

Mi-1-Mediated Nematode Resistance in Tomatoes is Broken by Short-Term Heat Stress but Recovers Over Time

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Abstract: Tomato (*Solanum lycopersicum* L.) is among the most valuable agricultural products, but *Meloidogyne* spp. (root-knot nematode) infestations result in serious crop losses. In tomato, resistance to root-knot nematodes is controlled by the gene *Mi-1*, but heat stress interferes with *Mi-1*-associated resistance. Inconsistent results in published field and greenhouse experiments led us to test the effect of short-term midday heat stress on tomato susceptibility to *Meloidogyne incognita* race 1. Under controlled day/night temperatures of 25°C/21°C, 'Amelia', which was verified as possessing the *Mi-1* gene, was deemed resistant (4.1 ± 0.4 galls/plant) and Rutgers, which does not possess the *Mi-1* gene, was susceptible (132 ± 9.9 galls/plant) to *M. incognita* infection. Exposure to a single 3 hr heat spike of 35°C was sufficient to increase the susceptibility of 'Amelia' but did not affect Rutgers. Despite this change in resistance, *Mi-1* gene expression was not affected by heat treatment, or nematode infection. The heat-induced breakdown of *Mi-1* resistance in 'Amelia' did recover with time regardless of additional heat exposures and *M. incognita* infection. These findings would aid in the development of management strategies to protect the tomato crop at times of heightened *M. incognita* susceptibility.

Key words: heat stress, *Meloidogyne incognita*, *Mi-1* gene, resistance, root-knot nematode, *Solanum lycopersicum*.

Tomato (*Solanum lycopersicum* L.) is among the most valuable agricultural products globally. More than 100 million tons are produced annually, and the USA is among the top five producers (Anonymous, 2012a). However, *Meloidogyne* spp. (root-knot nematode) infestations cause serious crop losses worldwide. Tomato yield reductions as severe as 40% have been reported (Reddy, 1985), particularly in warm climates such as Florida where 10% of U.S. tomatoes are grown and where conditions allow for high nematode population growth within a season (Lamberti, 1979; Williamson and Hussey, 1996; Koenning et al., 1999; Anonymous, 2012b).

Management of root-knot nematodes is challenging because their wide host range prevents use of crop rotation (Trudgill, 1991; Chen et al., 2006). Soil fumigants, contact and systemic nematicides, resistant cultivars, resistant rootstocks, and cultural practices are commonly employed to control root-knot nematodes (Lopez-Perez et al., 2006; Devran et al., 2010). The highly effective and widely used soil fumigant methyl bromide has been banned or phased out in most countries by international agreement because of its contribution to reducing stratospheric ozone (Roskopf et al., 2005) and human and animal health concerns (Oka et al., 2000; Ploeg, 2002; Devran et al., 2010). Thus, host-plant resistance to root-knot nematodes is a powerful and sustainable tool for crop protection

(Roberts and Thomason, 1986; Devran and Elekçioğlu, 2004).

The only commercially available source of resistance to root-knot nematodes in tomatoes is a single, dominant gene named *Mi-1*, which confers resistance, though not immunity, to three of the most damaging species: *Meloidogyne incognita*, *Meloidogyne javanica*, and *Meloidogyne arenaria* (Milligan et al., 1998). This gene was found in *Solanum peruvianum* L. and outcrossed into *S. lycopersicum* L. (Rodriguez, 2013). Resistance is associated with a hypersensitive response, characterized by localized cell death of host tissue near the invading nematode in the root tips (Williamson and Kumar, 2006). For this study, we consider resistance as a reduction in the ability of the nematode to reproduce on the plant, as quantified by the number of galls formed (Sasser et al., 1984). Interestingly, other root-knot nematode resistance genes have been found in the *S. peruvianum* complex, including *Mi-9*, a homologue of *Mi-1* unaffected by temperature, but these are not yet commercially available (Bleve-Zacheo et al., 2007).

The efficacy of the *Mi-1* gene varies with root-knot nematode species and population, tomato cultivar, and environmental conditions, particularly soil temperature (Araújo et al., 1982; Devran et al., 2010; Verdejo-Lucas et al., 2013). Increased gall formation has been shown in plants exposed to soil temperatures above 28°C, with higher temperatures associated with higher gall numbers (Dropkin, 1969; Ammati et al., 1986; Wang et al., 2009; Devran et al., 2010). However, there is some discrepancy in the literature. Several articles have reported complete loss of resistance at temperatures $\geq 32^\circ\text{C}$ (Dropkin, 1969; Williamson, 1998) whereas others have shown that *Mi-1*-conferred resistance was still effective in some cultivars at soil temperatures $\geq 34^\circ\text{C}$ (Abdul-Baki et al., 1996; Verdejo-Lucas et al., 2013).

Dropkin (1969) found that *Mi-1* resistance was lost after 4 d at temperatures $\geq 33^\circ\text{C}$ in 1- to 3-d-old seedlings exposed to heat treatment in constant-temperature

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tanks after inoculation with nematodes, and subsequently held at 27°C for 1 mo. In contrast, the *Mi-1* gene was still effective at soil temperatures $\geq 34^\circ\text{C}$ in excised plant roots in constant-temperature growth chambers (Abdul-Baki et al., 1996) and in greenhouses kept at ambient temperature, where heat occurred before and after inoculation (Verdejo-Lucas et al., 2013).

These studies differed in length of time of heat exposure and temporal sequence of heat and inoculation leading us to consider the possibility of resistance recovery. We hypothesized that *Mi-1*-resistant tomatoes exposed to spikes of high temperature will become susceptible, but resistance may return with time. Thus, the goal of our study was to (i) compare the level of *M. incognita* infection in a resistant tomato cultivar at ambient temperature (25°C) to the level of infection following a midday heat spike, (ii) evaluate the potential recovery of *Mi-1*-resistance post-heat stress, (iii) determine the impact of multiple consecutive days of midday heat spikes on resistance, and (iv) assess the susceptibility of infected plants to secondary nematode infection.

MATERIALS AND METHODS

Plant material and growth condition: All experiments were conducted using two commercial tomato cultivars: *Solanum lycopersicum* cv. Amelia, which is heterozygous for the *Mi-1* gene for resistance (Desaeger and Csinos, 2006), and *S. lycopersicum* cv. Rutgers, which does not have the *Mi-1* gene and is considered susceptible to *M. incognita* infection (Melakeberhan, 1998; Bendezu, 2004). ‘Amelia’ is a determinate hybrid with resistance to *M. incognita*, *M. javanica*, and *M. arenaria*; races 1, 2, and 3 of *Fusarium* wilt, *Verticillium* wilt, tomato spotted wilt; high temperatures; and cracking (Riley et al., 2011; Srinivasan et al., 2012). It was bred primarily for the southeast United States (Gardner and Panthee, 2009a, 2009b). Level of zygosity and, in general, genotypic background have been shown to affect the level of resistance provided by the *Mi-1* gene, and may also affect heat stability (Abdul-Baki et al., 1996; Jacquet et al., 2005).

Plants were grown from seed in autoclaved, sifted 80:20 sand:growing medium (Playsand No. 1113; QuickCrete Intl. Inc. Atlanta, GA and MetroMix 910; Sun Gro Horticulture Distribution, Inc., Bellevue, WA) in 0.5-liter plastic pots. Plants were watered daily with Hoagland’s complete nutrient solution (Gibeaut et al., 1997; Melakeberhan, 1998) and maintained at 25°C \pm 2°C (0700–1800 hr; day): 21°C \pm 1°C (1900–0600 hr; night), with 55% to 60% relative humidity for 3 wk in an environmentally controlled chamber. Before experimentation with heat treatments, the plants were transferred to two independent environmentally controlled rooms of an eight-room polycarbonate greenhouse in Gainesville, FL, as previously described (Zhang et al., 2014) and were allowed to acclimate for at least 2 hr.

Temperature conditions are described in the heat treatment section below.

Meloidogyne incognita nematode inoculation: The root-knot nematodes, *M. incognita* race 1, came from a culture maintained on the susceptible tomato cv. Rutgers, originally started from a single egg mass by Dr. J. Brito (Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL). Nematode eggs were extracted from infected roots using a 10% NaOCl solution (Hussey and Barker, 1973). The plants were inoculated with 500 J2 of *M. incognita*, a quantity of nematodes commensurate with the small size of the 3-wk-old plants (Cooper et al., 2005). A suspension of J2 nematodes in water was distributed among three holes in the soil (0.5 cm deep), 2 to 3 cm from the stem. In all cases, inoculation occurred after heat treatment, such that nematodes were not exposed to the heat (Figs. 1A; 2A). Also, within an experiment, planting dates and heat treatments were manipulated to enable inoculation to occur on the same day, using the same inoculum.

Verification of Mi-1 gene genotype in tested cultivars: To initially verify the presence and absence of the *Mi-1* gene in ‘Amelia’ and ‘Rutgers’, respectively, we collected root tissue from 3-wk-old plants of each cultivar and isolated DNA using a Zymo columns kit (Zymo Research Co., Irvine, CA), following manufacturer’s instructions. *Mi-1* gene-specific primers (C2S4/VIGS_F; Table 1) and two sets of control gene primers (HsfA2 and SP), which should be present in both cultivars, were used to amplify DNA fragments with Invitrogen Platinum Taq DNA polymerase (Life Technologies, Grand Island, NY), following manufacturer’s instructions. Thermal cycling conditions were 94°C for 2 min, followed by 34 cycles of 94°C for 30 sec, 52°C for 30 sec, and 72°C for 5 min. Thermal cycling conditions for HsfA2 and SP control gene primers were similar, except annealing temperatures were set at 58°C for 30 sec and 57 for 1 min, respectively. PCR products were cloned into the pGEM-T Easy Vector System (Promega, Madison, WI) and sequenced for validation (University of Florida Interdisciplinary Center for Biotechnology Research, Gainesville, FL).

Mi-1 gene transcript analysis: To assess whether changes in ‘Amelia’ resistance with heat treatment were related to transcriptional changes of the *Mi-1* gene, the relative amount of *Mi-1* mRNA in tomato cv. Amelia was estimated by real-time quantitative PCR (qPCR). Heat treated 3-wk-old plants (3 hr at 35°C) were inoculated with 500 *M. incognita* eggs 30 min after the heat treatment ended, and the roots were collected 21 hr later (at 1030 hr). RNA was isolated using the RNeasy mini kit (QIAGEN, Hilden, Germany), following the manufacturer’s instructions. Three primer pairs (qMi-1, qMi-2, and qMi-3; Table 1) were developed using the software MacVector 12.6 (MacVector, Inc., Oxford Molecular Group, Cary, NC). To test for primer pair efficiency,

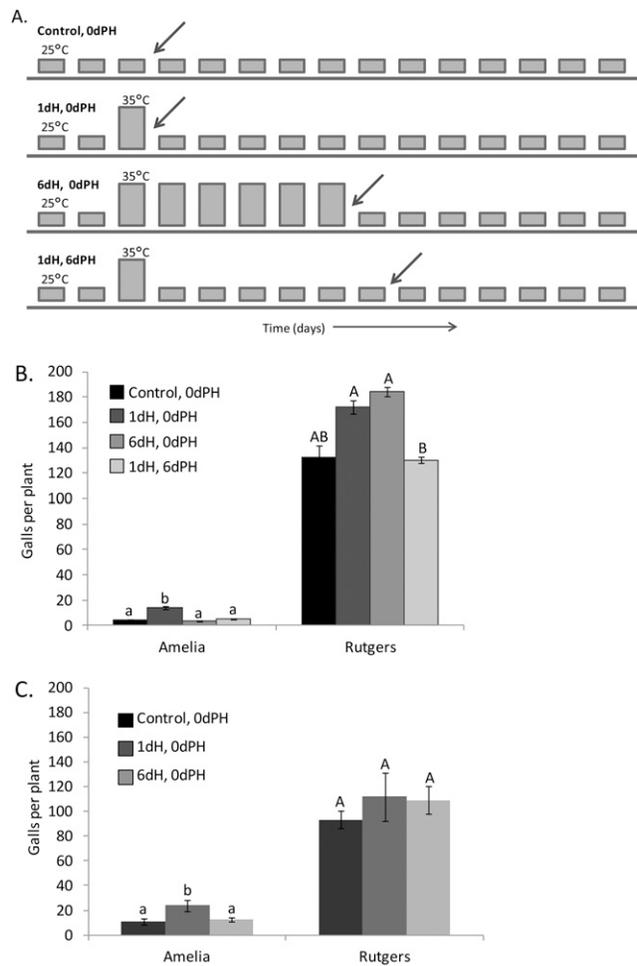


FIG. 1. Effect of heat treatment on the number of *Meloidogyne incognita* galls. A. Graphical representation of treatments, where each square represents 1 d, and treatment name is in bold above the sequence of days with heat spike (taller bars) and nematode inoculation (diagonal arrows) indicated. Heat spike consisted of 3 hr at 35°C. Inoculation with 500 J2 nematodes occurred after heat treatment. Roots were collected and stained for gall quantification 2 weeks after nematode inoculation. B. Tomato cv. Amelia ($N = 8$ plants) and cv. Rutgers ($N = 3$ plants, except the control, which had 5 plants). Different letters indicate statistical differences ($P < 0.05$) using a non-parametric distribution. C. Tomato cv. Amelia ($N = 8$ plants) and Rutgers ($N = 5$ plants). dH = days of heat treatment, dPH = days post-heat treatment. Different letters indicate statistical differences ($P < 0.033$) using a nonparametric distribution and different letter case indicates a separate statistical analysis for 'Amelia' and 'Rutgers'. Errors bars indicate \pm standard error.

a fivefold dilution series of pure 'Amelia' DNA was used to generate a standard curve by plotting the C_t values obtained by qRT-PCR against the log [DNA] (Nicolaisen et al., 2009). The most efficient primer pair, qMi-1, was selected as well as housekeeping genes *LeEF1* and *Elf-1a* to conduct q-PCR (Table 1). Thermal cycling conditions were 95°C for 2 min, followed by 40 cycles of 95°C for 5 sec and 60°C for 30 sec, then 95°C for 5 sec, and finally a melt curve of 65°C to 95°C with an increment of 0.5°C per 5 sec.

Heat treatment experiments: To compare differences between the level of *M. incognita* infection in plants at

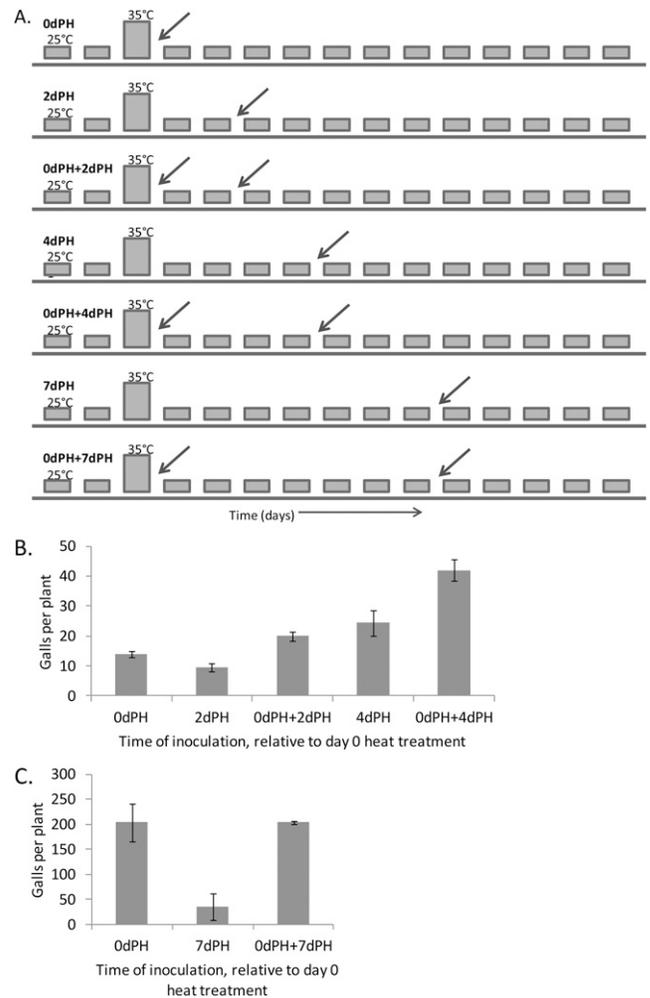


FIG. 2. Effect of time since heat treatment and of previous inoculation on the number of root-knot nematode galls. A. Graphical representation of treatments, where each square represents 1 d, and treatment name is in bold above the sequence of days with heat spike (taller bars) and nematode inoculation (diagonal arrows) indicated. Heat spike consisted of 3 hr at 35°C. Inoculation with 500 J2 nematodes occurred after heat treatment. Roots were collected and stained for gall quantification 2 wk after last nematode inoculation of each treatment. B. Tomato cv. Amelia ($N = 10$ plants). Using a non-parametric distribution, 0 dPH and 2 dPH combined are not significantly different from 0 dPH + 2 dPH ($P = 0.1122$), and 0 dPH and 4 dPH combined are not significantly different from 0 dPH + 4 dPH ($P = 1.0362$). C. Tomato cv. Amelia ($N = 6$ plants). Using a nonparametric distribution, 0 dPH and 7 dPH combined are not significantly different from 0 dPH + 7 dPH ($P = 0.0802$). dH = days of heat treatment, dPH = days post-heat treatment. Errors bars indicate \pm standard error.

ambient temperatures to the level of infection following a single midday heat spike, subsets of 'Amelia' and 'Rutgers' plants were transferred to separate rooms: one designated as control and the other as heat treatment. The control greenhouse room was maintained at 25°C \pm 2°C (0700–1800 hr; day): 21°C \pm 1°C (1900–0600 hr; night), with 55% to 60% relative humidity. The heat-treatment room was maintained at the same conditions except for a single heat treatment day (1 d heat; 1 dH) of 35°C from 1200 to 1500 hr, preceded and followed by 1 hr of temperature transition (Fig. 1A). Each

TABLE 1. Primers used for 5' and 3' PCR and qPCR.

Primer	Sequence	Temperature (°C)	Primer pair efficiency (%)	Source
PCR primers				
Mi-1				
C2S4	5'-CTAAGAGGAATCTCATCACAGG-3'	55.2	–	Li et al., 2006
VIGS_F	5'-CTTGCGTCTACTGACTCTTTCC-3'	52.1	–	
Heat stress transcription factor A2				
HsfA2-F	5'-GGCGACCATAACTCTATCCTTCCC-3'	58.7	–	Giorno et al., 2010
HsfA2-R	5'-GCCTCCTCCACTATTCCAGTATCC-3'	52.9	–	
Mi 900-bp fragment				
SP_F	5'-AGATGTCGGTAAAGAAATAGCC-3'	57.4	–	Chen et al., 2006
SP_R	5'-ATCCACGGACAGCACTCG-3'	52.4	–	
qPCR primers				
Mi-1				
qMi-F1	5'-CGCTGAAGATATGAGAGGAGG-3'	54.4	98.4	MacVector 12.6, MacVector, Inc., Oxford Molecular Group, Cary, NC
qMi-R1	5'-CACCAAGCAAAGTTCAACCA-3'	53.9		
<i>Lycopersicon esculentum</i> elongation factor-1				
LeEF1-F	5'-TGATCAAGCCTGGTATGGTTGT-3'	56.4	107.88	Giorno et al., 2010
LeEF1-R	5'-CTGGGTCATCCTTGGAGTT-3'	54.2		
Elongation factor-1 alpha				
EIF-1AF	5'-GAACTGTCCCAGTTGGTCGT-3'	57.2	92.38	MacVector 2.6, MacVector, Inc., Oxford Molecular Group, Cary, NC
EIF-1AFR	5'-GTCAAACCAGTAGGGCCAAA-3'	55.3		

treatment was replicated with 10 plants of 'Amelia' and five of 'Rutgers'. Plants were watered as needed with Hoagland's solution maintained at the individual greenhouse room temperature. Soil temperature was monitored by StowAway TidbiT temperature data loggers (Onset Computer Corporation, Cape Cod, Bourne, MA) buried in pot substrate. One hour after the heat treatment, at which point the soil temperature had returned to $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$, the plants were inoculated with *M. incognita* as described above (0 d post heat; 0 dPH). Plants were then maintained at control conditions, described above, for 14 d to allow for nematode development and galls production. This postinoculation period is likely insufficient for development of egg masses and secondary *M. incognita* infection.

To determine whether additional days of heat treatment would intensify the level of susceptibility, an additional set of plants were subjected to the heat treatment of 35°C between 1200 and 1500 hr for six consecutive days (6 d heat; 6 dH) before nematode inoculation (0 dPH). Furthermore, to determine whether the enhanced susceptibility following a single heat treatment persisted or decreased with time, plants were exposed to the heat treatment (1 dH), but then inoculated 6 d after the treatment (6 dPH). The control, 1 dH, and 6 dH treatments of this experiment were replicated, using eight plants each of 'Amelia' and 'Rutgers'.

A separate experiment was conducted to better characterize changes in susceptibility. Plants were exposed to the heat treatment (1 dH), and then inoculated 2, 4, or 7 d after the treatment (2, 4, or 7 dPH). Again, the nematodes were allowed to develop for 14 d, and the number of galls were counted and compared to the gall production in other treatments.

Finally, the influence of previous *M. incognita* infection on secondary nematode infection was assessed. Plants were exposed to a single day of heat treatment (1 dH) and inoculated with *M. incognita* twice: on the same day as the heat treatment, and again 2, 4, 6, or 7 dPH (0 dPH + 2 dPH, 0 dPH + 4 dPH, or 0 dPH + 7 dPH).

Data collection and statistical analysis: Roots of the inoculated tomato plants were stained with acid fuchsin 14 d postinoculation to quantify the number of nematode galls (Barker, 1985). Stained galls were counted using a light microscope.

Statistical analyses: Quantitative PCR data was analyzed using the $\Delta\Delta C_t$ method (Livak and Schmittgen, 2001). The ΔC_t values were used to conduct an ANOVA to compare relative mRNA levels between tomato plants with or without heat treatment and with or without nematode inoculation.

A nonparametric bootstrap strategy was used to compare the number of galls (gall count) of any two treatments because the data were overdispersed and not normally distributed (Efron and Tibshirani, 1993). The difference between the mean gall counts of any two treatments was used as the test statistic. The null distribution of the difference was constructed by sampling with replacement from the mixed data from the two treatments. *P*-values were determined by comparing the observed difference to the constructed null distribution of the differences in mean gall counts. A Bonferroni correction was used in the case where multiple tests were conducted.

A parametric bootstrap approach was used to compare the sum of mean gall counts of two treatments run separately to the mean gall counts of another treatment within the same experiment (e.g., the sum of mean gall counts from singly inoculated 0 and 2 dPH plants

compared to the twice inoculated 0 dPH + 2 dPH plants). In this case, a nonparametric distribution of the gall counts was assumed. The null distribution was constructed using the singly inoculated treatments and assuming independence of their results, leading to the expectation that the expected mean gall counts of the twice inoculated treatments will be the sum of the two means of the singly inoculated treatments. That is, a previous inoculation at 0 dPH will not affect the infection rate of a second inoculation at 2 dPH, and hence, the resulting counts will match the sum of the counts when these two treatments were separate. The difference between the mean counts of the observed samples ([mean count of 0 dPH + mean count of 2 dPH] – [mean count of 0 dPH + 2 dPH]) were compared to those generated under the null distribution to determine the *P*-values.

RESULTS

The presence of the Mi-1 gene—conferred resistance to ‘Amelia’: Using *Mi-1* gene-specific primers, we confirmed the presence of the *Mi-1* gene in ‘Amelia’ and the absence in ‘Rutgers’ (Fig. 3). This was consistent with *M. incognita* resistance in ‘Amelia’, which only formed 4.1 ± 0.4 galls per plant and *M. incognita* susceptibility in ‘Rutgers’, which formed 132.0 ± 9.9 galls per plant when grown at 25°C (control, 0 dPH; Fig. 1B,C).

Heat treatment increased gall number in ‘Amelia’, but resistance recovered with time. This recovery was unaffected by additional consecutive heat treatments or previous infection: A single midday 3 hr heat spike of 35°C was sufficient to significantly reduce *Mi-1* gene-conferred resistance in ‘Amelia’. The number of nematode galls formed in heat-treated plants was 3.4-fold higher than unheated ‘Amelia’ control plants (Bonferonni corrected $P < 0.0001$) (1 dH, 0 dPH; Fig. 1B). Results were similar in a second replicate of the experiment (Bonferonni-corrected $P = 0.033$) (1 dH, 0 dPH; Fig. 1C). There was no significant difference between the heat-treated ‘Rutgers’ and the control ‘Rutgers’ plants (Bonferonni-corrected $P = 0.144$ and $P = 0.5124$, respectively, shown in Fig. 1B,C).

Contrary to the expectation that additional days of heat might increase the susceptibility of ‘Amelia’ to *M. incognita*, plants exposed to six consecutive daily heat spikes (3 hr at 35°C) with nematode inoculation on the 6th day of heat (6 dH, 0 dPH) formed fourfold fewer galls than ‘Amelia’ exposed to a single heat spike (Bonferonni-corrected $P = 0.0014$; Fig. 1B). In a second replicate of the experiment, there was no significant difference between plants exposed to 1 or 6 dH (Bonferonni-corrected $P = 0.6111$) (6 dH, 0 dPH; Fig. 1C). Thus, *Mi-1* resistance in ‘Amelia’ recovered with time, or at least, did not break down further, despite the additional days of heat treatment. Similarly, the number

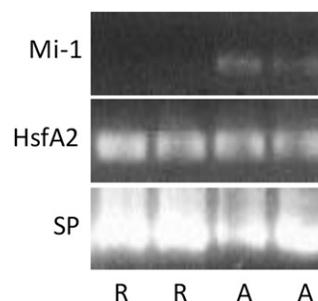


FIG. 3. Polymerase chain reaction product indicating the presence of the *Mi-1* gene in tomato cv. Amelia (A) and absence in cv. Rutgers (R), and the presence of housekeeping genes *HsfA2* and *SP* in both cultivars.

of galls that formed in ‘Amelia’ inoculated with nematodes 6 d after a single day of heat treatment (1 dH, 6 dPH) was not significantly different from ‘Amelia’ control plants (Bonferonni-corrected $P = 0.9$; Fig. 1B), nor from plants exposed to 6 consecutive days of heat spikes (Bonferonni-corrected $P = 0.4614$; Fig. 1B). Under the same treatment of 6 consecutive days of heat spikes (6 dH, 0 dPH), ‘Rutgers’ gall formation remained unaffected (Bonferonni-corrected $P = 1.386$ and $P = 1.6644$, respectively; Fig. 1B,C).

Furthermore, nematode infection did not appear to enhance *M. incognita* susceptibility nor reduce the ability of ‘Amelia’ to recover resistance with time (Fig. 2B,C). The average number of galls in ‘Amelia’ inoculated twice (0 dPH + 2 dPH) was not significantly different from the sum of those found in ‘Amelia’ inoculated solely on 0 or 2 dPH (Bonferonni-corrected $P = 0.1122$; Fig. 2B). Similarly, there was no significant difference when the second inoculation was 4 or 7 dPH (Bonferonni-corrected $P = 1.0362$, and 0.0802 , respectively; Fig. 2B,C).

Heat treatment and nematode infection did not influence Mi-1 gene transcription: Although the presence of *Mi-1* gene confers *M. incognita* resistance to ‘Amelia’, this resistance does not appear to be regulated at the transcriptional level. Transcript levels of *Mi-1* gene in ‘Amelia’ plants 21 hr after exposure to heat (35°C), inoculation with *M. incognita*, or both, remained similar to transcript levels in ‘Amelia’ plants grown at 25°C without *M. incognita* inoculation (Fig. 4).

DISCUSSION

To date, *Mi-1* is the only commercially available resistance gene for root-knot nematodes, one of the most damaging plant pathogens worldwide (Mantelin et al., 2013). Improved understanding of how and when heat exposure disrupts *Mi-1* resistance is essential to developing strategies to reduce agro-economical losses associated with root-knot nematodes. Our research has shown that a single midday heat exposure of 35°C for 3 hr is sufficient to break *Mi-1* resistance in *S. lycopersicum* cv. Amelia, leading to a significant increase in gall

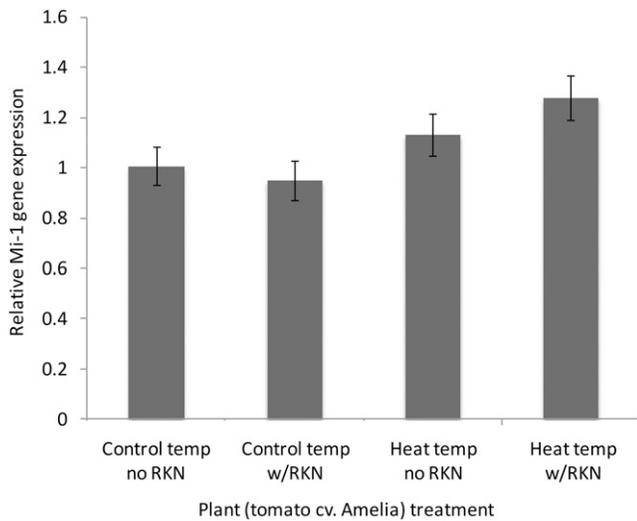


FIG. 4. Effect of a single day of 3 hr heat at 35°C, followed by inoculation with 500 J2 *Meloidogyne incognita*, on the relative *Mi-1* mRNA level in tomato cv. Amelia ($N = 4$ plants), using qMi-1 primers. Errors bars indicate \pm standard error. Analysis of variance, $F = 3.297$, $P = 0.0578$.

formation. However, this increase in susceptibility does not persist, and resistance is completely recovered 6 d after heat exposure. Recovery of *Mi-1* gene resistance is not affected by additional days of heat or previous nematode infection.

The presence of the *Mi-1* gene in ‘Amelia’ and absence in ‘Rutgers’ was consistent with resistance of ‘Amelia’ and susceptibility of ‘Rutgers’ to *M. incognita* race 1, as measured by number of galls formed. Similarly, other studies have found fewer *M. incognita* juveniles, egg masses, and galls in plants with the *Mi-1* gene than in susceptible cultivars (Vos et al., 1998; Tzortzakakis et al., 1999; Jacquet et al., 2005; Lopez-Perez et al., 2006). The presence of a few galls in the control is not surprising: multiple studies, including ours, show that the *Mi-1* gene confers resistance not immunity. Variation in *Meloidogyne* spp. reproduction on resistant tomato genotypes has been attributed to the plant’s genetic background, level of zygosity, and possibly an additional locus or other factor in the resistant genotype affecting resistance expression (Abdul-Baki et al., 1996; Jacquet et al., 2005; Verdejo-Lucas et al., 2013). These factors may also play a role in resistance stability and recovery, such that a different cultivar with the *Mi-1* gene may respond differently to heat exposure compared to the heterozygous cv. Amelia. The lower number of galls in ‘Amelia’ relative to ‘Rutgers’ is expected because of high *M. incognita* susceptibility of ‘Rutgers’ (Ammati et al., 1986).

Our study is unique in that it used a short (3 hr) midday heat treatment, which is more comparable to field-like heat exposures during the day. Previous studies have found that resistance to *M. incognita* breaks down at $\geq 32^\circ\text{C}$; however, these studies employed heat treatments of ≥ 24 hr (Abawi and Barker, 1984; Zacheo

et al., 1995; Devran et al., 2010). Zacheo et al. (1995) found that 2 d at 34°C was required to break resistance of cv. VFN8 to *M. incognita*. In addition to treatment timing, duration, and nematode exposure to heat, nematode species and plant cultivar differences may explain some of the conflicts with previous studies. For example, Verdejo-Lucas et al. (2013) found that *Mi-1* resistance to *M. arenaria* and *M. javanica* remained intact in three of five cultivars exposed for 65 d to ambient soil temperatures as high as 34.1°C for ≥ 7.5 hr daily.

We found that 6 d with 3 hr of heat before nematode inoculation actually produced fewer galls than a single day of heat, showing that resistance returns over time or with a longer heat treatment. This suggests that *Mi-1* resistance either acclimates to or recovers from exposure to high temperatures. Both of these hypotheses are also supported by a previous study, which found that 6 d of heat (constant 34°C) rendered seedlings susceptible for only 1 to 2 d after returning to control temperature (27°C) (Zacheo et al., 1995). Interestingly, the loss of susceptibility was accompanied by an increase in peroxidase activity, which is associated with the hypersensitive response, signifying the return of *Mi-1* resistance (Zacheo et al., 1995). Contrary to the recovery hypothesis, resistance to *M. incognita* and *M. arenaria* was lost or reduced at 37°C for 35 d in six cultivars (Abdul-Baki et al., 1996). However, this lack of recovery may be the result of a lack of daily temperature fluctuation: temperatures were kept constant throughout that study. Araújo et al. (1982) found that plants exposed to differentially low nocturnal temperatures (19°C–25°C) and high diurnal temperatures (31°C) were better able to maintain resistance than plants exposed to constantly high temperatures. Daily temperature fluctuation may also explain the maintenance of resistance in the study by Verdejo-Lucas et al. (2013), who suggested that differences in the literature regarding resistance to root-knot nematodes and high temperature could be explained by the level of stress, where plants that experience daily fluctuations in soil temperature could recover *Mi-1* resistance more easily than with longer heat treatments.

The reduction in gall number when ‘Amelia’ plants were inoculated 6 d after a single day of heat treatment suggests recovery of *Mi-1* resistance, rather than acclimation to high temperature. Also, previously *M. incognita*-infected plants inoculated with a second aliquot of *M. incognita* did not show any additional susceptibility to this second infection. The increase in galls in these twice inoculated plants was consistent with the number of galls found in a plant inoculated only at the second time point (2, 4, or 7 DPH). Again, this suggests that the plant’s overall resistance does recover. It is useful to note that Dropkin (1969) found that once an individual *M. incognita* initiates development in the root, nematode growth will continue, even after a shift in temperature.

Despite the break in *Mi-1* resistance, *Mi-1* gene transcription was not affected by the 35°C heat spike. This suggests that heat-associated breakdown in *Mi-1* resistance is affected by changes downstream of gene expression, such as protein conformational changes (Zhu et al., 2010).

From our results and previous studies, we suggest that soil temperatures ca. 35°C reduce the effectiveness of *Mi-1* resistance in tomatoes. We also show that *Mi-1* resistance can recover with time and that previous *M. incognita* infection does not make *Mi-1* resistant plants more susceptible to further *M. incognita* infection. Hence, planting during the hottest season should be avoided, but occasional short-term high temperatures in the field will likely not greatly increase *M. incognita* gall formation. Understanding the effect of heat on *Mi-1* resistance in tomatoes downstream of *Mi-1* gene expression may offer insight into how this resistance works, as well as avenues for ameliorating the effect of heat stress.

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