then extracted from pulverized root tissue with Trizol (Zymo) using
202 manufacturer's protocol. cDNA was synthesized using Powerscript reagent and
203 degenerate primers from 1 ug of RNA for each sample. Real-time quantification and Ct
204 values were obtained for transcripts encoding AAT_{Rhg1}, $WI12_{Rhg1}$, α-$SNAP_{Rhg1}$ (detects all
205 haplotypes), α-$SNAP_{Rhg1}$HC (detects only the HC/ *rhg1-b* haplotype), as well as for *AAT*
206 (*Glyma.10G109500*; JA response indicator), *NIMIN1* (*Glyma.10G010100*; SA response
207 indicator) and the internal standard gene *NREG1* (*Glyma.11G079600*) (Beyer et al.
208 2021).To determine the fold-change in transcript abundance in response to nematode
209 inoculations, the dCt values obtained using NREG1 as internal standard were normalized
210 to the average dCt values of all no-nematode samples for the same plant genotype within
211 the same experiment date to obtain ddCt values. Alternatively, to evaluate relative
212 transcript abundances across plant genotypes, the dCt values for all genotypes and
213 treatments were normalized to the average dCt values of no-nematode inoculated Sus.
214 NIL plants to obtain ddCt values.

215 **Statistical analyses**

216 All data were analyzed and visualized using R (version 4.3.0). Graphs show mean
217 and standard error of mean for the original non-transformed data. For statistical tests,
218 count data were square root transformed or log transformed to achieve normality,
219 multivariate analyses of variance were performed using the aov() function, and the means

were separated using TukeyHSD() in the package stats() with an α=0.05. Normality was confirmed using Shapiro.test() function for all variables.

The SCN cyst data for the experiments were analyzed using the formula:

$$\sqrt[2]{Cysts} \sim [(experiments|replicates) + Plant\ genotype * Nematode\ inoculation]$$

Similarly, Pp count data was analyzed using the formula:

$$\sqrt[2]{P.penetrans}\ or \log(P.penetrans + 1) \sim [(experiments|replicates) + Plant\ genotype * Nematode\ inoculation]$$

For the NIL nematode count evaluations, we used:

$$\log(SCN\ cysts) \sim [(experiments|replicates) + Plant\ genotype * Nematode\ inoculation]$$

$$\log(Total\ P.penetrans) \sim [(experiments|replicates) + Plant\ genotype * Nematode\ inoculation]$$

For the transcript abundance evaluations, statistical analyses were performed on the ddCt values since the fold-change (2^-ddCt) values did not pass the Shapiro-wilk normality test. The formula we used to evaluate transcript induction within a plant genotype in response to nematodes:

$$ddCt \sim \frac{Experiments}{root\ replicates} + Plant\ genotype * Nematode\ treatment$$

We then visualized the data and analyses using Microsoft Office and Adobe Illustrator.

239 ## Results

240 **Pp parasitism of soybean is enhanced by co-infection with SCN**

241 Controlled environment single-species inoculation and co-inoculation experiments
242 with Pp and SCN were carried out (Pp-only, SCN-only or Pp+SCN all within the same
243 experiment). Experiments were first conducted using soybean genotypes that differ at
244 hundreds of loci, including different SCN resistance haplotypes. Williams 82 (*rhg1-c*) is
245 SCN-susceptible while IL3025N (also known as LD11-2170) carries *rhg1-b* and IL3849N
246 (also known as LD07-3395bf) carries *rhg1-a* and *Rhg4*. Four independent experiments
247 were conducted, two using a 1:1 ratio and two using a 2:1 SCN:Pp ratio for co-
248 inoculations, with five replicates of each treatment within each experiment.

249 Upon co-inoculation with virulent SCN HG 2.5.7 IL, more Pp were recovered
250 compared to the Pp-only inoculations (($p < 0.001$), orange vs. blue bars in Figure 1A and
251 1B; ANOVA tables associated with all figures are provided as Supplementary Tables). The
252 experiments utilized an SCN HG 2.5.7 population that, as expected, reproduced well on
253 the susceptible *rhg1-c* (Williams 82) and substantially overcame *rhg1-b* resistance
254 (IL3025N) while exhibiting poor reproduction on a plant line carrying the *rhg1-a* and *Rhg4*
255 combination (IL3849N) (green bars in Figure 1C and 1D; all differences between SCN-only
256 inoculations (green bars) were significant at $p<0.001$). Within single soybean genotypes,
257 the increase in Pp populations upon co-inoculation with SCN (orange vs. blue bars) was
258 statistically significant only on the Williams 82 and IL3025N soybean genotypes that
259 support substantial SCN growth and reproduction (Figure 1A and 1B). On IL3849N, which
260 largely prevented growth of the HG 2.5.7 SCN population, the increases in Pp abundance
261 in the co-inoculations as opposed to the inoculations only with Pp were not statistically

262 significant (Figure 1A and 1B). Fewer Pp were recovered from IL3849N than from
263 IL3025N in both single and co-inoculations (Figure 1A and 1B). SCN:Pp inoculation ratios
264 of 2:1 and 1:1 were tested and gave similar results (Figure 1A and 1B). In all of the
265 experiments in Figure 1, about twice as many Pp were recovered 30 days after inoculation
266 when Pp were co-inoculated with SCN HG 2.5.7, compared to Pp-only inoculations (the
267 ratio on the same plant genotype in the same experiment ranged from 1.7 to 2.3, across
268 all plant genotypes and both ratios of co-inoculation).

269 Within the same experiments as above, there were no significant differences in SCN
270 HG 2.5.7 IL cyst numbers between SCN-only inoculations and co-inoculations with Pp on
271 the same plant genotype (green vs. blue bars, Figure 1C and 1D). Nearly 10-fold more
272 cysts were recovered across treatments when 1000 (Figure 1D) rather than 500 (Figure
273 1C) SCN J2s were inoculated, for both solo inoculations and co-inoculations with Pp.

274

275 ***rhg1-b* impedes the parasitism of Pp**

276 A separate phenomenon was observed in the experiments of Figure 1, suggesting
277 that soybean SCN resistance might also impact RLN. When inoculated by itself in these
278 experiments, significantly fewer Pp were recovered after 30 days from the SCN-resistant
279 variety IL3849N than from Williams 82 (blue bars in Figure 1A and 1B). Pp abundance on
280 IL3025N was intermediate, being significantly lower only in the 1:1 SCN:Pp experiments
281 (Figure 1A and 1B).

282 A more rigorous test for an effect of the SCN resistance *rhg1-b* haplotype on Pp
283 was conducted using near-isogenic soybean lines (NIL) expected to carry the same
284 genotype at >96% of all loci but carrying either *rhg1-c* (Sus. NIL) or *rhg1-b* (Res. NIL) at

285 the *Rhg1* locus. The results supported the conclusion that *rhg1-b* can negatively impact Pp
286 (Figure 2). Mirroring the experiments of Figure 1, significantly fewer Pp were recovered
287 from the resistant NIL compared to the susceptible NIL when singly inoculated with Pp
288 (Figure 2A blue bars). When co-inoculated with both nematodes (Figure 2A orange bars),
289 fewer Pp were again recovered from Res. NIL plants than Sus. NIL plants but with a
290 difference that was at the margin of statistical significance ($p = 0.056$).

291 For SCN, multiple studies have shown that the proportion of female to male
292 nematodes formed is often lower on soybean plants with effective SCN resistance than on
293 susceptible plants (Colgrove and Niblack 2005). Intriguingly, with migratory Pp we also
294 observed that the ratios of females to males recovered from the *rhg1-b* Res. NIL were
295 significantly lower than those for *rhg1-c* Sus. NIL plants (Figure 2B). This was true in both
296 Pp-only and SCN co-inoculated treatments. When the proportion of Pp found in roots vs.
297 the surrounding sand at 30 dpi was enumerated, the *rhg1-b* Res. NIL had a higher
298 proportion of the Pp in the sand than the Sus. NIL, but intriguingly, that was only in the Pp
299 + SCN co-inoculated samples (Figure 2C). For the Res. NIL line, a ~5-fold higher
300 proportion of Pp were in the sand than in the root upon co-inoculation with SCN, compared
301 to the Pp-only samples (Figure 2C). The relative presence of juvenile Pp 30 days after
302 inoculation was examined as another parasitism/resistance metric, because those
303 juveniles are predominantly progeny produced from the initial inoculum. At 30 days after
304 inoculation the number of juvenile-stage Pp (Figure 2D) and the proportion of juvenile-
305 stage vs. adult Pp (Figure 2E) again showed that presence of SCN shifted Pp population
306 demographics. Juvenile Pp were significantly less common on *rhg1-b* Res. NIL plants
307 compared to the *rhg1-c* Sus. NIL. For the juvenile:adult ratio they were less common on

308 Res. NIL plants only when Pp and SCN were co-inoculated (Figure 2E). The findings of
309 Figure 2 together indicate that *rhg1-b* can exert a negative impact on Pp, in the presence
310 of SCN but also in the absence of SCN.

311

312 **Pp co-inoculation reduces SCN cyst numbers only in the susceptible *rhg1-c* lines**

313 As expected, in SCN-only inoculations fewer SCN HG 2.5.7 IL cysts were
314 recovered from the resistant *rhg1-b* NIL plants compared to the susceptible *rhg1-c* NIL
315 plants or Wm82 (Figure 3A; data are from same experiments at Figure 2). Co-inoculation
316 of Pp with SCN reduced the number of cysts recovered on the SCN-susceptible *rhg1-c*
317 Williams 82 and Sus. NIL lines (Figure 3A). The difference was statistically significant only
318 for Williams 82 (Figure 3A), but a similar trend was also observed on Williams 82 in the
319 experiments of Figure 1A ($p = .094$) and on Sus. NIL in the experiments of Figure 3A ($p =$
320 $.070$). When the plants had partial resistance to SCN that reduced cyst production (Res.
321 NIL), co-inoculation with Pp did not further reduce cyst numbers (Figure 3A). In the same
322 experiments, the J2 and male SCN populations in roots and sand at 30 dpi were also
323 enumerated. The results were not consistent across the two SCN-susceptible plant
324 genotypes, as fewer total SCN J2s were recovered after co-inoculation with Pp only in the
325 case of the Sus. NIL plants (Figure 3B). Co-inoculation with Pp did not reduce J2 SCN
326 numbers for Williams 82 and Res. NIL plants (Figure 3B). The number of SCN males
327 recovered exhibited substantial variation, and no statistically significant differences were
328 observed between plant genotypes or between plants inoculated with only SCN and those
329 inoculated with both nematodes (Figure 3C).

330

331 **No significant changes in *rhg1-b* transcript abundance detected in response to**
332 **nematode infection**

333 In light of the observed interactions between SCN, Pp and *rhg1-b*, we investigated
334 nematode induction of increases in transcript abundance of the three *rhg1-b* genes as well
335 as indicator genes of SA-response and JA-response activity in soybeans, at 3dpi and 7dpi
336 (Figures 4 and 5). As has previously been observed (Cook et al. 2014), *Rhg1* transcripts
337 were approximately 5- to 20-fold more abundant for the high-copy *rhg1-b* genotype than
338 for the single-copy *rhg1-c* genotype (Supplemental Figure 1). Transcript abundances are
339 reported in Figures 4 and 5 as fold-change relative to the mean of the abundance for the
340 non-inoculated control of the same plant genotype within the same experiment (ddCt).
341 Figure 4 A-F show that even with two independent experiments that each sampled
342 infected zones from three independent root systems, we did not detect any significant
343 nematode-induced transcript abundance of any of the *rhg1-b* genes when plants were
344 inoculated at VE stage (emerging seedlings, cotyledons not yet separated). We also
345 observed no significant differences with plants inoculated at the V1 stage (fully expanded
346 trifoliate; Supplemental Figure 2).

347

348 **Combined infection by SCN and Pp induces a stronger salicylic acid response**

349 At either 3 dpi or 7 dpi, neither SCN, Pp nor their combination induced a significant
350 change in transcript abundance (ddCt) for the JA-response indicator gene '*AAT*' (Figure 5A
351 and 5B). Note that this established soybean JA-response indicator gene *AAT* (Beyer et al.
352 2021) is *Glyma.10G109500* and is not the *Rhg1* gene for AAT_{Rhg1}. In contrast to the JA
353 result, transcript abundances for the soybean SA-response indicator gene *NIMIN1*

354 (*Glyma.10G010100*) did exhibit statistically significant increases at 3 dpi in many of the
355 nematode-inoculated samples (Figure 5C). Only dual inoculation with SCN and Pp
356 consistently caused significant elevation of the SA indicator in all three soybean genotypes
357 (Figure 5C). The elevated SA response indicated by *NIMIN1* transcript levels was gone by
358 7 dpi (Figure 5D).

359

360 Discussion

361 Although SCN is the most economically important nematode of soybeans in the US,
362 in more tropical soybean producing states such as Brazil, RLN (especially *Pratylenchus*
363 *brachyurus*) has been reported to be more consequential (Castanheira et al. 2020;
364 Nomura et al. 2024). No soybean varieties labeled as having strong resistance to RLN
365 have been deployed but searches are underway (e.g., Chowdhury et al. 2022; Hamawaki
366 et al. 2019; Machado and Araújujo 2016). SCN and RLN frequently co-occur. Given the
367 importance of the soybean crop, it is crucial to understand if there are interactions
368 between the two nematodes on soybean and if that could lead to a change in their relative
369 abundance over time. Only limited surveys and studies are available on the tripartite
370 interaction of Soybean-SCN-RLN (Melakeberhan and Dey 2003; Schumacher and Bird
371 2025). At the time of writing this manuscript, no published reports are available on how the
372 deployed *Rhg1* resistance against SCN factors into this tripartite interaction.

373 Many soybean fields are infested with both SCN and RLN and the limited research
374 reported suggests that their interaction is complicated. MacGuidwin et al. suggested a
375 competitive relationship (population densities of both SCN and RLN suppressed by co-

376 occurence) based on an analysis of field survey data (MacGuidwin et al. 2023).
377 Melakeberhan and Dey tested four 1000-nematode inocula with varying proportions of *H.*
378 *glycines* and *P. penetrans* and found evidence of competition when SCN outnumbered Pp
379 3:1. When both species were equally represented the interaction appeared to be
380 antagonistic, as numbers of Pp increased and SCN numbers decreased as compared to
381 the single-species case (Melakeberhan and Dey 2003). Those experiments used HG 0
382 SCN on an SCN-susceptible soybean host. In the present study we also observed that
383 presence of SCN increased Pp numbers on susceptible hosts. This was true both on
384 Williams 82, which lacks SCN resistance, and on IL3025N, which carries *rhg1-b* but is
385 susceptible to the HG 2.5.7 IL (FI ~70) population that was used. Hypotheses for how SCN
386 assists Pp parasitism include effector-mediated suppression host defenses, and/or simply
387 by providing established entry points into the root.

388 We used HG 2.5.7 SCN both because it is informative with respect to *Rhg1* (it
389 overcomes *rhg1-b* but not *rhg1-a*), and because it is becoming the most common type of
390 SCN found in North Central U.S. soybean fields. The positive effect of SCN on Pp
391 population numbers was not observed on IL3849N, which carries *rhg1-a* + *Rhg4* SCN
392 resistance that is effective against HG 2.5.7 IL. The failure of SCN to increase Pp
393 numbers on IL3849N is a related topic for future study - it may for example be due to the
394 reduced number of SCN present on the resistant host after the first few days of infection,
395 and/or to the plant defense responses induced by SCN on the resistant host.

396 In our previous studies with *Rhg1* genes we had shown their capacity to increase
397 resistance to other cyst nematode species and genera (*Heterodera schactii*, *Globodera*

398 *pallida*, *Globodera rostochiensis*) when transgenically expressed in Arabidopsis or potato
399 (Butler et al. 2019). Similarly, the combined presence of SCN resistance loci *rhg1-a* and
400 *rhg2* was shown to also provide quantitative resistance against reniform nematodes
401 (*Rotylenchulus reniformis*) (Usovsky et al. 2021; Usovsky et al. 2022). For the present
402 study we hypothesized that SCN-resistant soybeans deployed in fields may have been
403 partially controlling RLN. Our data support that early (30 dpi) establishment of Pp is
404 impeded by *rhg1-a*, as evidenced by the reduction of Pp population sizes in IL3849N
405 relative to Williams 82. Early Pp establishment was also impeded on IL3025N (*rhg1-b*)
406 when initial SCN and Pp population densities were similar, but was not significant when
407 SCN outnumbered Pp. We further hypothesized that the interaction between SCN and Pp
408 could reduce the efficacy of *Rhg1*. In support of that, relative to inoculation of the same
409 plants with just Pp, we found an increase in Pp recovery when simultaneous SCN
410 pressure was applied on plants that are susceptible to the SCN population (Pp + HG 2.5.7
411 SCN on Williams 82 and IL3025N). Interestingly, for the virulent SCN HG 2.5.7 IL
412 population that we tested, counts of SCN cysts were not reduced by co-inoculation with Pp
413 except at 1:1 ratio when *rhg1-a* or *rhg1-b* were absent. A similar dynamic between SCN
414 and Pp was observed by Melakeberhan and Dey on multiple soybean cultivars that lacked
415 the *rhg1-a* or *rhg1-b* resistance (Melakeberhan and Dey 2003). We have not investigated
416 full-season resistance or yield protection (see also Pedro et al. 2024), but the reductions in
417 parasitism observed in the present study and that of Melakeberhan and Dey suggest a
418 potential contribution to full-season disease resistance against Pp. The field studies of
419 Saikai and MacGuidwin (Saikai and MacGuidwin 2022) were done using varieties with
420 *rhg1-b* resistance, so a yield loss impact of Pp on SCN-resistant soybean varieties has

421 been demonstrated. The present study suggests that Pp damage could be even worse if
422 use of *rhg1-b* soybeans was not prevalent, but further field-level and full-season or multi-
423 season studies are needed to investigate this point.

424 As noted above, in our experiments and those of Melakeberhan and Dey (2003) co-
425 inoculation with SCN helped Pp or was neutral, in all cases tested. Even on resistant
426 hosts, we observed no reduction of Pp at 30 dpi when co-inoculated with SCN. In
427 contrast, with wheat, Laserre et al. 1994 and Mokrini et al. 2018 both observed that on
428 susceptible hosts co-inoculation with cereal cyst nematode tended to reduce *Pratylenchus*
429 population sizes (Lasserre et al. 1994; Mokrini et al. 2018). Intriguingly Laserre and
430 colleagues also showed, on a susceptible host, a reduction in eggs and vermiform *P.*
431 *neglectus* upon inoculation of *H. avenae* onto a separate part of the root system. However,
432 on wheat accessions with resistance to *H. avenae* they observed that co-inoculation with
433 *H. avenae* led in some cases to higher numbers of *Pratylenchus*, similar to the trend in our
434 experiments (Lasserre et al. 1994).

435 The *rhg1-b* haplotype SCN resistance, unlike the *rhg1-a* haplotype resistance, is not
436 co-dependent on the *Rhg4* locus on chromosome 08 (Patil et al. 2019). We were able to
437 obtain near-isogenic lines for *rhg1-b* that, after four backcrosses, should be genetically
438 >96% identical, for a more well-controlled test of the contribution of *rhg1-b* to resistance
439 against Pp. As with IL3025N and IL3849N, we observed fewer Pp on Res. NIL plants
440 compared to the Sus. NIL plants when only Pp was inoculated, strongly linking *rhg1-b*
441 SCN resistance to partial Pp resistance as well. In addition to the overall reduction in Pp
442 numbers in the Res. NIL, we also observed reductions in the ratios of females to males
443 and juveniles to adults. RLN males and females differ in how they feed on the roots and

444 how much damage they can subsequently cause (Saikai and MacGuidwin 2020). Females
445 make larger lesions and burrow more frequently into the roots than the males. Hence
446 having overall fewer Pp and a population that is skewed towards adults, especially males,
447 may be less damaging to the crop. When considered together, these observations indicate
448 that *rhg1-b* imposes stress on Pp populations and further support the indication that *rhg1-b*
449 may be reducing the severity of damage to soybean caused by Pp.

450 In an earlier pair of studies, we had observed AAT_{Rhg1} protein abundance increasing
451 along SCN penetration path (Han et al. 2023), unlike the α-$SNAP_{Rhg1}$ that exhibited a
452 strong abundance increase in the syncytium (Bayless et al. 2016). Because Pp do not
453 form syncytia, in the present study we hypothesized that *Rhg1-GmAAT* could be one of the
454 early response genes that functions against diverse nematodes. However, for the genes
455 encoding AAT_{Rhg1}, α-$SNAP_{Rhg1}$ or $WI12_{Rhg1}$ we did not observe any statistically significant
456 transcript induction at 3 dpi for plants inoculated with Pp at the VE or the V1 stage, or at 7
457 dpi after VE stage inoculation. It is possible that any transcript induction is sufficiently local
458 to infected cells that the vast majority of non-infected cells in the root samples diluted out
459 any signal from cells at or immediately adjacent to the site of nematode infection. It is also
460 possible that expression of the *Rhg1* gene products is regulated in part at the post-
461 transcriptional level. This absence of an observed transcript induction is consistent with a
462 previous study where we saw no significant differences in transcript induction for α -
463 $SNAP_{Rhg1}$HC at 7 dpi but did observe significant changes in protein abundance (Haarith et
464 al. 2025).

465 Transcript abundances for *NIMIN1*, a preferred soybean salicylic acid response
466 pathway indicator (Beyer et al. 2021), were upregulated significantly at 3 dpi in response

467 to SCN, and were more reliably upregulated when Pp co-infected alongside SCN. Perhaps
468 of greater interest, Pp alone did not reproducibly elicit this SA response while Pp+SCN did
469 (Figure 5), and this Pp co-infection with SCN enhanced both SA responses and Pp
470 population sizes (Figure 1). Antagonism between SA- and JA-mediated defense
471 responses has been widely reported (Glazebrook et al. 2003; Verma et al. 2025). There
472 are multiple instances of a "host mis-direction" virulence strategy in which pathogens or
473 pests that are more effectively defended against by the one set of defense responses
474 cause the plant to instead activate the other defense responses (Schweiger et al. 2014).
475 The non-sedentary (migratory) parasitism exhibited by Pp may be more similar to plant
476 attack by herbivorous insects than it is to a haustorium-forming biotrophic fungus or
477 oomycete, or a syncytium-forming cyst nematode. Our results suggest that Pp may lose
478 more to JA-mediated plant defenses than to SA-mediated plant defenses; future studies
479 could investigate if Pp in fact benefits from stronger elicitation of SA-mediated defenses.

480 The findings of (Usovsky et al. 2021) correlating α-SNAP-encoding genetic loci with
481 resistance to both SCN and reniform nematode support speculation that α-SNAP protein
482 variants may also contribute to resistance against Pp. A large body of evidence has
483 implicated SNAP proteins in SCN resistance, including major roles for the α-SNAP$_{Rhg1}$HC
484 protein encoded by *rhg1-b* and the α-SNAP$_{Rhg1}$LC/GmSNAP18 protein encoded by *rhg1-a*,
485 and more subtle contributions from a γ-SNAP (gamma-SNAP) encoded at *cqSCN-006*
486 (*Glyma.15G191200*) and loss of the α-SNAPs encoded at *rhg2*/*GmSNAP02*
487 (*Glyma.02G260400*), and *GmSNAP11* (*Glyma.11g234500*) (Bayless et al. 2016; Bent
488 2022; Butler et al. 2021; Cook et al. 2014; Cook et al. 2012a; Haarith et al. 2025;
489 Lakhssassi et al. 2017; Liu et al. 2017; Matsye et al. 2012; Usovsky et al. 2023) SNAP

490 proteins interact with each other as they form multimers on SNARE protein bundles that
491 mediate subcellular vesicle trafficking (Zhao et al. 2015). Future research could reveal that
492 combinations of SCN resistance-associated α-SNAP-encoding loci may provide stronger
493 Pp resistance than *rhg1-b*, and/or provide diversified resistance mediators that impose
494 distinct selection pressures on Pp populations. However, a leading hypothesis for the
495 impact on cyst nematodes of the non-canonical SCN resistance-associated α-SNAPs is
496 their cytotoxicity and elevated abundance in syncytia (Bayless et al. 2016; Bayless et al.
497 2018). Pp are not sedentary feeders that induce syncytia formation in the host. The *Rhg1*-
498 encoded AAT_{Rhg1} and $WI12_{Rhg1}$ proteins also contribute to SCN resistance and are equally
499 strong candidates for contributing to Pp resistance.

500 Taken together, the present results indicate that the *rhg1-b* haplotype impedes the
501 parasitism of Pp as well as SCN – a pair of plant parasitic nematode species that are
502 highly distinct taxonomically and behaviorally. The present controlled environment results
503 also show, in agreement with a past study, that on SCN-susceptible soybean genotypes
504 SCN can enhance Pp population sizes. There is measurable interaction between the two
505 nematodes regarding the efficacy of *rhg1-b* resistance, and in their induction of salicylic
506 acid responses within the plant. The findings suggest multiple avenues for future study and
507 manipulation of soybean resistance to *Pratylenchus* root lesion nematodes.

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Author Contributions

DH conceptualized, designed, and performed the experiments, analyzed the data, and authored the manuscript. AEM provided Pp inoculum, methods and expertise in *Pratylenchus penetrans*, and edited the manuscript. AFB co-designed the experiments, and analyzed and co-authored the manuscript. AFB and DH are co-PIs on the grant that funded this study.

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525 ## Figure Captions

526 **Figure 1.** Nematode counts on Wm82, IL3849N (*rhg1*-a) and IL3025N (*rhg1*-b) plants at
527 30 dpi with either or both SCN HG 2.5.7 IL and Pp. (A) Pp counts at 30 dpi at 1:1 SCN: Pp
528 inoculation (B) Pp counts at 30 dpi at 2:1 SCN:Pp inoculation. Blue bars correspond to Pp-
529 only inoculation and orange bars represent combined inoculation of both SCN and Pp. (C)
530 Numbers of SCN cysts recovered at 30 dpi at 1:1 SCN:Pp inoculation. (D) Numbers of
531 SCN cysts recovered at 30 dpi at 2:1 SCN:Pp inoculation. Green bars represent SCN-only
532 inoculation and the light-blue bars represent combined inoculation of both SCN and Pp.
533 Values shown are mean +/- standard error of mean. Statistical significance for differences
534 between means are displayed as p-values from ANOVA Tukey HSD tests performed on
535 data transformed to achieve normality (see Methods). The SCN HG 2.5.7 IL used partially
536 overcomes *rhg1-b* resistance and represents the most prevalent HG type in the USA. All
537 data are for two independent experiments with five replicates within each experiment,
538 using distinct batches of seedlings and fresh nematode inoculum.

539

540 **Figure 2.** Pp counts on Wm82, Sus. NIL and Res. NIL plants at 30 dpi in either Pp-only
541 inoculations (blue bars) or a combination of SCN and Pp at 1:1 ratio (orange bars). (A)
542 Counts of all Pp. (B) Ratio of Pp females to males. (C) Ratio of Pp in sand fraction to root
543 fraction. (D) Ratio of Pp juveniles (J1 through J4) to adults (male + female). (E) Counts of
544 Pp juveniles (J1 through J4). Values shown are mean +/- standard error of mean.
545 Instances of possible significant differences of means are marked with p-values from
546 ANOVA Tukey HSD tests performed on data transformed to achieve normality (see

547 Methods; p > 0.2 if no p value is shown). The SCN HG 2.5.7 IL used partially overcomes
548 *rhg1-b* resistance and represents the most prevalent HG type in the USA. All data are for
549 two independent experiments with five replicates within each experiment, using distinct
550 batches of seedlings and fresh nematode inoculum.

551

552 **Figure 3.** SCN cyst counts on Wm82, Sus. NIL and Res. NIL plants at 30 dpi in either
553 SCN-only inoculation (green bars) or a combination of SCN and Pp at 1:1 ratio (light-blue
554 bars). (A) SCN cyst counts. (B) SCN J2 counts (sand + root). (C) SCN male counts (sand
555 + root). Values are mean +/- standard error of mean. Instances of possible significant
556 differences of means are marked with p-values from ANOVA Tukey HSD tests performed
557 on data transformed to achieve normality (see Methods) (p > 0.2 if no p value is shown).
558 The SCN HG 2.5.7 IL used partially overcomes *rhg1-b* resistance and represents the most
559 prevalent HG type in the USA. All data are for two independent experiments with five
560 replicates within each experiment, using distinct batches of seedlings and fresh nematode
561 inoculum.

562

563 **Figure 4.** *Rhg1* transcript induction (fold change) relative to (w.r.t.) no-nematode controls
564 within each plant genotype. The protein encoded by the analyzed transcript is noted in the
565 upper-left corner of each graph. Wm82, Sus. NIL and Res. NIL plants were inoculated at
566 VE stage and root samples from infection areas taken at: (A, C and E) 3 dpi, or (B, D and
567 F) 7 dpi. No statistically significant differences between samples were observed using
568 ANOVA and Tukey HSD test. The SCN HG 2.5.7 IL used partially overcomes *rhg1-b*

resistance and represents the most prevalent HG type in the USA. All data are for two independent experiments with four replicate roots assessed within each experiment, using distinct batches of seedlings and fresh nematode inoculum.

Figure 5. Induction (fold change) of JA-response indicator gene *Glyma.10G109500* and SA-response indicator gene *Glyma.10G010100* transcripts at 3 dpi and 7dpi, relative to (w.r.t.) no-nematode controls within each plant genotype within the same experiment. Wm82, Sus. NIL and Res. NIL soybean accessions inoculated at VE stage; transcript measured and sample dpi indicated in upper left corner of graph. Significant differences of means are marked with p-values from ANOVA Tukey HSD tests ($p > 0.2$ if no p value is shown). The SCN HG 2.5.7 IL used partially overcomes *rhg1-b* resistance and represents the most prevalent HG type in the USA. All data are for two independent experiments with four replicate roots assessed within each experiment, using distinct batches of seedlings and fresh nematode inoculum.

Supplemental Figure 1. Same data as in Figure 4 but with data for all genotypes normalized to mean Sus. NIL no nematode control within same experiment, to show relative abundances between genotypes. *Rhg1* transcript induction (fold change) compared to no-nematode controls within each plant genotype. The protein encoded by the analyzed transcript is noted in the upper-left corner of each graph. Wm82, Sus. NIL and Res. NIL plants were inoculated at VE stage and root samples from infection areas taken at: (A, C, E and G) 3 dpi, or (B, D, F and H) 7 dpi. No statistically significant

differences between samples were observed using ANOVA and Tukey HSD test. The SCN HG 2.5.7 IL used partially overcomes *rhg1-b* resistance and represents the most prevalent HG type in the USA. All data are for two independent experiments with four replicate roots assessed within each experiment, using distinct batches of seedlings and fresh nematode inoculum.

Supplemental Figure 2. *Rhg1* transcript induction (fold change) for V1 plants at 3 dpi compared to no-nematode controls within each plant genotype. The protein encoded by the analyzed transcript is noted in the upper-left corner of each graph. Wm82, Sus. NIL and Res. NIL plants were inoculated at V1 stage. (A, C, E) Data normalized to mean for no-nematode control of same genotype in same experiment. (B, D, F) Same data as A, C, E with data for all genotypes normalized to mean Sus. NIL no nematode control within same experiment, to show relative abundances between genotypes. Instances of possible significant differences of means are marked with p-values from ANOVA Tukey HSD tests ($p > 0.2$ if no p value is shown). The SCN HG 2.5.7 IL used partially overcomes *rhg1-b* resistance and represents the most prevalent HG type in the USA. All experiments were repeated twice with three replicate roots assessed within each experiment, using distinct batches of seedlings and fresh nematode inoculum.

611 **Literature Cited**

612 Allen, T. W., Bradley, C. A., Sisson, A. J., Byamukama, E., Chilvers, M. I., Coker, C. M.,
613 Collins, A. A., Damicone, J. P., Dorrance, A. E., Dufault, N. S., Esker, P. D., Faske,
614 T. R., Giesler, L. J., Grybauskas, A. P., Hershman, D. E., Hollier, C. A., Isakeit, T.,
615 Jardine, D. J., Kelly, H. M., Kemerait, R. C., Kleczewski, N. M., Koenning, S. R.,
616 Kurle, J. E., Malvick, D. K., Markell, S. G., Mehl, H. L., Mueller, D. S., Mueller, J. D.,
617 Mulrooney, R. P., Nelson, B. D., Newman, M. A., Osborne, L., Overstreet, C.,
618 Padgett, G. B., Phipps, P. M., Price, P. P., Sikora, E. J., Smith, D. L., Spurlock, T. N.,
619 Tande, C. A., Tenuta, A. U., Wise, K. A., and Wrather, J. A. 2017. Soybean Yield
620 Loss Estimates Due to Diseases in the United States and Ontario, Canada, from
621 2010 to 2014. Plant Health Progress 18:19-27.
622 Baermann, G. 1917. Eine einfache Methode zur Auffindung von Anklostomum
623 (Nematoden) Larven in Erdproben. Geneeskundig tijdschrift voor Nederlandsch-
624 Indië 57:131-137.
625 Basnet, P., Meinhardt, C. G., Usovsky, M., Gillman, J. D., Joshi, T., Song, Q., Diers, B.,
626 Mitchum, M. G., and Scaboo, A. M. 2022. Epistatic interaction between Rhg1-a and
627 Rhg2 in PI 90763 confers resistance to virulent soybean cyst nematode
628 populations. Theor Appl Genet 135:2025-2039.
629 Bayless, A. M., Zapotocny, R. W., Grunwald, D. J., Amundson, K. K., Diers, B. W., and
630 Bent, A. F. 2018. An atypical N-ethylmaleimide sensitive factor enables the viability
631 of nematode-resistant Rhg1 soybeans. Proc Natl Acad Sci U S A 115:E4512-E4521.
632 Bayless, A. M., Zapotocny, R. W., Han, S., Grunwald, D. J., Amundson, K. K., and Bent, A.
633 F. 2019. The rhg1-a (Rhg1 low-copy) nematode resistance source harbors a copia-
634 family retrotransposon within the Rhg1-encoded alpha-SNAP gene. Plant Direct
635 3:e00164.
636 Bayless, A. M., Smith, J. M., Song, J., McMinn, P. H., Teillet, A., August, B. K., and Bent, A.
637 F. 2016. Disease resistance through impairment of alpha-SNAP-NSF interaction
638 and vesicular trafficking by soybean Rhg1. Proc Natl Acad Sci U S A 113:E7375-
639 E7382.
640 Bent, A. 2022. Exploring Soybean Resistance to Soybean Cyst Nematode. Annu Rev
641 Phytopathol 60:379-409.
642 Beyer, S. F., Bel, P. S., Flors, V., Schultheiss, H., Conrath, U., and Langenbach, C. J. G.
643 2021. Disclosure of salicylic acid and jasmonic acid-responsive genes provides a
644 molecular tool for deciphering stress responses in soybean. Sci Rep 11:20600.
645 Bradley, C. A., Allen, T. W., Sisson, A. J., Bergstrom, G. C., Bissonnette, K. M., Bond, J.,
646 Byamukama, E., Chilvers, M. I., Collins, A. A., Damicone, J. P., Dorrance, A. E.,
647 Dufault, N. S., Esker, P. D., Faske, T. R., Fiorellino, N. M., Giesler, L. J., Hartman,
648 G. L., Hollier, C. A., Isakeit, T., Jackson-Ziems, T. A., Jardine, D. J., Kelly, H. M.,
649 Kemerait, R. C., Kleczewski, N. M., Koehler, A. M., Kratochvil, R. J., Kurle, J. E.,
650 Malvick, D. K., Markell, S. G., Mathew, F. M., Mehl, H. L., Mehl, K. M., Mueller, D.
651 S., Mueller, J. D., Nelson, B. D., Overstreet, C., Padgett, G. B., Price, P. P., Sikora,
652 E. J., Small, I., Smith, D. L., Spurlock, T. N., Tande, C. A., Telenko, D. E. P., Tenuta,
653 A. U., Thiessen, L. D., Warner, F., Wiebold, W. J., and Wise, K. A. 2021. Soybean
654 Yield Loss Estimates Due to Diseases in the United States and Ontario, Canada,
655 from 2015 to 2019. Plant Health Progress 22:483-495.

656 Brucker, E., Carlson, S., Wright, E., Niblack, T., and Diers, B. 2005. Rhg1 alleles from
657 soybean PI 437654 and PI 88788 respond differentially to isolates of Heterodera
658 glycines in the greenhouse. Theor Appl Genet 111:44-49.
659 Brzostowski, L. F., and Diers, B. W. 2017. Pyramiding of Alleles from Multiple Sources
660 Increases the Resistance of Soybean to Highly Virulent Soybean Cyst Nematode
661 Isolates. Crop Science 57:2932-2941.
662 Butler, K. J., Chen, S., Smith, J. M., Wang, X., and Bent, A. F. 2019. Soybean Resistance
663 Locus Rhg1 Confers Resistance to Multiple Cyst Nematodes in Diverse Plant
664 Species. Phytopathology 109:2107-2115.
665 Butler, K. J., Fliege, C., Zapotocny, R., Diers, B., Hudson, M., and Bent, A. F. 2021.
666 Soybean Cyst Nematode Resistance Quantitative Trait Locus cqSCN-006 Alters the
667 Expression of a gamma-SNAP Protein. Mol Plant Microbe Interact 34:1433-1445.
668 Castanheira, C. M., Falcão, H. G., Ida, E. I., Dias-Arieira, C. R., and Bolanho Barros, B. C.
669 2020. Pratylenchus brachyurus parasitism on soybean: effects on productivity,
670 vegetative and nematological parameters and chemical properties. European
671 Journal of Plant Pathology 157:651-661.
672 Chen, S., Shaeffer, C. C., Wyse, D. L., Nickel, P., and Kandel, H. 2012. Plant-parasitic
673 Nematode Communities and Their Associations with Soil Factors in Organically
674 Farmed Fields in Minnesota. Journal of Nematology 44:361-369.
675 Chowdhury, I. A., Yan, G. P., Kandel, H., and Plaisance, A. 2022. Population Development
676 of the Root-Lesion Nematode on Soybean Cultivars. Plant Disease 106:2117-2126.
677 Colgrove, A. L., and Niblack, T. L. 2005. The effect of resistant soybean on male and
678 female development and adult sex ratios of. Journal of Nematology 37:161-167.
679 Cook, D. E., Bayless, A. M., Wang, K., Guo, X., Song, Q., Jiang, J., and Bent, A. F. 2014.
680 Distinct Copy Number, Coding Sequence, and Locus Methylation Patterns Underlie
681 Rhg1-Mediated Soybean Resistance to Soybean Cyst Nematode. Plant Physiol
682 165:630-647.
683 Cook, D. E., Lee, T. G., Guo, X., Melito, S., Wang, K., Bayless, A. M., Wang, J., Hughes, T.
684 J., Willis, D. K., Clemente, T. E., Diers, B., Jiang, J., Hudson, M., and Bent, A. F.
685 2012a. Copy Number Variation of Multiple Genes at Rhg1 Mediates Nematode
686 Resistance in Soybean. Science 338:1206-1209.
687 Cook, D. E., Lee, T. G., Guo, X., Melito, S., Wang, K., Bayless, A. M., Wang, J., Hughes, T.
688 J., Willis, D. K., Clemente, T. E., Diers, B. W., Jiang, J., Hudson, M. E., and Bent, A.
689 F. 2012b. Copy number variation of multiple genes at Rhg1 mediates nematode
690 resistance in soybean. Science 338:1206-1209.
691 Dong, J., and Hudson, M. E. 2022. WI12(Rhg1) interacts with DELLAs and mediates
692 soybean cyst nematode resistance through hormone pathways. Plant Biotechnol J
693 20:283-296.
694 Endo, B. Y. 1967. Comparitive Population Increase of Pratylenchus brachyurus and P.
695 zeae in Corn and in Soybean Varieties Lee and Peking. Phytopathology 57:118-
696 120.
697 Favoreto, L., Meyer, M. C., Dias-Arieira, C. R., Machado, A. C. Z., Santiago, D. C., and
698 Riberiro, N. R. 2019. Diagnosis and Management of Nematodes In Soybean Crop.
699 Informe Agropecuario 40:18-29.
700 Glazebrook, J., Chen, W. J., Estes, B., Chang, H. S., Nawrath, C., Metraux, J. P., Zhu, T.,
701 and Katagiri, F. 2003. Topology of the network integrating salicylate and jasmonate

signal transduction derived from global expression phenotyping. Plant Journal 34:217-228.

Haarith, D., Das, S., Nelson, E., Zapotocny, R., and Bent, A. F. 2025. Overexpression of α-SNAPRhg1 Can Improve rhg1-a Mediated Soybean Resistance to Soybean Cyst Nematode. Phytopathology.

Hamawaki, O. T., Hamawaki, R. L., Nogueira, A. P. O., Glasenapp, J. S., Hamawaki, C. D. L., and da Silva, C. O. 2019. Evaluation of soybean breeding lineages to new sources of root-knot nematode resistance. Cienc Agrotec 43.

Han, S., Smith, J. M., Du, Y., and Bent, A. F. 2023. Soybean transporter AAT Rhg1 abundance increases along the nematode migration path and impacts vesiculation and ROS. Plant Physiol 192:133-153.

Jones, J. T., Haegeman, A., Danchin, E. G., Gaur, H. S., Helder, J., Jones, M. G., Kikuchi, T., Manzanilla-Lopez, R., Palomares-Rius, J. E., Wesemael, W. M., and Perry, R. N. 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. Mol Plant Pathol 14:946-961.

Kleczewski, N., Colsgrove, A., Bowman, D., Harbach, C., and Plewa, D. 2023. A Survey of Soybean Cyst Nematode Population Densities and Phenotypes in Illinois: 2018 and 2020. Plant Health Progress 24:221-225.

Lakhssassi, N., Liu, S., Bekal, S., Zhou, Z., Colantonio, V., Lambert, K., Barakat, A., and Meksem, K. 2017. Characterization of the Soluble NSF Attachment Protein gene family identifies two members involved in additive resistance to a plant pathogen. Sci Rep 7:45226.

Lasserre, F., Rivoal, R., and Cook, R. 1994. Interactions between Heterodera-Avenae and Pratylenchus-Neglectus on Wheat. Journal of Nematology 26:336-344.

Liu, S., Kandoth, P. K., Lakhssassi, N., Kang, J., Colantonio, V., Heinz, R., Yeckel, G., Zhou, Z., Bekal, S., Dapprich, J., Rotter, B., Cianzio, S., Mitchum, M. G., and Meksem, K. 2017. The soybean GmSNAP18 gene underlies two types of resistance to soybean cyst nematode. Nat Commun 8:14822.

Lopez-Nicora, H. D., Ralston, T. I., Diers, B. W., Dorrance, A. E., and Niblack, T. L. 2023. Interactions Among Heterodera glycines, Macrophomina phaseolina, and Soybean Genotype. Plant Dis 107:401-412.

MacGuidwin, A. E., and Bender, B. E. 2012. Estimating Population Densities of Root Lesion Nematodes, Pratylenchus spp., from Soil Samples Using Dual Active and Passive Assays. Plant Health Progress 13.

MacGuidwin, A. E., and Bender, B. E. 2016. Development of a Damage Function Model for Pratylenchus penetrans on Corn. Plant Dis 100:764-769.

MacGuidwin, A. E., Smith, D. L., Conley, S. P., and Saikai, K. A. 2023. Prevalence of Pest Nematodes Associated with Soybean (*Glycine max*) in Wisconsin from 1998 to 2021. J Nematol 55:20230053.

Machado, A. C. Z., and Araújujo, J. V. 2016. Broad-sense heritability and variance component estimates for resistance in Brazilian soybean genotypes. Tropical Plant Pathology 41:390-396.

Matsye, P. D., Lawrence, G. W., Youssef, R. M., Kim, K. H., Lawrence, K. S., Matthews, B. F., and Klink, V. P. 2012. The expression of a naturally occurring, truncated allele of an alpha-SNAP gene suppresses plant parasitic nematode infection. Plant Mol Biol 80:131-155.

McCarville, M. T., Marett, C. C., Mullaney, M. P., Gebhart, G. D., and Tylka, G. L. 2017. Increase in Soybean Cyst Nematode Virulence and Reproduction on Resistant Soybean Varieties in Iowa From 2001 to 2015 and the Effects on Soybean Yields. Plant Health Progress 18:146-155.

Melakeberhan, H., and Dey, J. 2003. Competition between *Heterodera glycines* and *Meloidogyne incognita* or *Pratylenchus penetrans*: Independent Infection Rate Measurements. Journal of Nematology 35:1-6.

Mokrini, F., Viaene, N., Waeyenberge, L., Dababat, A. A., and Moens, M. 2018. Investigation of resistance to *Pratylenchus penetrans* and *P. thornei* in international wheat lines and its durability when inoculated together with the cereal cyst nematode *Heterodera avenae*, using qPCR for nematode quantification. European Journal of Plant Pathology 151:875-889.

Niblack, T. L., Hussey, R. S., and Boerma, H. R. 1986. Effects of Interactions among Heterodera-Glycines, Meloidogyne-Incognita, and Host Genotype on Soybean Yield and Nematode Population-Densities. Journal of Nematology 18:436-443.

Niblack, T. L., Lambert, K. N., and Tylka, G. L. 2006. A model plant pathogen from the kingdom animalia: Heterodera glycines, the soybean cyst nematode. Annual Review of Phytopathology 44:283-303.

Nomura, R. B. G., Lopes-Caitar, V. S., Hishinuma-Silva, S. M., Machado, A. C. Z., Meyer, M. C., and Marcelino-Guimarães, F. C. 2024. Pratylenchus brachyurus: status and perspectives in Brazilian agriculture. Tropical Plant Pathology 49:573-589.

Patil, G. B., Lakhssassi, N., Wan, J., Song, L., Zhou, Z., Klepadlo, M., Vuong, T. D., Stec, A. O., Kahil, S. S., Colantonio, V., Valliyodan, B., Rice, J. H., Piya, S., Hewezi, T., Stupar, R. M., Meksem, K., and Nguyen, H. T. 2019. Whole-genome re-sequencing reveals the impact of the interaction of copy number variants of the rhg1 and Rhg4 genes on broad-based resistance to soybean cyst nematode. Plant Biotechnol J 17:1595-1611.

Pedro, M. S., da Silva, S. A., Picoli, L. H., da Cunha, L. S., and Machado, A. C. Z. 2024. Experimental and statistical approaches to evaluate the reaction of soybean to. Tropical Plant Pathology 49:612-621.

Rosa de Araújo, T., de Assis dos Santos Diniz, F., Mendes, B. L., Almeida, L. C., and Santiago, T. R. 2025. Pratylenchus zeae is the main root lesion nematode affecting sugarcane in the world's most important producing regions. Physiological and Molecular Plant Pathology 136.

Saikai, K., and MacGuidwin, A. E. 2020. Difference in Lesion Formation by Male and Female *Pratylenchus penetrans*. Journal of Nematology 52.

Saikai, K., and MacGuidwin, A. E. 2022. Impact of Pratylenchus penetrans on Soybean Grown in Wisconsin, U.S.A. Plant Dis 106:2904-2910.

Schmitt, D. P., and Barker, K. R. 1981. Damage and Reproductive Potentials of Pratylenchus-Brachyurus and Pratylenchus-Penetrans on Soybean. Journal of Nematology 13:327-332.

Schumacher, L. A., and Bird, G. W. 2025. Distribution of *Pratylenchus* spp. and *Heterodera glycines* in Michigan and Combined Reproduction on Peking and PI 437654 Soybean. Plant Health Progress.

792 Schweiger, R., Heise, A. M., Persicke, M., and Muller, C. 2014. Interactions between the
793 jasmonic and salicylic acid pathway modulate the plant metabolome and affect
794 herbivores of different feeding types. Plant Cell Environ 37:1574-1585.
795 Townshend, J. L. 1984. Anhydrobiosis in *Pratylenchus penetrans*. Journal of Nematology
796 16:282-289.
797 Tylka, G. L., Gebhart, G. D., Marrett, C. C., and Mullaney, M. P. 2012. Evaluation of
798 soybean varieties resistant to soybean cyst nematode in Iowa in 2012. Iowa State
799 University Extension and Outreach.
800 Usovsky, M., Robert T. Robbins, Juliet Fultz Wilkes, Devany Crippen, Vijay Shankar, Tri D.
801 Vuong, Paula Agudelo, and Nguyen, H. T. 2022. Classification methods and
802 identification of reniform nematode resistance in known soybean cyst nematode
803 resistant soybean genotypes. Plant Disease 106:382-389.
804 Usovsky, M., Lakhssassi, N., Patil, G. B., Vuong, T. D., Piya, S., Hewezi, T., Robbins, R. T.,
805 Stupar, R. M., Meksem, K., and Nguyen, H. T. 2021. Dissecting nematode
806 resistance regions in soybean revealed pleiotropic effect of soybean cyst and
807 reniform nematode resistance genes. Plant Genome 14:e20083.
808 Usovsky, M., Gamage, V. A., Meinhardt, C. G., Dietz, N., Triller, M., Basnet, P., Gillman, J.
809 D., Bilyeu, K. D., Song, Q., Dhital, B., Nguyen, A., Mitchum, M. G., and Scaboo, A.
810 M. 2023. Loss-of-function of an alpha-SNAP gene confers resistance to soybean
811 cyst nematode. Nat Commun 14:7629.
812 Verma, K., Kumari, K., Rawat, M., Devi, K., and Joshi, R. 2025. Crosstalk of Jasmonic acid
813 and Salicylic acid with other Phytohormones Alleviates Abiotic and Biotic Stresses
814 in Plants. Journal of Soil Science and Plant Nutrition 25:4997-5019.
815 Yu, N., Lee, T. G., Rosa, D. P., Hudson, M., and Diers, B. W. 2016. Impact of Rhg1 copy
816 number, type, and interaction with Rhg4 on resistance to Heterodera glycines in
817 soybean. Theor Appl Genet 129:2403-2412.
818 Zhao, M., Wu, S., Zhou, Q., Vivona, S., Cipriano, D. J., Cheng, Y., and Brunger, A. T. 2015.
819 Mechanistic insights into the recycling machine of the SNARE complex. Nature
820 518:61-67.

821

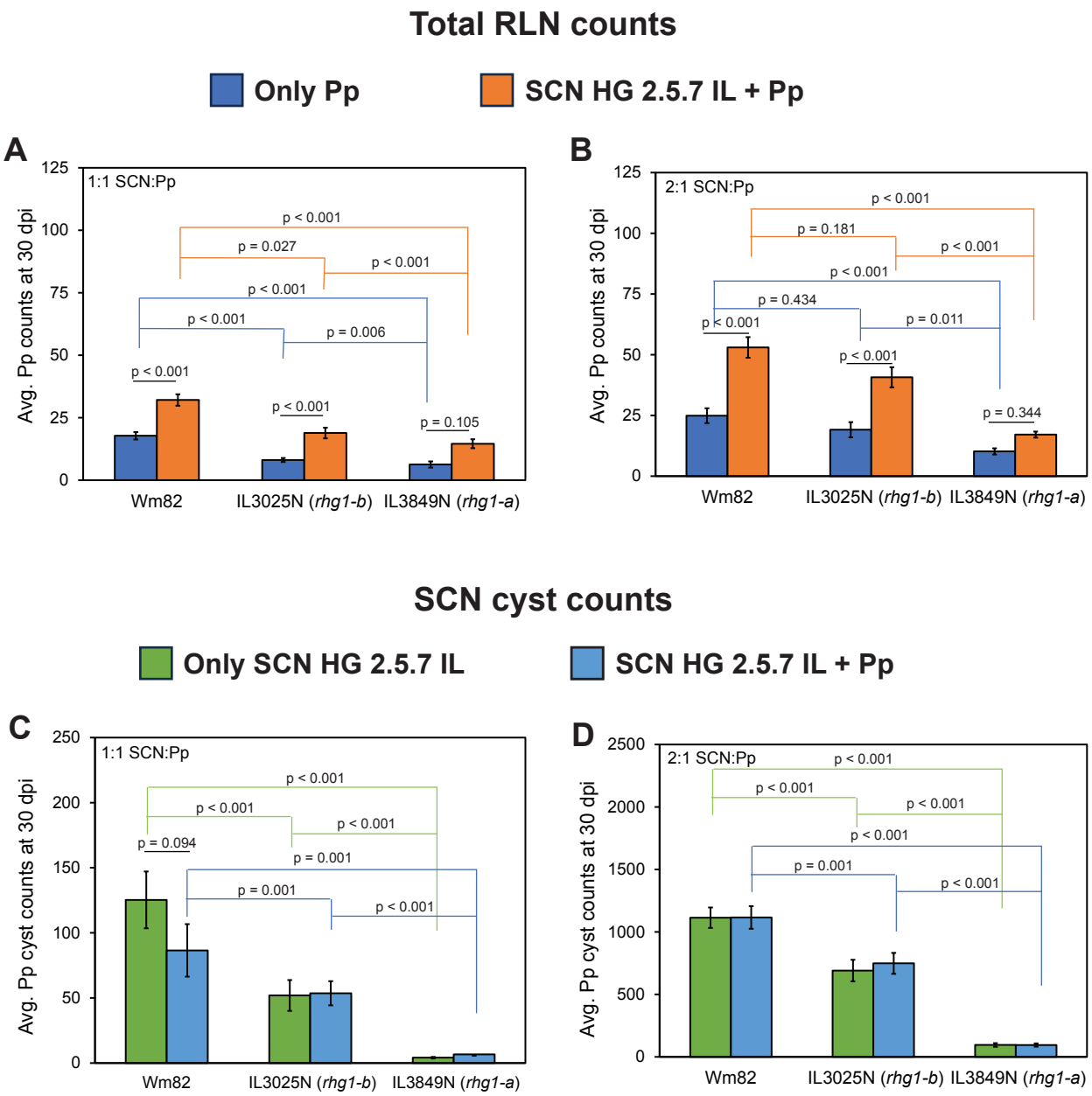

Figure 1. Nematode counts on Wm82, IL3849N (*rhg1-a*) and IL3025N (*rhg1-b*) plants at 30 dpi with either or both SCN HG 2.5.7 IL and Pp. (A) Pp counts at 30 dpi at 1:1 SCN: Pp inoculation (B) Pp counts at 30 dpi at 2:1 SCN:Pp inoculation. Blue bars correspond to Pp-only inoculation and orange bars represent combined inoculation of both SCN and Pp. (C) Numbers of SCN cysts recovered at 30 dpi at 1:1 SCN:Pp inoculation. (D) Numbers of SCN cysts recovered at 30 dpi at 2:1 SCN:Pp inoculation. Green bars represent SCN-only inoculation and the light-blue bars represent combined inoculation of both SCN and Pp. Values shown are mean +/- standard error of mean. Statistical significance for differences between means are displayed as p-values from ANOVA Tukey HSD tests performed on data transformed to achieve normality (see Methods). The SCN HG 2.5.7 IL used partially overcomes *rhg1-b* resistance and represents the most prevalent HG type in the USA. All data are for two independent experiments with five replicates within each experiment, using distinct batches of seedlings and fresh nematode inoculum.

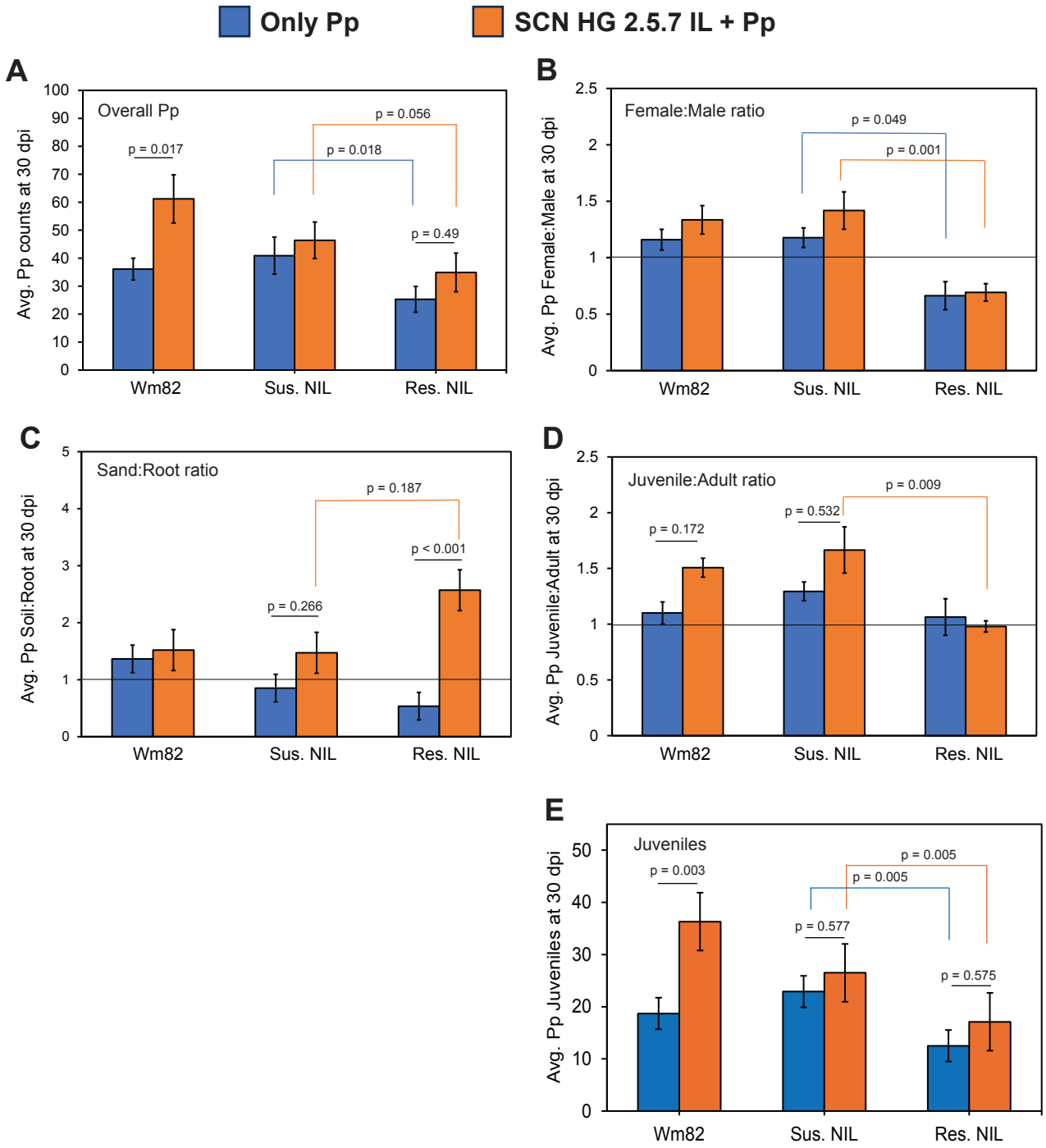

Figure 2. Pp counts on Wm82, Sus. NIL and Res. NIL plants at 30 dpi in either Pp-only inoculations (blue bars) or a combination of SCN and Pp at 1:1 ratio (orange bars). (A) Counts of all Pp. (B) Ratio of Pp females to males. (C) Ratio of Pp in sand fraction to root fraction. (D) Ratio of Pp juveniles (J1 through J4) to adults (male + female). (E) Counts of Pp juveniles (J1 through J4). Values shown are mean +/- standard error of mean. Instances of possible significant differences of means are marked with p-values from ANOVA Tukey HSD tests performed on data transformed to achieve normality (see Methods; $p > 0.2$ if no p value is shown). The SCN HG 2.5.7 IL used partially overcomes *rhg1-b* resistance and represents the most prevalent HG type in the USA. All data are for two independent experiments with five replicates within each experiment, using distinct batches of seedlings and fresh nematode inoculum.

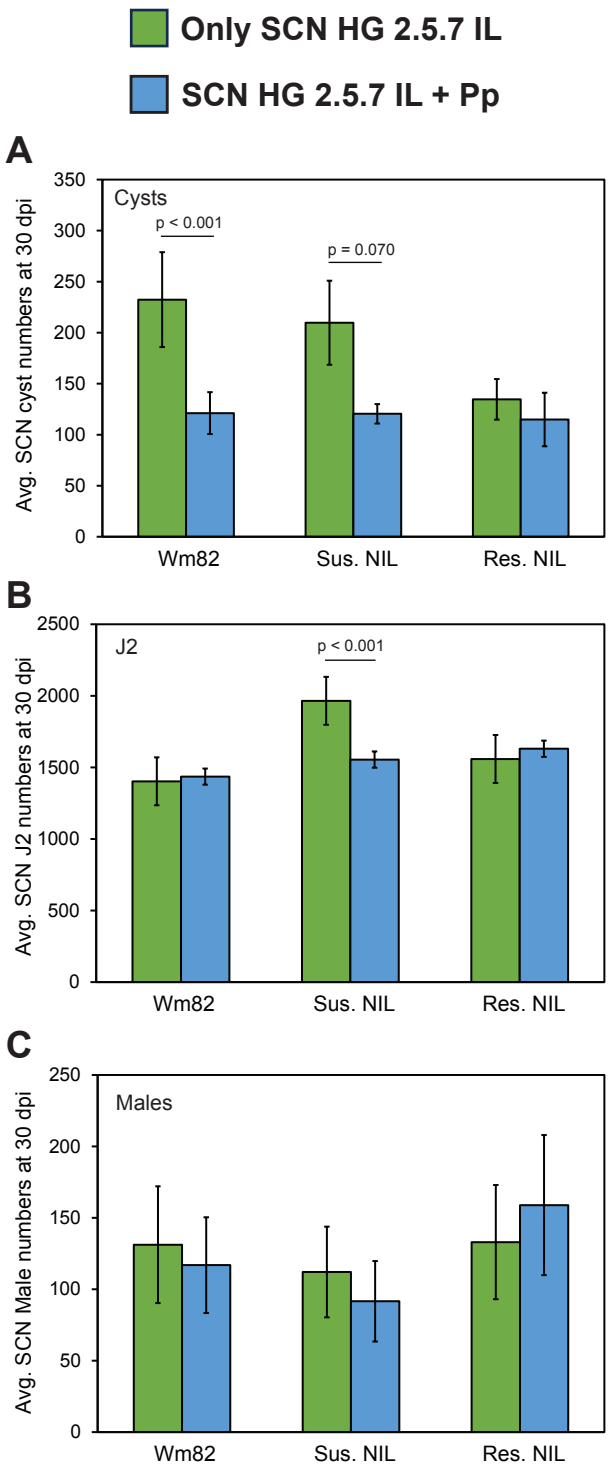

Figure 3. SCN cyst counts on Wm82, Sus. NIL and Res. NIL plants at 30 dpi in either SCN-only inoculation (green bars) or a combination of SCN and Pp at 1:1 ratio (light-blue bars). (A) SCN cyst counts. (B) SCN J2 counts (sand + root). (C) SCN male counts (sand + root). Values are mean +/- standard error of mean. Instances of possible significant differences of means are marked with p-values from ANOVA Tukey HSD tests performed on data transformed to achieve normality (see Methods) ($p > 0.2$ if no p value is shown). The SCN HG 2.5.7 IL used partially overcomes *rhg1-b* resistance and represents the most prevalent HG type in the USA. All data are for two independent experiments with five replicates within each experiment, using distinct batches of seedlings and fresh nematode inoculum.

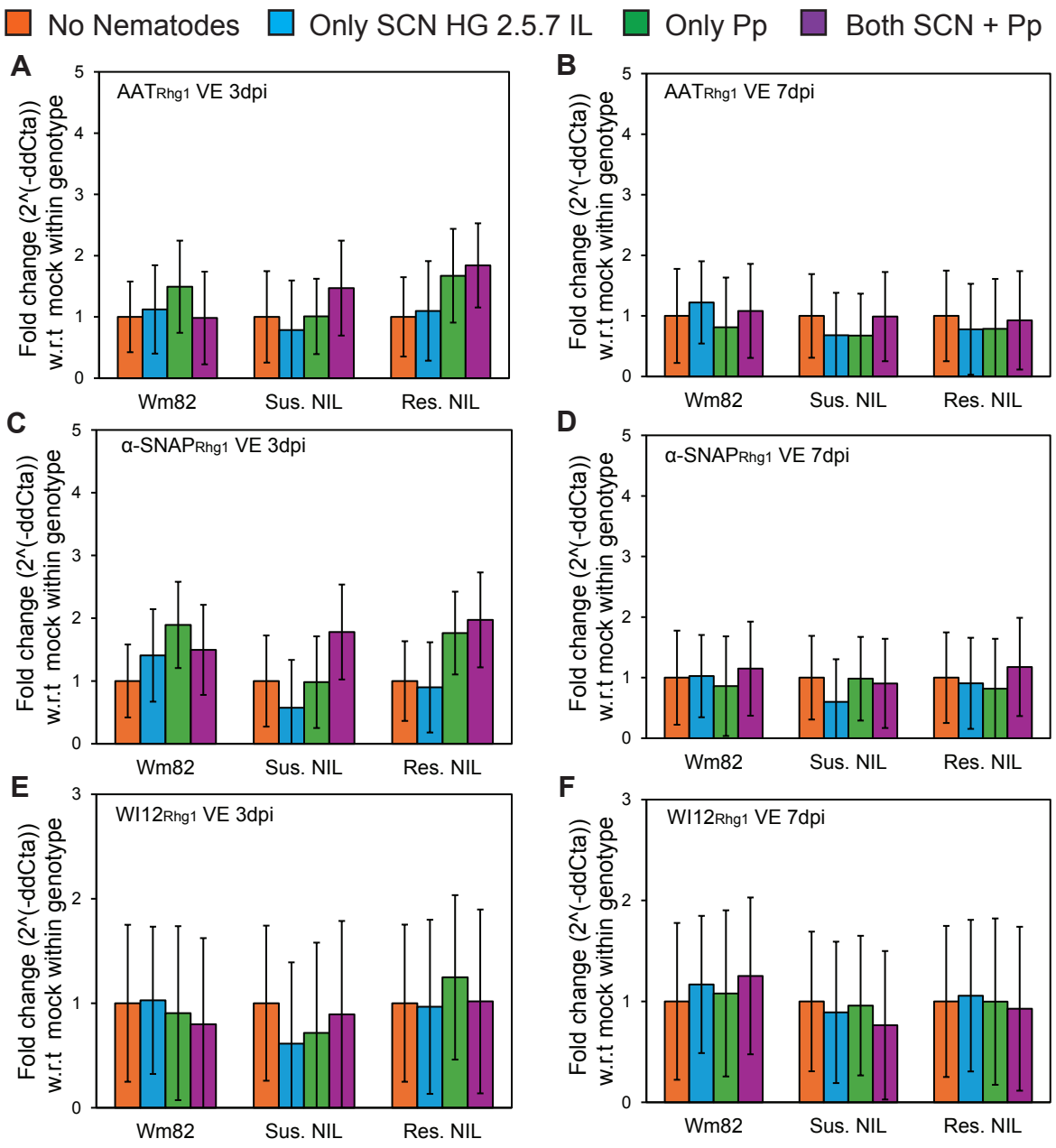

Figure 4. *Rhg1* transcript induction (fold change) relative to (w.r.t.) no-nematode controls within each plant genotype. The protein encoded by the analyzed transcript is noted in the upper-left corner of each graph. Wm82, Sus. NIL and Res. NIL plants were inoculated at VE stage and root samples from infection areas taken at: (A, C and E) 3 dpi, or (B, D and F) 7 dpi. No statistically significant differences between samples were observed using ANOVA and Tukey HSD test. The SCN HG 2.5.7 IL used partially overcomes *rhg1-b* resistance and represents the most prevalent HG type in the USA. All data are for two independent experiments with four replicate roots assessed within each experiment, using distinct batches of seedlings and fresh nematode inoculum.

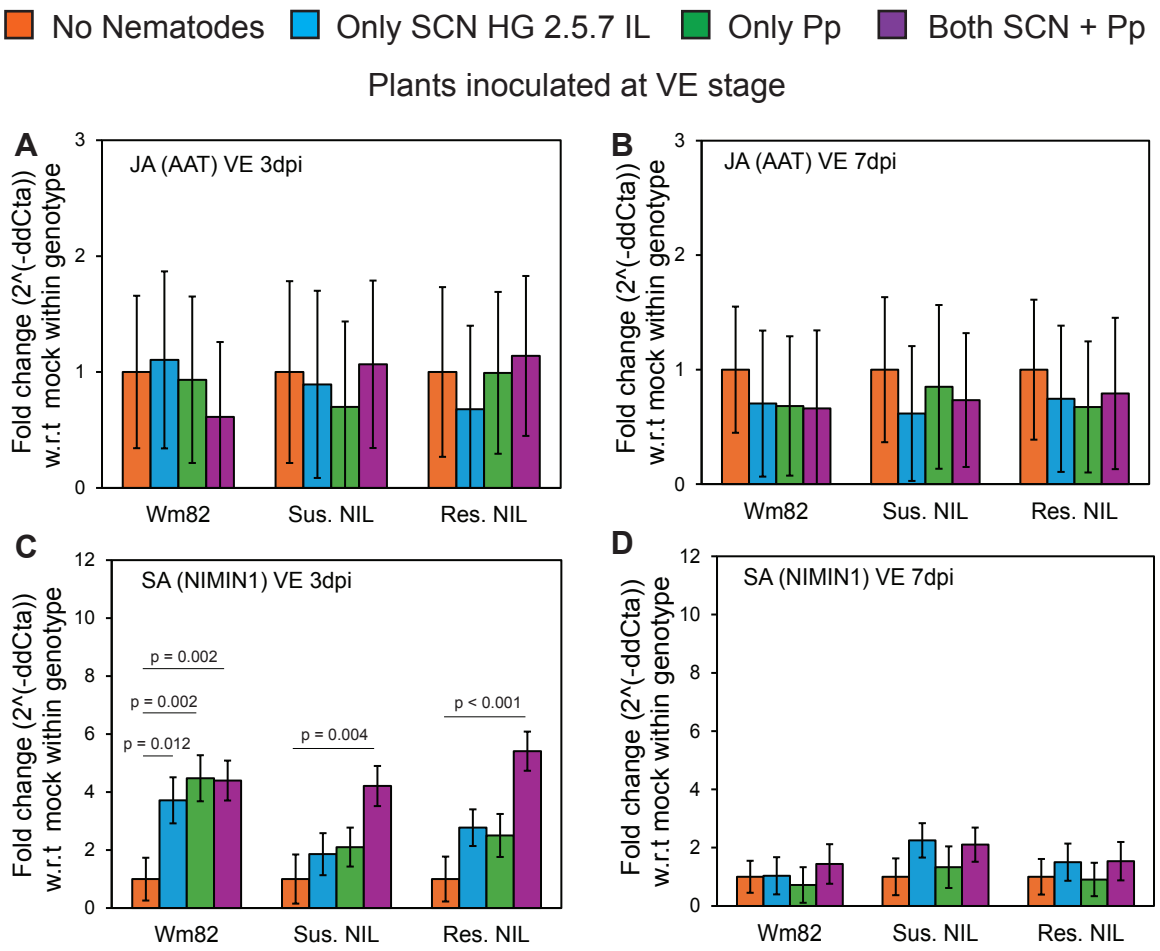

Figure 5. Induction (fold change) of JA-response indicator gene *Glyma.10G109500* and SA-response indicator gene *Glyma.10G010100* transcripts at 3 dpi and 7dpi, relative to (w.r.t.) no-nematode controls within each plant genotype within the same experiment. Wm82, Sus. NIL and Res. NIL soybean accessions inoculated at VE stage; transcript measured and sample dpi indicated in upper left corner of graph. Significant differences of means are marked with p-values from ANOVA Tukey HSD tests ($p > 0.2$ if no p value is shown). The SCN HG 2.5.7 IL used partially overcomes *rhg1-b* resistance and represents the most prevalent HG type in the USA. All data are for two independent experiments with four replicate roots assessed within each experiment, using distinct batches of seedlings and fresh nematode inoculum.