Soybean Resistance Locus *Rhg1* Confers Resistance to Multiple Cyst Nematodes in Diverse Plant Species

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ABSTRACT

Cyst nematodes consistently threaten agricultural production, causing billions of dollars in losses globally. The *Rhg1* (resistance to *Heterodera glycines* 1) locus of soybean (*Glycine max*) is the most popular resistance source used against soybean cyst nematodes (*H. glycines*). *Rhg1* is a complex locus that has multiple repeats of an ≈30-kilobase segment carrying three genes that contribute to resistance. We investigated whether soybean *Rhg1* could function in different plant families, conferring resistance to their respective cyst nematode parasites. Transgenic *Arabidopsis thaliana* and potato (*Solanum tuberosum*) plants expressing the three soybean *Rhg1* genes were generated. The recipient Brassicaceae and Solanaceae plant species exhibited elevated resistance to *H. schachtii* and *Globodera rostochiensis* and to *G. pallida*, respectively. However, some negative consequences including reduced root growth and tuber biomass were observed upon *Rhg1* expression in heterologous species. One of the genes at *Rhg1*

encodes a toxic version of an alpha-SNAP protein that has been demonstrated to interfere with vesicle trafficking. Using a transient expression assay for *Nicotiana benthamiana*, native *Arabidopsis* and potato alpha-SNAPs (soluble NSF [N-ethylamine sensitive factor] attachment protein) were found to compensate for the toxicity of soybean *Rhg1* alpha-SNAP proteins. Hence, future manipulation of the balance between *Rhg1* alpha-SNAP and the endogenous wild-type alpha-SNAPs (as well as the recently discovered soybean NSF-RAN07) may mitigate impacts of *Rhg1* on plant productivity. The multispecies efficacy of soybean *Rhg1* demonstrates that the encoded mechanisms can function across plant and cyst nematode species and offers a possible avenue for engineered resistance in diverse crop species.

Keywords: genetics and resistance, nematology

Cyst nematodes in the *Heteroderinae* subfamily consistently threaten agricultural production and annually cause substantial crop losses (Nicol et al. 2011). Cyst nematodes dramatically reprogram certain host root cells to form a syncytium, a feeding structure characterized by fusion of adjacent root cells to form a large multinucleate cell with dense cytoplasm and substantially smaller vacuoles (Endo 1964; Kyndt et al. 2013).

Most major crops have co-adapted species of cyst nematodes that can limit production; in a number of cropping systems, cyst nematodes are considered one of the most threatening pathogens (Turner and Rowe 2006). For example, the soybean cyst nematode (SCN; *Heterodera glycines*) is the most yield-reducing pathogen of soybean, and it is now present in all major soybean-producing areas in the United States (Allen et al. 2017; Niblack 2005). Potato cyst nematodes (PCN; *Globodera rostochiensis* and *G. pallida*) are a similarly major issue in potato production in Europe and South America, where the pathogen originated (Plantard et al. 2008). As a

worldwide-recognized quarantine pest, PCN can infect several Solanaceous crops, including potato, tomato, and eggplant, and it can cause significant yield losses if proactive disease management practices are not utilized (Evans and Rowe 1998; Turner and Rowe 2006). Beet cyst nematode (BCN; *H. schachtii*) is an ongoing issue in sugar beet production that can also infect cruciferous vegetables such as cabbage (Evans and Rowe 1998). Additionally, BCN can infect *Arabidopsis thaliana*, which has made it a widely adopted model of cyst nematode species for studies of interactions between plants and nematodes (Siddique and Grundler 2015; Sijmons et al. 1991).

Cyst nematodes are particularly difficult to manage because of their long persistence as unhatched eggs encased in cysts in the soil, as well as their resistance to chemical treatments. Quarantine, crop rotation, and host resistance are the primary means of control for cyst nematodes, with resistance being the most economically and environmentally favored practice (Turner and Rowe 2006). Accordingly, plant genes for resistance to cyst nematodes are valuable traits in crop breeding, and significant efforts have been expended to identify resistance to the major cyst nematode pests. Resistance to *G. rostochiensis* pathotypes Ro1 and Ro4 is available from the *H1* resistance gene, which has been widely introgressed into many commercial potato cultivars (Brodie and Mai 1989; Finkers-Tomczak et al. 2011). However, other *G. rostochiensis* pathotypes and *G. pallida* can reproduce on potato cultivars containing the *H1* gene. Additionally, there is no gene or single locus that confers complete resistance to *G. pallida* (Gebhardt 2013; Minnis et al. 2002). Resistance to BCN in sugar beet is an important trait for beet growers. Several sources of strong resistance were identified in wild beet species; however, integration of these traits

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into cultivated varieties has been troublesome (reviewed in Zhang et al. 2008). Traits have been introduced to create commercial varieties with resistance or tolerance to BCN, but their performance has varied (Hauer et al. 2015). One major gene, *Hs1^{pro-1}*, has been cloned and encodes a protein with leucine-rich repeats and a transmembrane domain associated with typical disease resistance proteins (Cai 1997).

The *Rhg1* (resistance to *H. glycines* 1) locus from soybean accession PI 88788 is the primary locus deployed for SCN management. This locus comprises a ≈ 30 -kb block with four genes tandemly repeated up to 10 times to comprise a ≈ 300 -kb resistance locus (Cook et al. 2012; Lee et al. 2015). Three of the repeated genes contribute to full resistance in soybean (Cook et al. 2012). The three genes conferring resistance encode a putative amino acid transporter (*Glyma.18G022400*), an α -SNAP (soluble NSF [N-ethylmaleimide sensitive fusion]-associated protein; *Glyma.18G022500*), and a protein with minimal annotation other than a putative wound-inducible domain (*Glyma.18G022700*) (Cook et al. 2012). The mechanisms through which the transporter and wound-inducible protein contribute to resistance remain unclear (Guo et al. 2019), but the function of the α -SNAP has been, at least partially, elucidated. The α -SNAP proteins usually are highly conserved housekeeping proteins required for cellular vesicular trafficking. However, the α -SNAPs encoded by the resistance-conferring *Rhg1* loci (α -SNAP_{*Rhg1*}) have amino acid polymorphisms at otherwise highly conserved residues, causing interference with proper vesicular trafficking (Bayless et al. 2016; Cook et al. 2014). These aberrant α -SNAP_{*Rhg1*} proteins were found to accumulate to high levels in developing syncytia. It is thought that high levels of toxic α -SNAP_{*Rhg1*} interfere with vesicular trafficking and block nematode development by interfering with syncytium health (Bayless et al. 2016).

Knowledge to date regarding *Rhg1* suggests that the locus represents an unusual evolutionary event that evolved to directly interfere with conserved mechanisms required for cyst nematode parasitism, rather than encoding a protein that recognizes specific nematode pathogens and activates host defenses (Bayless et al. 2016; Cook et al. 2012, 2014; Liu et al. 2017). In the present study, we tested the hypothesis that *Rhg1* efficacy can be extended beyond SCN to function in other plant families against other cyst nematode species or genera. Transgenic *Arabidopsis* and potato expressing *Rhg1* were generated and challenged with BCN and PCNs, respectively. Excitingly, *Rhg1* was found to confer resistance in these diverse plant families against their respective cyst nematode parasites. We also completed initial studies regarding the potential consequences of expressing a foreign and toxic α -SNAP_{*Rhg1*} protein in these plant species. Some negative consequences of *Rhg1* expression in *Arabidopsis* and potato such as reduced root growth and tuber biomass were observed. In separate experiments, the strong toxicity of α -SNAP_{*Rhg1*} in *Nicotiana benthamiana* was found to be rescued by endogenous potato and *Arabidopsis* α -SNAP proteins. The PI 88788-derived haplotype of *Rhg1* has been used for soybean for decades without yield penalties, suggesting the presence of additional factors in the soybean genome that allow *Rhg1*-containing soybeans to thrive (Bayless et al. 2018). The present results demonstrate that *Rhg1* can function in other plant species and confer resistance against different cyst nematode species, thus providing a tool to further investigate both nematode virulence and novel host resistance. Our findings also support a proposed method for the development of engineered nematode resistance in crops where resistance is rare or ineffective.

MATERIALS AND METHODS

Oligonucleotides. The oligonucleotide primer sequences used in this study are listed in Supplementary Figure S8.

Generation of *Rhg1* gene constructs. *A. thaliana* ecotype Col-0 (Columbia) was transformed with three-gene *Rhg1*

overexpression constructs used previously by Cook et al. (2012). Briefly, coding sequences for each of the three genes were cloned into binary vector pSM101 (Melito et al. 2010). Both *Glyma.18G022500* and *Glyma.18G022700* were expressed under a soybean ubiquitin promoter (Hernandez-Garcia et al. 2010), and *Glyma.18G022400* was expressed under the *nos* promoter. A 3 $\times$ -HA tag was added to the C-terminus of *Glyma.18G022500* and *Glyma.18G022700*, and a 4 $\times$ myc tag was added to the C-terminus of *Glyma.18G022400* (Cook et al. 2012). For transformation of *Solanum tuberosum* ‘*Désirée*’, the binary vector pCambia2300 was PCR-amplified with Kapa HiFi polymerase (Kapa Biosystems, California) to add MluI and PspOMI restriction sites to the multicloning site using the polymerase incomplete primer extension (PIPE) method (Klock and Lesley 2009). In the potato overexpression plasmid, the aforementioned expression cassette contained epitope-tagged cDNAs of the three *Rhg1* genes *Glyma.18G022400*, *Glyma.18G022500*, and *Glyma.18G022700*; however, the HA tag was shifted to the N-terminus of *Glyma.18G022500*. The cassette was PCR-amplified with Kapa HiFi Polymerase to contain *AvrII* restriction overhangs, digested, and then ligated into the *XbaI* restriction site of pCambia2300 using T4 DNA ligase (Promega, Madison) to create plasmid pC23-Rhg1-OXCNC. For the pCambia2300 native genomic three-gene *Rhg1* construct, a native genomic region of the *Glyma.18G022700* gene (3.66 kb total, including 1.8 kb upstream of 5' UTR and 1.0 kb downstream of 3' UTR) was amplified from fosmid subclones of *Rhg1* derived from PI 88788 (Fosmid-32) (Cook et al. 2012) and blunt-cloned into the pTopo XL vector to make pTopo XL 2610 native by following the manufacturer's instructions (Invitrogen). Similarly, an 11.21-kb region containing the divergently transcribed *Glyma.18G022400* and *Glyma.18G022500* genes (including 1.5 kb downstream of 2400 3' UTR and 0.8 kb downstream of the 2500 3'UTR) was amplified from Fosmid-32, with *SbfI* and *AvrII* restriction sites incorporated at the 5' and 3' ends, and blunt-cloned into a pTopo XL vector. This pTopo XL 2580-2590 native vector was then digested with *SbfI* and *AvrII*, gel-purified, and ligated into the pTopo XL 2610 native vector, which was digested with *PstI*-*XbaI*, thereby generating pTopo XL 2580-2590-2610 with the three native *Rhg1* genes. The combined pTopo XL 2580-2590-2610 native genomic fragment was digested using *NotI* and *MluI* sites present in the pTopo XL vector, gel-purified, and ligated into pCambia2300 MluI-PspOMI vector cut with *NotI* and PspOMI, thereby generating pC23-Rhg1-N. The combined total native genomic region of pC23-Rhg1-N is ≈ 14.9 kb.

Generation of transgenic potato. Transgenic potato lines expressing the three *Rhg1* genes under the control of their native soybean promoters or the overexpression cassette already described were generated as previously described (Chronis et al. 2014a). Briefly, potato internode segments from in vitro-grown plants (*Solanum tuberosum* ‘*Désirée*’) were cut, incubated in a cell suspension of *Agrobacterium tumefaciens* strain LBA4404 containing the specified binary plasmid, and transferred onto callus and shoot induction medium. After ≈ 4 weeks, emerging shoots were dissected and transferred to culture tubes containing propagation medium. Plantlets were cultivated in a growth chamber at 24°C under a 16 h light/8 h dark cycle. To confirm transgene expression in independent transgenic lines, mRNA was isolated from plant roots using a Dynabeads mRNA DIRECT Kit (Life Technologies, Carlsbad, CA). DNA contamination was removed by treatment with DNase I (Life Technologies). First-strand cDNA was synthesized from 50 ng of mRNA using ProtoScript II reverse transcription (NEB, Ipswich, MA). Primers specific to *Glyma.18G022400* of *Rhg1* (*Rhg1_F* and *Rhg1_R*) were used for PCR detection of gene expression. The *StUbi* gene (XM_010326024.1) was used as an internal reference in the PCR assay. The PCR cycling conditions were 94°C for 40 s, followed by 25 cycles (for the *StUbi* gene) or 30 cycles (for the *Rhg1* gene) of 94°C

for 40 s, 60°C for 40 s, 72°C for 1 min, and a final reaction of 72°C for 10 min.

Generation of transgenic *Arabidopsis*. *A. thaliana* accession Columbia (Col-0) plants were transformed by the floral dip method (Clough and Bent 1998), and transgenic T1 seedlings were selected on 1/2× MS plates containing 25 µg/ml hygromycin. Transgenic T2 seedlings were identified by germination on the aforementioned media prior to use. Homozygous transgenic T3 lines were established by single-seed descent from T2 plants and testing for uniform germination of the progeny family on the aforementioned media.

Quantitative PCR to determine the copy number of the *Arabidopsis* transgene. Genomic DNA of *Arabidopsis* seedlings was extracted using a MoBio Power Plant DNA Extraction kit including RNase treatment (MoBio, St. Louis, MO). Six seedlings were pooled per replicate, and three replicates were run in parallel. Quantitative PCR (qPCR) was performed for 10 ng genomic DNA with Bullseye Evagreen 2× Supermix (MidSci, St. Louis, MO) using a CFX96 real-time PCR detection system (Bio-Rad, Hercules, CA) and the manufacturer-suggested Bullseye Evagreen cycling parameters. Each sample had two technical replications and each primer set had a no template control, which displayed no amplification. Known single-copy *Arabidopsis* genes *At4g03280* and *At1g06570* were used as the reference and calibrator gene, respectively. Primers specific to the *Rhg1* gene *Glyma.18G022400* were used to determine the copy number of the *Rhg1* transgene. All primers were validated with efficiency curves. Amplification was normalized to *At4g03280*, and *Glyma.18G022400* amplification was normalized to the calibrator *At1g06570*. The copy number was calculated using the $\Delta\Delta C_q$ method (Livak and Schmittgen 2001).

Reverse-transcription PCR of *Arabidopsis* to detect the expression of *Rhg1* genes. Total RNA was extracted from a pooled group of 10 *Arabidopsis* seedlings that were 10 days old and grown under 12 h days at 22°C on 1/2× MS and then flash-frozen in liquid nitrogen. RNA was extracted with the Direct-zol RNA extraction kit with on-column DNase treatment (Zymo Research, Irvine, CA) and analyzed for quality on a 1.5% agarose gel stained with ethidium bromide. cDNA was synthesized from 1000 ng of RNA with the iScript cDNA synthesis kit (Bio-Rad), and the 20-µl reaction was diluted to 100 µl; 3 µl of cDNA was used per reverse-transcription PCR (RT-PCR) with the gene-specific primers for the *Rhg1* transgene. The *Arabidopsis* gene *At3g18780* (*ACT2*) was used as a positive control. Amplification reactions were performed with DNA polymerase (Ampliqon, Odense, Denmark). The PCR cycling conditions were 95°C for 2 min, followed by 30 cycles of 95°C for 30 s, 54°C for 30 s, and 72°C for 45 s, and then a final extension step at 72°C for 5 min. Then, 5 µl of each reaction was analyzed on a 1% agarose gel stained with ethidium bromide.

Defense gene analysis. To test for constitutive defense gene expression, seedlings were grown on 1/2× MS without sucrose for 16 h/day at 24°C. Ten-day-old seedlings were sprayed with 1 mM salicylic acid (Sigma-Aldrich) or water as a control. For jasmonic acid-induced defenses, 14-day-old seedlings were grown in 1/2× MS and 2% sucrose and treated with 100 µM methyl-jasmonate (Sigma-Aldrich) or water as control. Twenty-four hours after treatment, seedling leaf tissues from approximately three to five seedlings were pooled and flash-frozen in liquid nitrogen. RNA extraction and cDNA synthesis were performed as described. RT-PCR was performed with GoTaq DNA polymerase (Promega, Madison, WI) with gene-specific primers. The PCR cycling conditions were 95°C for 2 min, followed by 25 cycles of 95°C for 30 s, 48°C for 30 s, and 72°C for 30 s, and then a final extension step of 72°C for 5 min. An equal amount of each reaction was analyzed on a 1% agarose gel stained with ethidium bromide.

Immunoblots for *Rhg1* protein detection. To detect *Rhg1* protein levels in *Arabidopsis*, root tissue was collected from 1-month-old seedlings grown on 1/2× MS media and immediately frozen in liquid nitrogen. Frozen samples were homogenized using

a PowerLyzer 24 (MO BIO, Carlsbad, CA) for one cycle at 30 s, flash-frozen, and homogenized again in 50 mM Tris-HCl (pH 6.5), 100 mM NaCl, 5 mM EDTA, 0.2% Triton X-100, and 10% (vol/vol) glycerol during an additional cycle of 30 s. After protein separation by SDS-PAGE and blotting, immunoblots were incubated overnight at 4°C with an antibody that detects myc tags (Sigma-Aldrich, St. Louis, MO) or a conjugated antibody that detects HA tags (Sigma-Aldrich) at 1:5,000 or 1:10,000, respectively, in 5% nonfat dry milk TBS-T (50 mM Tris, 150 mM NaCl, 0.05% Tween 20). Secondary horseradish peroxidase-conjugated goat antirabbit IgG was added at 1:10,000 to myc blots and incubated for 1 h at room temperature, followed by five washes of TBS-T. Detection was performed with Supersignal West Pico or Dura chemiluminescent substrate (Thermo Scientific, Waltham, MA) and developed using a ChemiDoc MP chemiluminescent imager (Bio-Rad). Similar protocols were used with potato root tissue to detect *Rhg1* proteins.

For cell fraction work, infected potato root tissue was ground on ice using a mortar and pestle, and cytosolic and microsomal fractions were isolated as described previously (Zhang and Peck 2011). Immunoblots were performed and imaged as described. An MPK6 antibody (Agrisera, Vännäs Sweden AS12 2633) was used as a cytosolic marker, and calnexin antibody (Agrisera, Vännäs Sweden AS12 2365) was used as a microsomal marker.

Potato PCN assays. *G. rostochiensis* (pathotype Ro1) or *G. pallida* (pathotype Pa2/3) PCN was propagated and hatched as previously described (Chronis et al. 2014b; Lu et al. 2008). *Rhg1* transgenic plantlets were planted in six-well plates with propagation medium containing timentin (100 µg/ml). Plants were grown at 24°C under 16-h light/8-h dark conditions for 14 days. Approximately 300 freshly hatched and surface-sterilized second-stage juveniles (J2) were inoculated on each plantlet as described by Chronis et al. (2014b). Twelve seedlings were included for each transgenic line. Six weeks after nematode inoculation, the number of nematode females formed on plant roots was counted under a dissecting microscope (Carl Zeiss Discovery V8). Data were analyzed by analysis of variance (ANOVA) using R software (R Core Team).

***Arabidopsis* BCN infection assay.** *H. schachtii* were propagated on collard greens and harvested using sucrose gradient centrifugation. Cysts were stored in sand at 4°C prior to use. To obtain infective J2s, cysts were crushed and eggs were collected and hatched as described by (Cook et al. 2012). Prior to hatching, eggs were surface-sterilized by treatment with 0.02% sodium azide for 20 min and rinsing with deionized water for 3 min. Approximately 5 days after hatching, infective J2s were surface-sterilized using HgCl₂ as described by (Cook et al. 2012). *Arabidopsis* seeds were surface-sterilized by vortexing for 2 min in 95% ethanol and allowed to air-dry in a laminar flow sterile hood before plating. Individual seeds were plated in sterile 12-well plates filled with 4 ml of modified Knop's media (Sijmons et al. 1991) supplemented with 2% sucrose. Each seed was randomly assigned to a numbered well to blind the investigator during subsequent nematode counting. Plates were wrapped with micropore tape and placed in the dark at 4°C for 3 days before being submitted to 22°C for 16-h/day. Twelve-day-old seedlings were inoculated with 100 to 200 sterile *H. schachtii* J2s at the base of the plant. Inoculated seedlings were placed in the dark at 28°C for 25 days before females on the roots were counted. Three independent experiments were performed. Data were analyzed by an ANOVA (genotype and experiment) using R (R Core Team).

***Arabidopsis* root knot nematode infection assay.** *Meloidogyne incognita* root knot nematodes were propagated and hatched as described previously (Jaouannet et al. 2013). Seeds of *Arabidopsis* ecotype Col-0 and transgenic Col-0 lines expressing *Rhg1* genes were surface-sterilized and planted in six-well plates containing 0.5× MS medium, 2% sucrose, and 0.8% agar. Plants were grown at 24°C under 16-h light/8-h dark conditions for

2 weeks. Approximately 300 freshly hatched and surface-sterilized juveniles (J2) were then inoculated on each seedling as described by Hamamouch et al. (2012). Galls formed on roots were counted under a microscope 4 weeks after inoculation.

Phenotypic analysis of *Rhg1*⁺ potato plants. Potato seedlings from in vitro tissue culture were transplanted into 2-gallon pots, and plants were grown in a greenhouse at 22 to 28°C with a 16-h light/8-h dark cycle for 4 months. Five replicates of each transgenic line were included. Tubers from each line were collected and weighed at the end of plant growth, and data were analyzed by an ANOVA using R software (R Core Team).

***Arabidopsis* root length assay.** *Arabidopsis* seeds were surface-sterilized in the same manner used for the BCN infection assays. Seeds were plated by genotype on 1/2× Murashige and Skoog medium with 1× B5 vitamins, 1% sucrose, and 0.5% agar. Plated seeds were covered with an additional layer of the same media and vernalized in the dark at 4°C for 3 days. Plates were then moved to a growth chamber (22°C, 16-h light/8-h dark cycle) and placed on edge at a 90° angle. After 4 days, the length of the root was marked and plates were shifted to 60° angles. Four days later, the final root length was marked and growth was measured between these two points by capturing seedling images and measuring root growth with the NeuronJ plugin (Fridman et al. 2014; Meijering et al. 2004) for ImageJ (NIH). This experiment was repeated three separate times. Statistical analysis by an ANOVA was performed using R (R Core Team).

***Arabidopsis* nematode penetration assay.** To measure nematode penetration, 10-day-old seedlings were inoculated with 50 *H. schachtii* J2 nematodes. Plants were stained 48 h after infection (Bybd et al. 1983), and the number of nematodes penetrated per root was determined using an Olympus DP73 microscope. Penetration data were analyzed by an ANOVA using R software (R Core Team).

Generation of plasmids for transient protein expression. Vectors for transient overexpression of *Arabidopsis* and potato α -SNAPs were constructed using the pSM101 binary vector (Melito et al. 2010). Coding sequences for *Arabidopsis* α -SNAP At03g56190.1 and potato α -SNAP Sotub06g011010.1.1 were amplified from the plant genotypes Col-0 and Désirée, respectively, used for transformation. RNA was extracted from plant tissues using a Direct-Zol RNA extraction kit with on-column DNase treatment (Zymo Research, Irvine, CA) and analyzed for quality by gel electrophoresis on a 1.5% agarose gel. cDNA was synthesized with iScript cDNA Synthesis kit (Bio-Rad). α -SNAP coding sequences were amplified from cDNA with KAPA HiFi Polymerase with specific primers (KAPA Biosystems, Wilmington, MA). Amplified ORFs were then placed between the soybean ubiquitin promoter (Hernandez-Garcia et al. 2010) and *nos* terminator in the vector pBluescript using Gibson assembly (Gibson et al. 2009) and sequence-verified. The overexpression cassettes were digested from pBluescript using *Xba*I/*Pst*I, gel-extracted using the QIAquick Gel Extraction kit (Qiagen, Hilden, Germany), and ligated into the binary vector pSM101 using T4 DNA ligase (Promega, Madison, WI). Final constructs were transformed into *Agrobacterium tumefaciens* strain GV3101 (pMP90) using the freeze–thaw method.

Transient gene expression in *Nicotiana benthamiana*. *Agrobacterium*-mediated transient expression was performed as described by (Bayless et al. 2016). Within each experiment, two leaves on multiple plants were used for infiltration, and the infiltration location was randomized between leaves. Images were collected 8 to 9 days after infiltration using a Canon Rebel T3i and de-identified (masked to rater); then, each infiltrated area was rated using a standardized scale of 0 to 5 scale, with 0 indicating no visible signs of chlorosis or necrosis and 5 indicating complete death of the infiltrated tissues. Ratings from two independent experiments (for potato α -SNAPs) or three independent experiments (for *Arabidopsis* α -SNAPs) were analyzed using the pairwise Wilcoxon rank-sum test in R (R Core Team).

RESULTS

Transgenic potato lines expressing *Rhg1* proteins are more resistant to potato cyst nematodes. To determine the efficacy of *Rhg1* in other plant species, transgenic potato lines expressing the set of three resistance-associated genes of the *Rhg1* locus (*Glyma.18g022400*, *Glyma.18g022500*, and *Glyma.18g022700*) were generated. Four independent transgenic potato lines were chosen for further study. Two lines received the three *Rhg1* genes with added epitope tags and under the control of the following strong promoters: the *Glycine max* ubiquitin promoter for HA-*Glyma.18g022500* and *Glyma.18g022700*-HA and an *Agrobacterium nos* promoter for *Glyma.18g022400*-MYC. Two other transgenic potato lines received the three *Rhg1* genes surrounded by their native *Rhg1* promoter and terminator sequences. The four independent potato lines were selected for study after examining *Glyma.18G022400* expression by RT-PCR (Supplementary Fig. S1, samples 10, 13, 2, and 9).

Transgenic potato lines were challenged with *G. rostochiensis* and *Globodera pallida*. All four *Rhg1*⁺ lines were found to be more resistant to both PCN species, as observed by significantly fewer females developing on *Rhg1*⁺ lines 6 weeks after inoculation (ANOVA, $P < 0.01$) relative to the empty-vector control lines (Fig. 1). Relative resistance levels between the independent lines were similar for both PCN species. Results from independent experiments can be found in Supplementary Figure S2.

Subsequent studies confirmed expression of the three *Rhg1* proteins in the transgenic potato lines that received epitope-tagged constructs (Supplementary Fig. S3). Proteins approximately matching the predicted sizes were first observed for *Glyma.18G022400* and *Glyma.18G022500*. Although the *Glyma.18G022700* protein was not detected in initial immunoblot experiments that detected the other two *Rhg1* proteins, it was detectable using a refined immunoblot protocol with PCN-infected roots of constitutive expression *Rhg1*⁺ lines in membrane fractionation experiments.

***Arabidopsis* lines expressing *Rhg1* are resistant to *H. schachtii*.** In *Arabidopsis*, the three *Rhg1* genes were overexpressed using epitope-tagged three-gene DNA constructs (all three genes on one *Agrobacterium* vector). Although the transformation rates were not recorded, *Arabidopsis* transformants were obtained at a lower-than-normal frequency for the *Rhg1* gene construct, suggesting possible toxicity of the *Rhg1* gene construct in *Arabidopsis*. Two independent *Arabidopsis* transgenic lines were used for nematode tests. They were subsequently determined to contain either one or ≈ 10 copies of the transgenic *Rhg1* locus as determined by qPCR (copy number $\pm$ SE: 0.92 ± 0.09 for line SI; 10.12 ± 0.69 for line MI; the parental *Arabidopsis* Col-0 line was confirmed to have an *Rhg1* copy number of zero). The single-copy and multi-copy *Arabidopsis* *Rhg1*⁺ lines were challenged with *H. schachtii* BCN. *Arabidopsis* plants expressing *Rhg1* were significantly more resistant to BCN, with fewer females developing on *Rhg1*⁺ lines relative to the wild-type *Arabidopsis* line (Fig. 2) (ANOVA, $P < 0.001$). These results were further supported by comparisons with the performance of two *Arabidopsis* mutant lines for which altered resistance to BCN was previously reported. *ETHYLENE INSENSITIVE 2-1* (*ein2-1*) and *SALICYLIC ACID INDUCTION DEFICIENT 2-2* (*sid2-2*), which were previously demonstrated to have increased and decreased resistance, respectively (Wubben et al. 2001, 2008), also exhibited those phenotypes under our experimental conditions (Fig. 2). Results from individual experiments can be found in Supplementary Figure S4.

Semiquantitative RT-PCR and immunoblot results for tissue samples from *Arabidopsis* *Rhg1*⁺ transgenic lines are shown in Supplementary Figure S5. Expression of all three *Rhg1* proteins was detected in the single copy insertion line. However, for the ≈ 10 -copy line, only the *Glyma.18G022400* (amino acid permease) mRNA and protein were readily detectable. In the ≈ 10 -copy line,

there was little or no detectable *Glyma.18G22500* or *Glyma.18G22700* mRNA or protein, consistent with silencing of those genes. The *Glyma.18G22400* mRNA and protein appeared to be expressed at a higher level in the multi-copy line than the single-copy line, consistent with the presence of multiple copies of *Rhg1*. Interestingly, the multi-copy *Arabidopsis Rhg1*⁺ line with primarily *Glyma.18G22400* expression showed resistance to BCN, providing evidence that a high level of *Glyma.18G22400* expression effectively conferred nematode resistance in this heterologous system.

Although not the primary focus of the present study, experiments to test the virulence of a *M. incognita* root knot nematode population were also performed using the aforementioned *Arabidopsis* single-copy and multi-copy *Rhg1*⁺ lines. As shown in Supplementary

Figure S6, the single-copy line that expresses the three distinct soybean *Rhg1* proteins exhibited increased resistance to root knot nematodes, but no significantly increased resistance was observed for the multi-copy *Rhg1*⁺ *Arabidopsis* line (which, as presented, was found by protein immunoblot to be expressing a high level of only the *Glyma.18G22400* protein product and low or undetectable amounts of the other two *Rhg1*-encoded protein products).

We also explored whether the observed *Rhg1*-mediated nematode resistance in *Arabidopsis* was likely to be the result of constitutively upregulated general defenses (Supplementary Fig. S7). RT-PCR was used to monitor the expression of *PR1*, an established indicator of increased defenses associated with salicylic acid (SA) signaling (Garner et al. 2016). *PR1* was not expressed in untreated control or *Rhg1*⁺ plants. As a positive control, *PR1* was found to be inducible in all three lines upon treatment with 1 mM SA. Jasmonic acid-associated gene expression (Kammerhofer et al. 2015) was monitored using the established indicator gene *PDF1.2* (Garner et al. 2016). Low levels of *PDF1.2* expression were observed in all lines, including the untreated wild-type control plants, possibly caused by growth in tissue culture plates, which were the context of the *Arabidopsis* BCN infection assays. Positive control treatment with 100 μ M methyl-jasmonate (mJA) induced expression of *PDF1.2* in all lines.

Heterologous expression of *Rhg1* impacts the growth of *Arabidopsis* roots but does not impact nematode penetration. *Arabidopsis* plants transformed with the *Rhg1* genes generated rosettes and roots that closely resembled nontransformed plants in their overall appearance. To more carefully test for impacts on root growth, the rate of root elongation was measured. Four days after germination on agar media in vertical petri dishes, the location of the root tip of growing seedlings was noted, and subsequent growth from this point was measured after another 4 days. Across three independent experiments, the *Rhg1*⁺ transgenic

Fig. 1. Expression of *Rhg1* in potato confers resistance to potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*). Relative susceptibility of transgenic *Solanum tuberosum* 'Desirée' lines to **A**, *G. rostochiensis* Ro1 and **B**, *G. pallida* Pa 2/3 shown as the mean of nematode female numbers per plant normalized to the mean of female numbers per plant of the empty vector (EV) control line (EV: **A**, $n = 20$, **B**, $n = 14$). Error bars display SEM. Means with different letters indicate statistically significant differences as determined by an analysis of variance (ANOVA) ($P < 0.01$). Within each of the three independent experiments (two for *G. rostochiensis* and one for *G. pallida*), the relative resistance of the transgenic lines with respect to each other were similar. Native-2 (**A**, $n = 16$, **B**, $n = 3$) and Native-9 (**A**, $n = 15$, **B**, $n = 7$) are two independent lines expressing the three *Rhg1* genes under the control of their native soybean promoters and terminators. OX-10 (**A**, $n = 16$, **B**, $n = 5$) and OX-13 (**A**, $n = 14$, **B**, $n = 8$) are independent lines expressing the three *Rhg1* genes under the control of constitutive plant promoters.

Fig. 2. Expression of *Rhg1* in *Arabidopsis* confers resistance to BCN. Susceptibility of transgenic and mutant *Arabidopsis* to *Heterodera schachtii* shown as mean cysts per plant 25 days after infection normalized to the mean for Col-0 controls within the same experiment. Data from three independent experiments are shown. Error bars display SEM. Letters indicate statistically significant differences as determined by an analysis of variance (ANOVA) ($P < 0.001$). Col-0 (wild-type): total $n = 64$; *Rhg1* SI (Col-0 with single transgenic insertion of the three *Rhg1* genes): $n = 58$; *Rhg1* MI (Col-0 with multiple insertion of *Rhg1* cassette): $n = 57$; *ein2-1* (ETHYLENE INSENSITIVE-2; mutant previously demonstrated to have increased resistance to *H. schachtii*): $n = 52$; and *sid2-2* (SALICYLIC ACID INDUCTION DEFICIENT-2; mutant previously demonstrated to have increased susceptibility to *H. schachtii*): $n = 61$.

lines exhibited a slight, but statistically significant, reduction in root growth (ANOVA, $P < 0.05$) as compared with the wild-type plants (Fig. 3A). In a separate set of experiments, the rate of penetration of BCN J2 nematodes in *Arabidopsis* roots was determined. Despite the subtle but significant reduction in root growth, we did not detect an impact of *Rhg1* on J2 penetration of the *Arabidopsis* roots (Fig. 3B).

Transgenic potato expressing *Rhg1* have reduced tuber mass. To determine if overexpression of *Rhg1* has an effect on potato tuber growth and development, transgenic potato lines were grown to maturity in greenhouses, and the tuber weight of each plant was determined. When comparing the average total tuber production per plant for the transgenic lines to the empty vector (EV) control plants, both types of *Rhg1* overexpression lines exhibited a statistically significant reduction in tuber biomass (Fig. 4) (ANOVA, $P < 0.1$). Although parameters such as the root elongation rate were not measured for potato, no overt macroscopic root

Fig. 3. Expression of *Rhg1* reduces *Arabidopsis* root growth but does not affect BCN nematode penetration. **A**, Normalized mean *Arabidopsis* seedling tap root growth over 4 days. Error bars display SEM; letters indicate statistically significant differences as determined by an analysis of variance (ANOVA) ($P < 0.05$). Wild-type *Arabidopsis* Col-0 (Col-0): $n = 87$; *Rhg1* SI (Col-0 with single transgenic insertion of the three *Rhg1* genes): $n = 46$; and *Rhg1* MI (Col-0 with multiple insertion of *Rhg1* cassette): $n = 70$. **B**, Mean $\pm$ SEM for the number of J2 BCN penetrating *Arabidopsis* roots 48 h after inoculation. Col-0: $n = 47$; *Rhg1* SI: $n = 48$; and *Rhg1* MI: $n = 43$. According to the ANOVA, means are not significantly different ($P > 0.1$).

Fig. 4. Expression of *Rhg1* reduces tuber production of greenhouse-grown potatoes. Average tuber biomass per plant as measured by total tuber weight per plant of transgenic lines (native and OX) ($n = 10$) relative to that of the empty vector (EV) ($n = 5$) control lines is shown. Letters indicate statistically significant differences in the mean as determined by an analysis of variance (ANOVA) ($P < 0.10$). Error bars display SEM.

phenotypes were observed for the *Rhg1*⁺ potato roots compared with the control roots.

Endogenous α -SNAPS can compensate for toxicity of *Rhg1* α -SNAPS. Our previous research revealed that one of the *Rhg1* genes, *Glyma.18G22500*, encodes an aberrant and toxic α -SNAP (α -SNAP_{*Rhg1*}) that apparently obstructs nematode development by interfering with vesicular trafficking in syncytia (Bayless et al. 2016; Cook et al. 2014). As such, overexpression of α -SNAP_{*Rhg1*} could have a negative effect on other plant species; a severe form of this effect has been confirmed when transiently expressing α -SNAP_{*Rhg1*} in *N. benthamiana* leaves after *Agrobacterium*-mediated delivery (Bayless et al. 2016). Several previous studies have indicated that cell death evident in *N. benthamiana* at ≈ 4 to 5 days after expression of α -SNAP_{*Rhg1*} (or synthetic derivatives) is a result of the α -SNAP_{*Rhg1*} impact on α -SNAP/NSF/SNARE complexes and cellular vesicle trafficking (Bayless et al. 2016, 2018). The ancestrally polyploid soybean genome encodes α -SNAPS at four loci, and the α -SNAPS encoded at three of those loci (wild-type α -SNAPS lacking the α -SNAP_{*Rhg1*} amino acid polymorphisms) have been shown to exhibit dose-dependent mitigation of the cytotoxicity caused by α -SNAP_{*Rhg1*} (Bayless et al. 2016). Our success generating transgenic *Rhg1*⁺ *Arabidopsis* and potato lines in the present study suggested the presence of mechanisms in both Brassicaceae and Solanaceae that are able to modulate α -SNAP_{*Rhg1*} toxicity. Therefore, we used the *N. benthamiana* expression assay to test the hypothesis that endogenous *Arabidopsis* and potato α -SNAPS can, at least partially, rescue the cytotoxicity caused by α -SNAP_{*Rhg1*}.

For the α -SNAP_{*Rhg1*} cytotoxicity assay, wild-type *Arabidopsis* and potato α -SNAP genes were transiently co-expressed with α -SNAP_{*Rhg1*} in *N. benthamiana* leaves. The genes encoding the proteins with the highest similarity to the soybean chromosome 18 wild-type α -SNAP were selected out of two total from *Arabidopsis* and four total from potato. *Arabidopsis At3g56190.1* encoding an α -SNAP protein with 77.9% similarity to the soybean chromosome 18-encoded wild-type α -SNAP was cloned downstream of a soybean ubiquitin promoter. As previously reported, the expression of soybean α -SNAP_{*Rhg1*} caused cell death and tissue collapse in the treated leaf sector (Fig. 5A). Co-expression of *At3g56190.1* with α -SNAP_{*Rhg1*} in *N. benthamiana* reduced the macroscopic chlorosis and necrosis indicative of α -SNAP_{*Rhg1*} cytotoxicity (Fig. 5A). Lesion severity scoring revealed a statistically significant reduction in cell death caused by α -SNAP_{*Rhg1*} when co-expressed with the *Arabidopsis* α -SNAP (Fig. 5C) (Wilcoxon rank-sum test, $P < 0.001$).

Potato *Sotub06g011010.1* encoding an α -SNAP protein with 76.5% similarity to the wild-type soybean chromosome 18-encoded α -SNAP was also tested for its ability to compensate for α -SNAP_{*Rhg1*} toxicity. The protein was co-expressed with α -SNAP_{*Rhg1*}. As seen with *At3g56190.1*, no visible signs of stress were observed even 8 to 9 days after infiltration at the infiltration sites where potato α -SNAP and α -SNAP_{*Rhg1*} were co-expressed (Fig. 5B), indicating that this potato α -SNAP can rescue α -SNAP_{*Rhg1*}-induced cytotoxicity. Lesion severity scoring documented statistically significant reductions of α -SNAP_{*Rhg1*}-induced cell death upon co-expression of the potato α -SNAP with the cytotoxic α -SNAP_{*Rhg1*} (Fig. 5D) (Wilcoxon rank-sum test, $P < 0.001$).

DISCUSSION

In the present study, we demonstrated the efficacy of the soybean cyst nematode resistance locus *Rhg1* in plants from two other taxonomic families compared to multiple cyst nematode species. The efficacy of *Rhg1* in *Arabidopsis* and potato suggested that *Rhg1* has evolved to interfere with one or more core or shared cyst nematode infection processes. Future work involving *Rhg1* could provide broadly applicable insight into cyst nematode parasitism as well as a novel form of host resistance.

The transfer of the nematode resistance function across plant families has not been commonly observed. The movement of resistance has been reported across related plant species, such as the *CaMi* root knot nematode resistance gene from hot pepper that can confer root knot nematode resistance in tomato (Chen et al. 2007) and the *Mi-1.2* gene from tomato that conferred root knot nematode resistance in eggplant (Goggin et al. 2006). In those *Mi-1.2* experiments, the gene transfer remained within *Solanaceae*, but even that type of transfer is not always successful. *Hero A* is a broad-spectrum tomato resistance gene encoding a nucleotide-binding/leucine-rich repeat (NLR) protein active against *Globodera* nematodes; however, when it was heterologously expressed in potato, it no longer conferred resistance (Sobczak et al. 2005). Our results confirmed the transfer of disease resistance when a cyst nematode resistance locus was moved from *Fabaceae* (soybean) into *Solanaceae* (potato) and *Brassicaceae* (*Arabidopsis*).

Previous research has shown that resistance conferred by some plant R genes that encode NLR proteins or pattern recognition receptors can be transferred across plant families (Boutrot and Zipfel 2017; Collinge et al. 2010; Narusaka et al. 2013). Less success has been observed in the transfer across plant families of other types of plant resistance traits with proven strong efficacy, with the notable exception of *mlo*, a loss-of-function mutation associated with powdery mildew resistance in a wide variety of plants (Kusch and Panstruga 2017). NB-LRR and pattern recognition receptor genes target specific pathogen effectors or microbial-associated molecular patterns (MAMPs); therefore, their functional range typically relies on the presence of pathogens that share a particular effector or effector target or a conserved pathogen marker. A resistance locus such as *Rhg1*, which does not encode a canonical receptor kinase or NLR protein, does not immediately imply pathogen detection as a mode of action. The capacity of *Rhg1* to function against both *Heterodera* and *Globodera* cyst nematodes suggested activity against one or more broadly conserved nematode infection strategies or modes of action. Elements of pathogen strain specificity of resistance remain because widespread use of *Rhg1* in soybean has led to the gradual development of SCN populations that partially overcome *Rhg1*-mediated resistance (McCarville et al. 2017), and virulence on different *Rhg1*-resistant sources appears to be unique for each source (Gardner et al. 2017). However, our results still suggest a broad resistance mechanism encoded at *Rhg1* that targets one or more infection processes that are conserved across diverse cyst nematode genera.

No constitutive expression of the plant defense indicator gene *PR1* was observed in the tested *Rhg1*⁺ *Arabidopsis* lines. A background level of *PDF1.2* expression was observed without mJA treatment in both control and transgenic lines, possibly as a result of growth in tissue culture plates, which comprised the environment where BCN infection assays were performed. Although the wild-type and *Rhg1*⁺ lines not treated with SA or mJA exhibited similar amounts of *PR1* or *PDF1.2*, we observed significant differences in nematode resistance between the plant lines; therefore, it appears unlikely that chronic up-modulation of defenses indicated by *PR1* or *PDF1.2* mRNA abundance caused the nematode resistance that was observed in *Arabidopsis* lines expressing *Rhg1* genes.

Our unanticipated identification of an *Arabidopsis* line that confers nematode resistance through high expression of only the *Glyma.18G24400*-encoded putative amino acid permease supports previous work indicating that α -SNAP_{*Rhg1*} is not the only protein contributing to resistance at the *Rhg1* locus (Cook et al. 2012). This topic merits deeper analysis in future studies. We were also intrigued when observing increased resistance against root knot nematodes in the *Arabidopsis* line expressing all three *Rhg1*-encoded proteins, but not the line expressing only the *Rhg1* amino acid permease-like protein. Because chronic upregulation of canonical markers of defenses was not observed, it is possible that the *Rhg1* locus more directly interferes with general nematode parasitism, not just cyst nematode infection processes. However, in

multiple studies of soybean resistance to *M. incognita*, the *Rhg1* locus has never been detected as making a contribution to soybean root knot nematode resistance (Kim et al. 2016). Although all three *Rhg1* genes have been demonstrated to be required for full resistance in soybean (Cook et al. 2012), the function of the encoded proteins may not be completely similar when ectopically expressed in heterologous plant species.

The mechanisms of resistance mediated by two of the three *Rhg1* genes remain poorly understood, but the mechanism of the *Rhg1* α -SNAP has been partly elucidated. This gene confers resistance through the accumulation of a toxic version of a housekeeping gene in the complex and essential biotrophic interface, the nematode-induced syncytium (Bayless et al. 2016). Overexpression of such a toxic gene in other plants could be a cause for concern regarding overall toxicity. The present study showed that, as in soybean, the native α -SNAP proteins of *Arabidopsis* and potato can at least partially compensate for the inherent toxicity of the α -SNAP_{*Rhg1*}.

Although native α -SNAP proteins can help compensate for the inherently toxic α -SNAP_{*Rhg1*}, there were still observable consequences of *Rhg1* expression in the transgenic lines. *Rhg1*-expressing *Arabidopsis* root systems appeared relatively normal, but the main tap roots of young noninoculated *Rhg1*-expressing *Arabidopsis* plants did add less length over a 4-day period in both the single insertion line expressing all three *Rhg1* proteins and in the multiple insertion lines primarily expressing *Glyma.18g24400*. The *Rhg1*-expressing *Arabidopsis* roots were accessed at a similar rate by infective juveniles, suggesting that the resistance phenotype was not caused by a decrease in nematode penetration, but rather because of responses occurring after infection. This is consistent

Fig. 5. Potato and *Arabidopsis* α -SNAP proteins relieve cytotoxicity caused by soybean α -SNAP_{*Rhg1*}. *Nicotiana benthamiana* agro-infiltration α -SNAP_{*Rhg1*} cytotoxicity assay of functional competition between wild-type α -SNAPs and toxic α -SNAP_{*Rhg1*} (*Rhg1*) is shown. Wild-types are from **A**, *Arabidopsis* (Ath; At3g56190) or **B**, potato (Stu; St006g011010). Both panels also contain empty vector (EV) and wild-type soybean α -SNAP (GmaWT; *Glyma.18G022500*) controls. All co-expression used a 3:1 ratio of the two *Agrobacterium* strains expressing the indicated proteins unless otherwise noted. Images were taken 8 to 9 days after infiltration, and treatment-blinded leaves were rated using a lesion severity scale of 0 to 5. **C and D**, Mean lesion severity ratings for leaves treated as in A or B, respectively. Error bars display SEM. Letters indicate significant differences (Wilcoxon rank-sum test, $P < 0.001$). **C**, $n = 17$. **D**, $n = 12$.

with soybean studies that showed that *Rhg1* blocks nematode development within roots rather than reducing the rate of initial root penetration (Chen et al. 2004). It was previously reported that certain *Arabidopsis* mutants with shorter root systems have increased susceptibility to BCN (Wubben et al. 2008). Hence, root length and susceptibility are not consistently correlated phenotypes.

A negative effect of *Rhg1* expression was also observed as a reduction in tuber biomass of *Rhg1*-expressing greenhouse-grown potatoes. The expression of *Rhg1* may interfere with general plant health, for example, by generic disruption of vesicle trafficking because of inefficient SNARE recycling by α -SNAP and NSF (Bayless et al. 2016). However, general negative impacts on plant health have not been observed during decades of use of the PI88788 haplotype of *Rhg1* by soybean breeders who have routinely achieved high efficacy against SCN with no evidence of yield drag (Brucker et al. 2005). Targeted elevation of the *Rhg1* α -SNAP in soybean syncytia is one apparent explanation for this, but the recent discovery of NSF_{RAN07} offers another explanation (Bayless et al. 2018).

The negative phenotypic effect of *Rhg1* expression on potato tuber production might initially suggest that it is a less promising biotechnological approach to cyst nematode management in potato, sugar beet, or other crops. However, our research group discovered that soybean lines with SCN resistance—conferring *Rhg1*⁺ haplotypes also have a unique NSF protein variant, NSF_{RAN07}, because of an alternative allele of *Glyma.07G195900*. NSF_{RAN07} is always co-inherited when a resistance-associated *Rhg1* locus is inherited, despite being encoded on a separate chromosome (Bayless et al. 2018). This NSF allele at least partially restores the α -SNAP/NSF interaction that was reduced between wild-type NSF and α -SNAP^{*Rhg1*}. This suggested that soybeans have co-evolved NSF_{RAN07} to overcome pleiotropic negative effects of *Rhg1* in other tissues or stages of plant development. In the future, co-transformation of *Rhg1* with the gene encoding NSF_{RAN07} may improve the agronomic potential of *Rhg1* for engineering other plant species toward improved resistance to cyst nematodes.

Overall, our results suggested that *Rhg1* has evolved to interfere with a conserved cyst nematode infection process, and that soybeans have been able to adapt to the expression of this locus. Because of the role that *Rhg1* has been shown to have in interfering with vesicular trafficking, it can be postulated that cyst nematode parasitism relies on healthy host trafficking systems, which may be a target of conserved cyst nematode effectors. However, the other two genes of *Rhg1* are likely to interfere with other cyst nematode infection processes. Further studies of *Rhg1* resistance mechanisms and the associated mechanisms of cyst nematode parasitism will be useful to increase the understanding of how cyst nematodes cause disease and how we might thwart their efforts. Independent of future improvements in our understanding of resistance and virulence mechanisms, the present work demonstrated that the complex *Rhg1* resistance locus may have relevance beyond the management of SCN in soybean.

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