horticulturae

MDPI

Article

Divergent Responses of Tomato Cultivars with Resistance to *Tomato Yellow Leaf Curl Virus* as Infected by *Meloidogyne javanica*

Soledad Verdejo-Lucas [1,2]

[1] Sustainable Plant Protection Program, IRTA, Crta. de Cabrils km 2, 08348 Cabrils, Spain; soledadverdejo@gmail.com
[2] c/Gerona 24, Pl 4 P2, 04002 Almería, Spain

Abstract: Commercial tomatoes are usually complex F1 hybrids with multiple resistances genes from different wild *Solanum* species. The response of tomato cultivars with resistance to root-knot nematodes (RKN) and *Tomato yellow leaf curl virus* (TYLCV) as infected by *Meloidogyne javanica* was determined in a controlled environment and field conditions. Four treatments were tested, viz. tomato cultivars with (i) RKN resistance alone; (ii) combination of RKN and TYLCV resistance (RKN + TYLCV); (iii) TYLCV resistance alone; and (iv) control (susceptible to the nematode and virus). The RKN-resistant plants effectively suppressed nematode infection and reproduction both in a controlled environment and in field conditions. The RKN + TYLC-resistant plants were less effective ($p < 0.001$) than the RKN plants in a controlled environment, and their resistance levels were significantly reduced in the field. Nonetheless, the RKN + TYLCV plants supported lower ($p < 0.001$) nematode infection and reproduction than the susceptible control plants. The TYLCV-resistant plants reduced ($p < 0.001$) nematode infection and reproduction compared to the susceptible control in a controlled environment and in field conditions. The divergent response of tomato cultivars with resistance to TYLCV via infection by M. *javanica* can be attributed to the genetic background of the cultivars.

Keywords: root-knot nematodes; *Solanum lycopersicum*; interaction resistance genes; reduced resistance levels

Citation: Verdejo-Lucas, S. Divergent Responses of Tomato Cultivars with Resistance to *Tomato Yellow Leaf Curl Virus* as Infected by *Meloidogyne javanica*. *Horticulturae* **2023**, *9*, 777. https://doi.org/10.3390/horticulturae9070777

Academic Editor: Carmelo Peter Bonsignore

Received: 18 June 2023
Revised: 3 July 2023
Accepted: 5 July 2023
Published: 7 July 2023

1. Introduction

Root-knot nematodes (RKN: *Meloidogyne* spp.) are important pathogens for vegetable production worldwide, particularly in protected cultivation because of frequently available host plants, high temperatures under the protected covers, and short fallowing periods between successive crops [1,2].

Plant resistance is an effective and easily adoptable method to control pathogens. Commercial tomato cultivars are usually complex F1 hybrids with multiple resistances genes from different wild *Solanum* species. The resistance to the nematode is conferred by the single dominant gene *Mi-1*, which was introgressed from an accession of *Solanum peruvianum* into *S. lycopersicum* and is effective against *Meloidogyne incognita*, *Meloidogyne javanica*, and *Meloidogyne arenaria* [3]. *Mi-1* resistance is characterized by a hypersensitive reaction that causes cell death, preventing the formation of the feeding site needed for further nematode development. Seven *Mi-1* gene homologues, all from the *S. peruvianum* introgression, are located on the short arm of chromosome 6 [4], but only the *Mi-1.2* is functional and confers resistance to RKN [5].

Tomato Yellow Leaf Curl Virus (TYLCV) is a monopartite begomovirus transmitted by the whitefly *Bemisia tabaci* that can have a devastating effect on tomatoes. Several sources of resistance against this viral disease have been identified in wild *Solanum*, such as *S. chilense* (*Ty-1*, *Ty-3*, *Ty-4*, and *Ty-6*), *S. habrochaites* (*Ty-2*), and *S. peruvianum* (*ty-5*) [6,7]. The *Ty-1* and *Ty-3* genes are mapped to chromosome 6, very close to the *Mi* locus [8,9]. Tomato cultivars

with *Ty*-resistance genes are indeed tolerant rather than resistant to TYLCV because the *Ty*-mediated resistance results in the systemic infection of the plant with mild to moderate symptoms and low levels of virus accumulation [10]. For the purpose of this study, the term "TYLCV resistance" is used because this is the way seed companies categorized the response of tomato to viral infection.

The *Mi*- and *Ty*-1 loci have been introgressed into commercial tomatoes with a double purpose: to decrease damage caused by the nematode and the virus and to reduce final nematode population densities after their cropping. Incorporation of various resistance genes into a single genotype may provide some levels of measurable resistance to another pathogen. For example, the *Mi-1* gene, in addition to RKN resistance, also confers resistance to potato aphids (*Macrosiphum euphorbiae*) [11] and whiteflies (*Bemisia tabaci*) [12]. Tomatoes with resistance to powdery mildew (*Oidium neolycopersici*) are also resistant to RKN and aphids [13]. TYLCV-resistant tomatoes are also resistant to *Tomato curly stunt virus* ToCSV [14] and *Tomato mottle virus* (ToMoV) [9]. Interactions between the nematode-virus and the plant host may be influenced by the dominance of the genes conferring resistance. The outcome might be an enhancement or reduction of the disease. Severe TYLCV infection was related to high incidence of RKN [15].

Tomato cv. Tyrmes with intermediate resistance to TYLCV but susceptible to RKN decreased *M. javanica* reproduction under different experimental conditions [16]. Based on this information and previous report [15], experiments were designed to determine the response of tomato cultivars with TYLCV resistance alone and in combination with RKN resistance to nematode infection. Experiments were conducted in a controlled environment and in the field.

2. Material and Methods

The selected tomato cultivars had been described by the seed companies as having high or intermediate resistance to RKN and TYLCV or susceptibility to both pathogens (Table 1).

Table 1. Resistances to root-knot nematodes and *Tomato yellow leaf curl virus* (TYLCV) of tomato cultivars according to the descriptions of the seed companies and experiments where they were tested.

Treatment (Resistance)	Cultivar	Resistances	Experiments [1]
Nematode	Caramba, De Ruiter Seeds	(HR): Ma, Mi, Mj	1, 2, 3, 4, Field
Nematode + Virus	Yanira, Western Seed	(HR): TYLCV/Mi	3, 4
	Rey, Western Seed	TYLCV, Nematodes	2, 3, 4
	Cantynflas, Diamond	TYLCV, Nematodes	2, 3, 4
	Razymo, Rijk Zwaan	(HR): Mi/TYLCV	1, Field
	Pintyno, Gautier	(IR): Ma, Mi, Mj/TYLCV	2, Field
	Mayoral, De Ruiter Seeds	(IR): Ma, Mi, Mj/TYLCV	2, Field
Virus	Tyrmes, Syngenta Seeds	(IR): TYLCV	1, 3, 4, Field
	Martina, Western Seed	TYLCV	2, 3, 4, Field
	Birloque, De Ruiter Seeds	TYLCV	2, 3, 4, Field
	Denis, De Ruiter Seeds	TYLCV	2
	Verdial, Semillas Fitó	TYLCV	1
Control [2]	Elvirado, Gautier	Susceptible	1
	Durinta, Western Seed	Susceptible	1, 2, 3, 4, Field

HR: High resistance; IR: Intermediate resistance; Ma, Mi, Mj: *Meloidogyne arenaria, Meloidogyne incognita, Meloidogyne javanica*. [1] Experiments 1, 2, 3, and 4 were conducted in a controlled environment in a growth chamber. [2] Susceptible to the nematode and the virus.

The experimental design was a factorial 2 × 2. The primary factors were the nematode and the virus, and the secondary factor was the presence or absence of resistance. Four treatments were tested in all experiments, viz. tomato cultivars with (i) RKN resistance alone; (ii) combination of RKN and TYLCV resistance (RKN + TYLCV); (iii) TYLCV resistance alone; and (iv) control (susceptible to the nematode and virus). The RKN-resistant cv.

Caramba and the susceptible cv. Durinta were included in all experiments as the standard for resistance and susceptibility, respectively.

2.1. Experiments in a Controlled Environment

Experiments were conducted in a controlled environment to determine the response of tomato cultivars to an initial population density of the nematode. Four experiments were conducted in a growth chamber following similar experimental conditions, plant maintenance, and assessments of nematode infection and reproduction. To circumvent any possible effect due to the cultivar and to check the consistency of the results, six tomato cultivars were tested in the RKN + TYLCV treatment (Cantynflas, Mayoral, Pintyno, Razimo, Rey, and Yanira) and five cultivars in the TYLCV treatment (Birloque, Denis, Tyrmes, Martina, and Verdial) (Table 1). Cultivars were tested as they became available (Table 1) and each cultivar was replicated eight times in experiments 1, 2, and 3, and seven times, in experiment 4.

Tomato seeds were germinated in seed trays containing vermiculite no. 2, and seedlings were transplanted singly into 500-cm^3 pots containing steam-sterilized river sand at the third-true leaf stage. Plants were allowed to grow in the growth chamber for one week before they were inoculated with the nematode. An avirulent population of *M. javanica* (code MJ-05) was used in the experiments. Nematode inoculum was obtained from infected tomato (cv. Roma) roots collected from pot cultures maintained in a glasshouse. Roots were macerated in a 0.5% NaOCl solution in a food blender at *ca* 1000 rpm for 5 min [17]. The egg suspension was passed through a 74 µm aperture sieve to remove root debris, and the eggs collected on a 25 µm sieve were used as inoculum. Tomato plants were inoculated with 1000 eggs per plant. Aliquots of the egg suspension were added into two holes made in the soil 3 cm apart from the base of the plant. Plants were watered daily as needed and fertilized with a slow-release fertilizer (15% N + 10% P_2O_5 + 12% K_2O + 2% MgO_2 + microelements).

Plants were maintained in a growth chamber at 23 ± 2 °C and a photoperiod of 16 h light and 8 h dark. Plants were harvested 49 days after nematode inoculation once they had accumulated 540–589 degree-days, enough to complete one generation of the nematode (base 10 °C). At harvest, shoots were cut at ground level and the root systems were washed free of soil and weighed. Egg masses were stained for ease of counting by immersion of the entire root system into a 0.1 g L^{-1} erioglaucine solution (Aldrich Chemical Company) [18] for two hours. Eggs were extracted from the entire root system in a 0.5% NaOCl solution for 10 min. The multiplication rate (Pf/Pi) of the nematode was calculated as eggs per plant (Pf) divided by nematode inoculum (Pi). Based on the reproduction index (RI) of the nematode, the resistance level of the cultivars was estimated as eggs/g root on experimental cultivar divided by eggs/g root on susceptible cultivar Durinta × 100 [19]. The level of resistance was categorized as high resistance (RI < 10%), intermediate resistance (10% < RI < 40%), and susceptibility (RI > 40%).

2.2. Field Experiment

The response of tomato cultivars to continuous exposure to high population densities of the nematode was determined in an unheated field plastic house infested with *M. javanica*. The experiment was conducted from April to July. The soil was a sandy loam with 85.8% sand, 8.1% silt, and 6.1% clay; pH 8.1; 0.9% organic matter (*w/w*); and 0.40 dS/m electric conductivity. The experimental design was a stratified randomized block due to the uneven distribution of the nematode in the field. Four treatments were tested as previously described: tomato cultivars with (i) RKN resistance alone; (ii) combination of RKN and TYLCV resistance (RKN + TYLCV); (iii) TYLCV resistance alone; and (iv) control (susceptible to the nematode and virus). The RKN + TYLCV treatment included three cultivars, Mayoral, Pintyno, and Razimo. The TYLCV alone included another three cultivars, Birloque, Martina, and Tyrmes (Table 1). The resistant cv. Caramba and susceptible cv. Duritnta were used as controls for reference. Each tomato cultivar was replicated 12 times (4 plants/plot × 3 plots).

Tomato seeds were germinated in seed trays containing vermiculite no. 2, and seedlings at the fourth-true leaf stage were transplanted to plots within each of three blocks. Individual plots consisted of a row of four plants of the respective tomato cultivar that were planted 50 cm apart within the row and 55 cm between rows. Plants were watered through a drip irrigation system, and they were fertilized weekly with a solution consisting of NPK (15-5-30), iron chelate, and micronutrients at rates of 31 kg/ha and 0.9 kg/ha, respectively. Tomato plants were vertically trained. Soil temperatures were recorded daily at 30 min intervals with temperature probes placed in the soil at 15 cm deep. Plants were harvested 93 days after transplanting once they had accumulated 1395 degree-days, sufficient temperature for the nematode to complete two generations.

Composite soil samples were collected before transplanting the tomatoes to determine pre-planting population densities. Individual samples consisted of five cores taken to 25 cm depth with a sampling tube (2.5 cm diameter) per plot. Soil was mixed thoroughly, and nematodes were extracted from 250 cm^3 soil subsamples using Baermann trays. Second-stage juveniles were collected 72 h later, concentrated on a 25 µm-pore sieve, and counted. The average initial population densities were 2886 nematodes/250 cm^3 soil. Final population densities (Pf) were estimated in rhizosphere soil collected around the roots of individual plants (96 samples). Then, plants were dug from the soil and the disease severity was assessed using a root galling index on a scale of 0 to 10, where 0 = a complete and healthy root system and 10 = plants and roots were dead [20]. Eggs were extracted from 10-g root subsamples from individual plants of each cultivar (96 samples) by blender maceration in a 0.5% NaOCl solution for 10 min [17]. Eggs are expressed per gram of root. The reproduction index (RI) of the nematode was used to estimate the level of resistance as previously described for the experiments in the controlled environment.

2.3. Statistical Analyses

Statistical analyses were performed using the IBM SPSS Statistics package v. 21. The general linear model procedure was used to analyze the data from the experiments conducted in a controlled environment and field condition. Data were expressed as mean ± standard error. Two variables were considered, nematode and virus; each variable had two levels, with or without resistance, which resulted in a factorial experimental design with four treatments. Data from the experiments in a controlled environment were pooled since there was no difference between the four replicated experiments. The dependent variables analyzed were root weight, egg masses/plant, eggs/g root, Pf (eggs/plant), and multiplication rate. For the field experiment, the variables were gall index, eggs/g root, Pf, and multiplication rate. The Bonferroni correction for multiple comparisons was applied when needed to separate means.

3. Results

3.1. Experiments in a Controlled Environment

The resistances to the nematode and virus and their interactions had significant effects on the number of egg masses per plant, eggs/g root, final population densities, and multiplication rate (Table 2). Root weight was lower ($p < 0.003$) in treatments with RKN resistance (7.73 ± 0.31 g) than in those with no RKN resistance (9.00 ± 0.29 g). Treatments with TYLCV resistance did not differ in root weight.

The analysis of the nematode x virus interaction indicated that egg masses/plant, final population, and the multiplication rate had the lowest ($p < 0.001$) values in the treatment RKN-alone followed by the RKN + TYLCV, TYLCV alone, and the susceptible control in the last place (Table 3). Eggs/g root were higher ($p < 0.001$) in RKN + TYLCV than in RKN alone but lower ($p < 0.001$) than in the susceptible control (Table 3). Treatments RKN + TYLCV and TYLCV alone had similar eggs/g root (Table 3).

Table 2. Statistical significance and probability of primary factors, resistance to root-knot nematodes (RKN) and *Tomato yellow leaf curl virus* (TYLCV), and their interaction, on the infection (egg masses/plant), reproduction (eggs/g root), final population densities (eggs/plant), and multiplication rate (final population/initial population) of *Meloidogyne javanica* on tomato in controlled conditions in a growth chamber.

	Egg Masses/Plant		Eggs/g Root		Final Population		Multiplication Rate	
Resistance	**F Value**	**Probability**	**F Value**	**Probability**	**F Value**	**Probability**	**F Value**	**Probability**
Nematode (N)	62.87	0.001	14.71	0.001	28.97	0.001	77.66	0.001
Virus (V)	26.80	0.001	10.35	0.001	14.65	0.001	45.69	0.001
N × V	38.24	0.001	16.84	0.001	21.04	0.001	58.78	0.001

Table 3. Infection (egg masses/plant), reproduction (eggs/g root), final population densities (eggs/plant), multiplication rate (final population/initial population) and reproduction index (RI; eggs/g root on experimental cultivar divided by eggs/g root on susceptible cultivar Durinta ×100) of *Meloidogyne javanica* on tomato cultivars with resistance to root-knot nematodes (RKN) and *Tomato yellow leaf curl virus* (TYLCV) alone and in combination, inoculated with 1000 eggs per plant in a controlled environment in a growth chamber 49 days after nematode inoculation.

Resistance			Egg Masses		Final	Multiplication		Plants
RKN	**TYLCV**	**Treatment**	**/Plant**	**Eggs/g Root**	**Population**	**Rate**	**RI (%)**	**Tested**
Yes	No	RKN	1.6 ± 4.3 [a]	42 ± 58 [a]	306 ± 420 [a]	0.17 ± 0.19 [a]	1.8 ± 2.4 [a]	37
Yes	Yes	RKN + TYLCV	8 ± 14 [b]	496 ± 1750 [b]	2308 ± 6344 [b]	1. 60 ± 2.01 [b]	10.9 ± 16 [b]	87
No	Yes	TYLCV	19 ± 22 [c]	359 ± 713 [b]	4408 ± 8329 [c]	2.18 ± 2.48 [c]	19.0 ± 21 [b]	85
No	No	Control [1]	92 ± 106 [d]	4102 ± 86 [c]	26,603 ± 4384 [d]	16.35 ± 16.62 [d]		43

Values are mean ± standard error of four experiments, eight replicated plants per cultivar; cultivars tested in each experiment are indicated in Table 1. Values in the same column with different letters indicate statistical differences between treatments according to Bonferroni test ($p < 0.001$). [1] Susceptible to the nematode and virus.

Based on the *M. javanica* RI, 100% of the RKN plants showed high resistance (Figure 1A), 70% of the RKN + TYLCV plants showed high resistance, 23% intermediate resistance, and 7% susceptibility, and 44% of the TYLCV plants expressed high resistance, 45% intermediate resistance, and only 12% susceptibility (Figure 1A).

3.2. Field Experiment

The nematode and the nematode x virus interaction had significant effects on root galling, eggs/g root, and final population (Table 4).

Table 4. Statistical significance and probability of the primary factors, resistance to root-knot nematodes (RKN) and *Tomato yellow leaf curl virus* (TYLCV), and their interaction, on root galling (scale 0 to 10), nematode reproduction (eggs/g root), and final population densities (juveniles/250 cm^3 soil) of *Meloidogyne javanica* on tomato in a field plastic house infested with the nematode.

	Root Galling		Eggs/g Root		Final Population	
	F Values	**Probability**	**F Values**	**Probability**	**F Values**	**Probability**
Nematode (N)	50.67	0.001	8.63	0.005	7.12	0.009
Virus (V)	0.78	0.38	1.55	0.22	0.007	0.93
N × V	50.67	0.001	13.43	0.001	9.85	0.002

The analysis of the interaction showed that root galling, eggs/g root, and final population were lower ($p < 0.001$) in the RKN plants than RKN + TYLCV and TYLCV plants that did not differ from each other, and they were followed by the susceptible control plants in the last place (Table 5). In relation to the RI, 92% of the RKN-resistant plants showed high resistance and 8% intermediate resistance and no susceptibility (Figure 1B). Only 8% of the RKN + TYLCV plants expressed high resistance, 33% intermediate resistance, and

58% susceptibility; and 31% of the TYLCV plants showed high resistance, 8% intermediate resistance, and 61% susceptibility (Figure 1B).

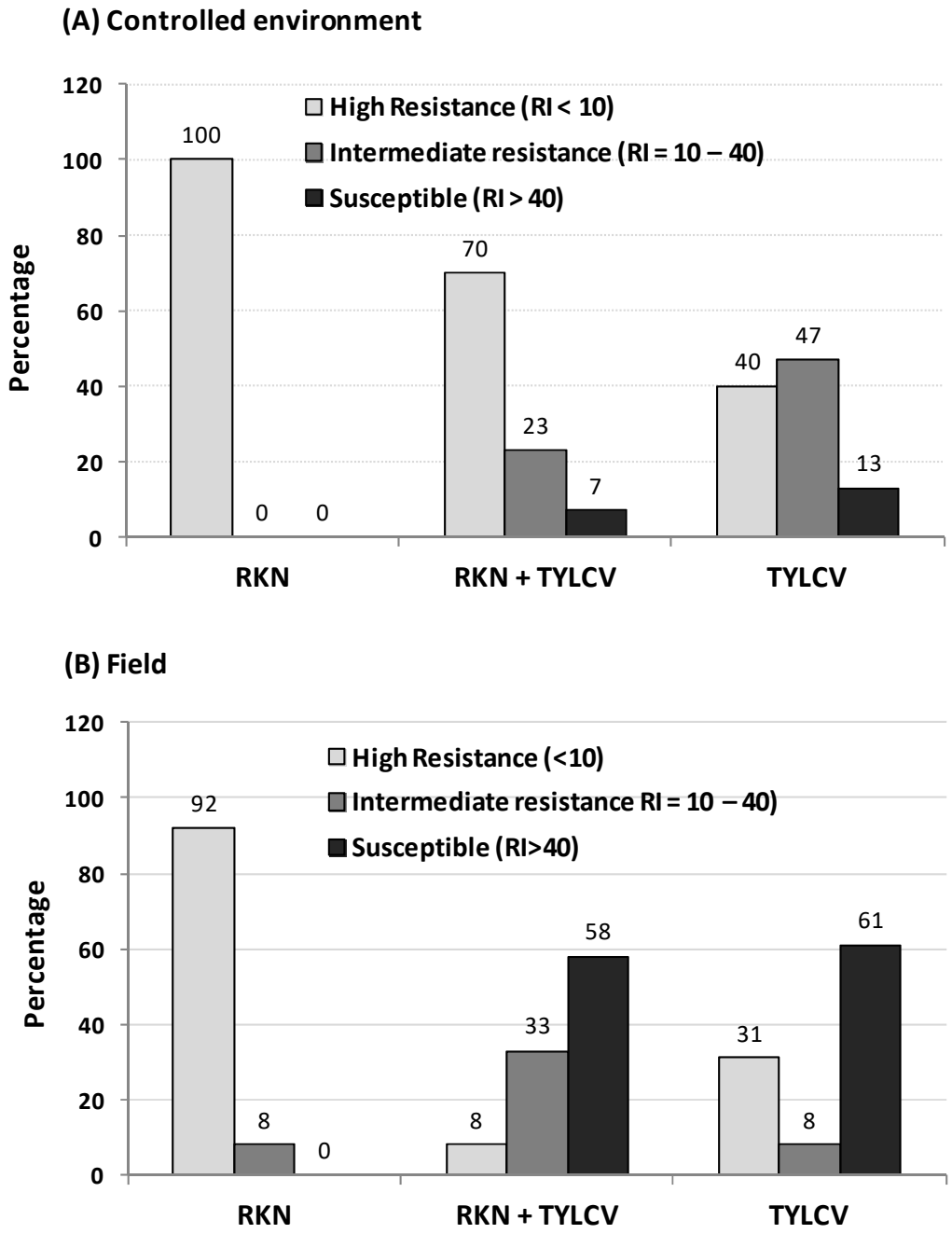

Figure 1. Percentage of plants with resistance to root-knot nematode (RKN) and *Tomato yellow leaf curl virus* (TYLCV) alone and in combination that showed high resistance, intermediate resistance, and susceptibility to *Meloidogyne javanica* according to the nematode reproduction index (eggs/g root on experimental cultivar divided by eggs/g root on susceptible cultivar Durinta ×100) in (**A**) controlled environment, and (**B**) field conditions.

Table 5. Root galling (scale 0 to 10), nematode reproduction (eggs/g root), final population densities (juveniles/250 cm^3 soil), and reproduction index (RI; eggs/g root on experimental cultivar divided by eggs/g root on susceptible cultivar Durinta ×100) of *Meloidogyne javanica* on tomato cultivars with resistance to root-knot nematodes (RKN) and *Tomato yellow leaf curl virus* (TYLCV) alone and in combination in a field plastic house infested with the nematode 93 days after transplanting.

Resistance							Plants
RKN	TYLCV	Treatment	Root Galling	Eggs/g Root	Final Population	RI (%)	Tested
Yes	No	RKN	0.67 ± 0.26 [a]	452 ± 216 [a]	1194 ± 881 [a]	3.23 ± 5.3 [a]	12
Yes	Yes	RKN + TYLCV	4.69 ± 0.32 [b]	10,523 ± 1693 [b]	11,481 ± 19,415 [b]	75 ± 72 [b]	36
No	Yes	TYLCV	4.69 ± 0.46 [b]	9031 ± 1428 [b]	9902 ± 10,454 [b]	61 ± 61 [b]	36
No	No	Control [1]	7.83 ± 0.30 [c]	14,000 ± 2434 [c]	20,756 ± 12,681 [c]		12

Values are mean ± standard error of 12 plants per cultivar (one cultivar for the resistant and susceptible controls and three cultivars for the RKN + TYLCV and TYLCV-resistant plants). Values in the same column with different letters indicate statistical differences between treatments according to Bonferroni test ($p < 0.001$). [1] Susceptible to the nematode and virus.

4. Discussion

The TYLCV resistance in the tomato cultivars provided opposing responses to nematode infection depending on whether the cultivars had RKN resistance or not. The efficacy of the RKN resistance was consistently reduced in cultivars with combined RKN + TYLCV resistance. The TYLCV resistance alone reduced nematode damage and population increases. All the cultivars included in the treatment provided similar levels of resistance or susceptibility to the nematode, which indicates that the response to the nematode was not cultivar dependent.

In a controlled environment, increased infection and reproduction occurred in the RKN + TYLCV in comparison to the RKN-resistant plants, which made the RKN + TYLCV plants less effective in suppressing the nematode. On the other hand, the TYLCV-resistant plants reduced nematode infection and reproduction compared to the susceptible control. In fact, 89% of the TYLCV plants suppressed nematode infection despite the lack of RKN resistance.

In field conditions, the RKN-resistant plants retained a high level of resistance even under continuous exposure to high population densities, which confirmed the high resistance level of cv. Caramba to the nematode [16]. The resistance genes to the nematode, virus, or to both made the plants more tolerant to nematode damage (root galling), and reduced population increases with respect to the susceptible control. However, the efficacy of the RKN + TYLCV plants in suppressing nematode disease and reproduction was greatly reduced under field conditions; indeed, 58% of these plants responded as susceptible. Cultivar Razymo, described as highly resistant, showed intermediate resistance in the field (RI = 38), whereas cv. Pintyno and Mayoral, described as intermediate resistant, showed susceptibility (RI = 108 and 62, respectively). Consequently, the RKN + TYLCV and TYLCV plants supported similar root galling and nematode reproduction.

The genealogy of the RKN + TYLCV tomatoes was unknown because we tested commercial cultivars but assumed that their resistance was due to background of the cultivars. Further genetic and molecular studies are needed to explain the opposing response of TYLCV-resistant plants to nematode infection in RKN-resistant and susceptible plants. The *Ty-1* gene may suppress the expression of the *Mi-1* gene resistance, and this hypothesis needs to be tested. The genetic base of TYLCV resistance depends on the introgressed *Solanum* species and ranges from a single incompletely dominant gene to a polygenic recessive pattern [21,22]. For example, the resistance derived from *S. chilense* accession LA1969 is conferred by a major incompletely dominant gene (*Ty-1* gene) and two or more modifier genes [8]. The RKN resistance is conferred by a single dominant gene (*Mi-1*) derived from *S. peruvianum*. The F1 tomato hybrids incorporate several genes from different wild *Solanum* species and these species often share the same allele for a

marker which can lead to false positives. In fact, begomovirus-resistant tomatoes with introgression from *S. habrochaites* and *S. chilense* that are susceptible to RKN gave false positives in screenings for RKN resistance with the PCR-based REX-1 marker [23]. The RKN + TYLCV plants may have been developed from a cross between one line carrying *Mi* and another one carrying *Ty-1* or from a cross between a line carrying both *Mi* and *Ty-1* and another line with *S. lycopersicum* [22]. Therefore, the complexities of the F1 hybrids affect the expression of nematode resistance. Thus, the commercial cultivar, Anastasia, heterozygous for *Mi* (*Mi/mi*) and *Ty-1* (*Ty-1/ty*-1) [22], showed susceptibility (RI = 59%) to *M. incognita* in biological tests (unpublished data). The RKN susceptible (*mi/mi*) but TYLCV-resistant (*Ty-1/Ty-1*) tomato SC showed the allele 2 from *S. peruvianum* for the REX-1 marker [22]. Lines that were homozygous for *Ty-1* and did not carry *Mi*, showed allele 1 (*S. lycopersicum* allele), allele 2 (*S. peruvinum* allele), or allele 3 (*S. lycopersicum* accession LA3473). The PCR-based marker PM3 [23] amplified a fragment of *ca* 500 bp representing PM3 in cv. Tyrmes but did not do so in the susceptible control Durinta [24], which suggests that Tyrmes may have resistance mediated by the *Mi-1* or *Mi* homologues. Moreover, factors such as a continuous source of high inoculum and the duration of the experiment (two nematode generations in field conditions) significantly lowered the resistance levels of the RKN + TYLCV plants. High continuous population densities reduced the resistance levels of tomato hybrid rootstocks [16]. Tomatoes with intermediate resistance might not provide sufficient nematode control under field conditions but they would perform better than completely susceptible plants. Although continuous cultivation of resistant tomatoes may select virulent individuals within the nematode population able to overcome the resistance [25], the RKN plants retained a high resistance level in the field; therefore, the hypothesis of virulence selection in the RKN + TYLCV plants was rejected.

TYLCV-resistant tomatoes were susceptible to the nematode as previously reported [8,16], but this study demonstrated that they reduced nematode damage and population densities which could be exploited in nematode management programs. The TYLCV disease cannot be effectively controlled with insecticides; therefore, pest advisors encourage growers to plant TYLCV-resistant cultivars in areas where the virus is prevalent. The protective effect of TYLCV resistance against *M. javanica* infection may lead to reduced yield losses. Nonetheless, where RKN and TYLCV occur simultaneously, effective control of both pathogens is necessary to obtain significant increases in yield [26]. It would be interesting to investigate if nematode-resistant plants affect the tolerance of the TYLCV plants to the viral disease.

5. Conclusions

The combination of RKN resistance and TYLCV resistance in a single genotype reduced the efficacy of the *Mi-1* gene in suppressing nematode reproduction. On the contrary, tomatoes with only TYLCV resistance reduced nematode infection and reproduction; therefore, they could be useful in nematode management programs.

Funding: This research received no external funding.

Data Availability Statement: Data will be made available on request.

Acknowledgments: The seeds of the cultivars were kindly provided by the seed companies. Thanks are given to A. Catena for statistical assistance, M. Talavera for reviewing the manuscript, and to M. Blanco, V. Barnes, and O. Jurado for technical assistance in the laboratory and field.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Talavera, M.; Sayadi, S.; Chirosa-Rios, M.; Salmerón, T.; Flor-Peregrin, E.; Verdejo-Lucas, S. Perception of the impact of nematode-induced diseases in horticultural protected crops of south-eastern Spain. *Nematology* **2012**, *14*, 517–527. [CrossRef]
2. Seid, A.; Fininsa, C.; Mekete, T.; Decraemer, W.; Wesemael, W.M.L. Tomato (*Solanum lycopersicum*) and root-knot nematodes (*Meloidogyne* spp.)—A century-old battle. *Nematology* **2015**, *17*, 995–1009. [CrossRef]

3. Kaloshian, I.; Yaghoobi, J.; Liharska, T.; Hontelez, J.; Hanson, D.; Hogan, P.; Jesse, T.; Wijbrandi, J.; Simons, G.; Vos, P.; et al. Genetic and physical localization of the root-knot nematode resistance locus *Mi* in tomato. *Mol. Gen. Genet.* **1998**, *257*, 376–385. [CrossRef]
4. Seah, S.; Williamson, V.M.; Garcia, B.E.; Mejia, L.; Salus, M.S.; Martin, C.T.; Maxwell, D.P. Evaluation of a co-dominant SCAR marker for detection of the *Mi-1* locus for resistance to root-knot nematode in tomato germplasm. *Tomato. Gen. Coop. Rep.* **2007**, *57*, 37–40.
5. Milligan, S.B.; Bodeau, J.; Yaghoobi, J.; Kaloshian, I.; Zabel, P.; Williamson, V.M. The root knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. *Plant Cell* **1998**, *10*, 1307–1319. [CrossRef]
6. Kıymacı, G.; Ari, B.Ç.; Uncu, A.T.; Uncu, A.Ö.; Issı, N.; Türkmen, Ö. Determination of resistance levels of selected tomato genotypes to *Meloidogyne incognita*, Tomato yellow leaf curling virus (TYLCV) *Verticillium wilt*, *Fusarium oxysporum radicis*, *Fusarium wilt*, Tomato spotted wilt virus (TSWV). *Selcuk J. Agric. Food. Sci.* **2023**, *37*, 86–94. [CrossRef]
7. Marchant, W.G.; Gautam, S.; Hutton, S.F.; Srinivasan, R. Tomato yellow leaf curl virus-resistant and -susceptible tomato genotypes similarly impact the virus population genetics. *Front. Plant. Sci.* **2020**, *11*, 599697. [CrossRef]
8. Zamir, D.; Ekstein-Michelson, I.; Zakay, Y.; Navot, N.; Zeidan, M.; Sarfatti, M.; Eshed, Y.; Harel, E.; Pleban, T.; Van-Oss, H.; et al. Mapping and introgression of a Tomato yellow leaf curl virus tolerance gene, *Ty-1*. *Theor. Appl. Genet.* **1994**, *88*, 141–146. [CrossRef] [PubMed]
9. Ji, Y.; Schuster, D.J.; Scott, J.W. *Ty-3*, a begomovirus resistance locus near the Tomato yellow leaf curl virus resistance locus *Ty-1* on chromosome 6 of tomato. *Mol. Breed.* **2007**, *20*, 271–284. [CrossRef]
10. Vidavsky, F.; Czosnek, H. Tomato breeding lines resistant and tolerant to Tomato yellow leaf curl virus issued from *Lycopersicon hirsutum*. *Phytopathology* **1998**, *88*, 910–914. [CrossRef]
11. Rossi, M.; Goggin, F.L.; Milligan, S.B.; Kaloshian, I.; Ullman, D.E.; Williamson, V.M. The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 9750–9754. [CrossRef]
12. Nombela, G.; Williamson, V.M.; Muniz, M. The root-knot nematode resistance gene *Mi-1.2* of tomato is responsible for resistance against the whitefly *Bemisia tabaci*. *Mol. Plant-Microbe Interact.* **2003**, *16*, 645–649. [CrossRef] [PubMed]
13. Seifi, A.; Kaloshian, I.; Vossen, J.; Che, D.; Bhattarai, K.K.; Fan, J.; Naher, Z.; Goverse, A.; Tjallingii, W.F.; Lindhout, P.; et al. Linked, if not the same, *Mi-1* homologues confer resistance to tomato powdery mildew and root-knot nematodes. *Mol. Plant-Microbe Interact.* **2011**, *24*, 441–450. [CrossRef] [PubMed]
14. Pietersen, G.; Smith, M.F. Tomato yellow leaf curl virus resistant tomatoes show resistance to Tomato curly stunt virus. *Plant Dis.* **2002**, *86*, 528–534. [CrossRef]
15. Mayee, C.D.; Mahajan, R.; Kanwar, J.S. Relationship of leaf curl virus and root-not nematode incidence in some tomato hybrids. *Nematol. Medit.* **1974**, *2*, 181–182.
16. Cortada, L.; Sorribas, F.J.; Ornat, C.; Kaloshian, I.; Verdejo-Lucas, S. Variability in infection and reproduction of *Meloidogyne javanica* on tomato rootstocks with the *Mi* resistance gene. *Plant Pathol.* **2008**, *57*, 1125–1135.
17. Hussey, R.S.; Barker, K.R. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Dis.* **1973**, *57*, 1025–1028.
18. Omwega, C.; Thomason, I.J.; Roberts, P.A. A non-destructive technique for screening bean germoplasm for resistance to *Meloidogyen incognita*. *Plant Dis.* **1988**, *72*, 970–972. [CrossRef]
19. Triantaphyllou, A.C. Genetic structure of races of *Heterodera glycines* and inheritance of ability to reproduce on resistant soybeans. *J. Nematol.* **1975**, *7*, 356–364.
20. Zeck, W.M. A rating scheme for field evaluation of root-knot nematode infestations. *Pflanzenschtz-Nach. Bayer* **1971**, *24*, 141–144.
21. Lapidot, M.; Friedmann, M. Breeding for resistance to whitefly-transmitted geminivirus. *Ann. App. Biol.* **2000**, *140*, 109–127. [CrossRef]
22. Pérez de Castro, A.; Blanca, J.M.; Díez, M.J.; Nuez Viñals, F. Identification of a CAPS marker tightly linked to the Tomato yellow leaf curl disease resistance gene *Ty-1* in tomato. *Eur. J. Plant Pathol.* **2007**, *117*, 347–356. [CrossRef]
23. El Mehrach, K.; Gharsallah Chouchane, S.; Mejia, L.; Williamson, V.M.; Vidavski, F.; Hatimi, A.; Salus, M.S.; Martin, C.T.; Maxwell, D.P. PCR-based methods for tagging the *Mi-1* locus for resistance to root-knot nematode in begomovirus-resistant tomato germplasm. *Acta Hortic.* **2005**, *695*, 263–270. [CrossRef]
24. Cortada, L.; Mantelin, S.; Verdejo-Lucas, S.; Kaloshian, I. Marker analysis for detection of the *Mi-1.2* resistance gene in tomato hybrid rootstocks and cultivars. *Nematology* **2012**, *14*, 631–642. [CrossRef]
25. Verdejo-Lucas, S.; Cortada, L.; Sorribas, F.J.; Ornat, C. Selection of virulent populations of *Meloidogyne javanica* by repeated cultivation of *Mi* resistance gene tomato rootstocks under field conditions. *Plant Pathol.* **2009**, *58*, 990–998. [CrossRef]
26. Mahajan, R.; Mayee, C.D. Combined control of root-knot nematodes and leaf curl disease in tomato. *Nematol. Medit.* **1977**, *5*, 141–142.