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SHORT COMMUNICATION

An update on the occurrence of resistance-breaking populations of root-knot nematodes (*Meloidogyne* spp.) on resistant tomato in Greece with six new records from Crete

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Summary The available published information on the occurrence of resistance-breaking populations of root-knot nematodes (*Meloidogyne* spp.) on resistant tomato in Greece is updated. Within the period 1994-2013, 13 populations (11 *M. javanica* and 2 *M. incognita*) able to reproduce on resistant tomato had been recorded in the regions of Crete, Epirus, Thrace, Peloponissos and Macedonia. In the present study six more resistance-breaking populations, four *M. javanica* and two *M. incognita*, were detected in the period 2013-2014, all originating from greenhouse vegetables in Crete. Four of these populations, two *M. javanica* and two *M. incognita*, originated from the region of Ierapetra. This is the first time that such populations are found in this major area of greenhouse vegetable production of Crete.

Additional keywords: *Meloidogyne javanica*, *M. incognita* , *Mi* gene, pathogenicity, pepper, virulence

Root-knot nematodes (RKN), *Meloidogyne* spp., are among the most economically important nematodes in agriculture with a broad host range (Karssen and Moens, 2006) and a wide distribution in the Mediterranean region (Lamberti, 1981) especially in greenhouses with vegetables. In tomato, there are commercially hybrids with resistance to RKN, which is conferred by the *Mi* gene being effective against *M. arenaria, M. javanica* and *M. incognita* at moderate soil temperatures (Williamson, 1998). However, there are several reports concerning virulent populations of these *Meloidogyne* species, able to reproduce on resistant tomato cultivars, occurring either naturally (Williamson, 1998) or after repeated selection on tomato plants with the *Mi* gene (Castagnone-Sereno *et al*., 1994).

A review on the occurrence of RKN in Greece has been published for the period 1996-2010 (Tzortzakakis *et al*., 2011); no information on the presence of virulent populations on resistant tomato was included. Since 1994, there have been 13 records of resistance-breaking populations of RKN from Greece (Tzortzakakis and Gowen, 1996; Tzortzakakis *et al*., 1999; Tzortzakakis *et al*., 2005; Tzortzakakis and Blok, 2007; Tzortzakakis *et al*., 2008; Tzortzakakis *et al*., 2014).

The aim of the current study was: a) to review the published records on the occurrence of RKN populations able to reproduce on resistant tomato in Greece and b) to evaluate the reproduction of 20 populations of RKN, collected randomly from greenhouses and outdoor crops of Crete during 2013-2014, on a resistant tomato and a susceptible pepper cultivar, and update the existing information.

Materials and Methods

From June 2013 until December 2014, 20 populations of RKN were collected from greenhouses and outdoor crops, from various areas of Crete. All originated from sus-

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ceptible crops, where no nematode resistant tomato hybrid had been used in the last 4-5 year crop rotation scheme. The nematode populations were established as cultures, by inserting pieces of root galls, in soil around seedlings of the susceptible tomato (*Solanum lycopersicum* L.) cv. ACE grown in pots. The RKN populations originating from pepper were established as cultures on the susceptible pepper (*Capsicum annuum* L.) cv. California Wonder. The plants were watered and fertilized as required; they grew in a controlled environment with 16 h photoperiod and soil temperature 24-26°C, at which the *Mi* gene is effective (Williamson, 1998). Every seven weeks, the plants were uprooted and ten egg masses were transferred to new plants to maintain the nematode populations.

Preliminary tests

Egg masses were used to inoculate resistant tomato plants cv. Silvana (with the *Mi* gene) and the susceptible pepper cv. California Wonder at a rate of 20 egg masses/plant, and were maintained in the conditions described above. Seven weeks after the inoculation, the roots of the resistant tomato and pepper plants were examined. If there were no egg masses, new plants of resistant tomato and pepper were inoculated again using inoculum from the original nematode cultures. In case that there was no reproduction of the nematodes in the second test, the populations were discarded.

From the 20 RKN populations tested, 12 were discarded as they did not reproduce on resistant tomato and pepper. The remaining eight populations, originating exclusively from greenhouse crops, were selected for subsequent studies according to the results of the preliminary tests:

1. Five populations which reproduced on resistant tomato but not on pepper (I1, I2, I3, S and V).
2. One population which reproduced on both resistant tomato and pepper (I4).
3. One population which reproduced on pepper but not on resistant tomato (K).
4. One population which did not reproduce in both, resistant tomato and pepper, was kept as control (I5).

RKN population virulence tests

The populations which reproduced on resistant tomato and pepper were maintained in the same cvs for 4-5 further generations, using each time ten egg masses originating from the tomato cv. Silvana or the pepper cv. California Wonder. All the original populations were simultaneously maintained in the susceptible tomato cv. ACE or in pepper cv. California Wonder.

Females of each population were extracted from the roots and used for identification. Protein extracts were obtained from females and the electrophoretic analysis was carried out using the Mini-Protean Tetra cell system (Bio-Rad) according to Esbenshade and Triantaphyllou (1985ab) and Pais *et al.* (1986), with some modifications. The origins and species of these eight *Meloidogyne* populations are presented in Table 2. In all cases the same species per population was identified in both the susceptible tomato and resistant tomato or pepper, indicating that selection in these plants did not alter the constitution of the initial nematode population.

The reproductive ability of all the original nematode populations, which were maintained either on the susceptible tomato cv. ACE or the susceptible pepper cv. California Wonder for at least four generations, was studied on the resistant tomato cv. Silvana and on the susceptible pepper cv. California Wonder in a pot experiment. Egg masses were left to hatch in extraction dishes and plants of both tomato cvs and pepper grown in 250 ml pots were inoculated with 400 second stage juveniles (J_{2s}) with five replicates per treatment. In each test, the population I5 was used as control to prove the resistance of the tomato cv. Silvana and the non host status of pepper cv. California Wonder, towards the avirulent *M. javanica*. Plants were maintained at 16 h photoperiod and soil temperature 24-26°C. The number of egg masses on roots was assessed seven weeks after inoculation. Egg masses

(c. 5-10) were randomly collected from roots of each plant, transferred into an aqueous solution of sodium hypochoride to release eggs (Hussey and Barker, 1973) and checked under the stereoscope for the presence of eggs. The number of egg masses produced on susceptible tomato, resistant tomato and pepper were compared by ANOVA. The experiment was conducted once.

Results and Discussion

A review of 13 described resistance-breaking populations of RKN from Greece during the period 1994-2013 (Table 1) reveals the following characteristics:

1. From 1994 until 2013, 13 RKN populations, from which 11 *M. javanica* and two *M. incognita*, able to reproduce on resistant tomato, have been recorded in five different regions of Greece: Crete, Epirus, Thrace, Peloponissos and Macedonia.
2. Four populations, three from Crete (*M. javanica* and *M. incognita*) and one from Peloponissos (*M. javanica*), collected from heavily infected roots of nematode resistant tomato, reproduced at high rates on resistant tomatoes in pot tests (Tzortzakakis *et al*., 1999, 2005, 2008).
3. Seven populations, two from Crete (*M. javanica*), four from Epirus (*M. javanica*) and one from Macedonia (*M. incognita*), collected from nematode susceptible crops with no recent history of resistant tomato cultivation in the field sites, reproduced at high rates on resistant tomatoes in pot tests (Tzortzakakis *et al*., 1999, 2005, 2014).
4. Two populations, one from Thrace (*M. arenaria* collected from balm) and one from Crete (*M. incognita* collected from susceptible tomato), were unable to reproduce on resistant tomato in pot tests, when six egg masses were inoculated per plant. However, when resistant tomatoes were inoculated with 30 egg masses per plant, a virulent *M. javanica* was revealed in both cases, composing a minor percentage in the original population which was undetected in the identification process (Tzortzakakis *et al*., 2008).
5. The only two resistance-breaking populations of *M. incognita* found in Greece,

Table 1. Root-knot nematode populations (*Meloidogyne* spp.) from Greece, virulent on resistant tomato hybrids with the *Mi* gene, reported within the period 1994-2013.

Code	Nematode species	Region	Host plant found	References
1HVa and HVb , 4/1 and 4/2[1]	*M. javanica*	Crete	Resistant tomato	Tzortzakakis and Gowen, 1996; Tzortzakakis *et al*., 1999
16, 17	*M. javanica*	Crete	Susceptible tomato	Tzortzakakis *et al*., 1999
*Mj*P1, *Mj*P2, *Mj*P3, *Mj*P4	*M. javanica*	Epirus	Susceptible tomato, cucumber	Tzortzakakis *et al*., 2005
*Mj*C1	*M. javanica*	Crete	Resistant tomato	Tzortzakakis *et al*., 2005
*Mi*C1	*M. incognita*	Crete	Resistant tomato	Tzortzakakis *et al*., 2005; Tzortzakakis and Blok, 2007
T	*M. javanica*[2]	Thrace	Balm	Tzortzakakis *et al*., 2008
C	*M. javanica*[3]	Crete	Susceptible tomato	Tzortzakakis *et al*., 2008
P	*M. javanica*	Peloponissos	Resistant tomato	Tzortzakakis *et al*., 2008
*Mi*NG	*M. incognita*	Macedonia	Beet	Tzortzakakis *et al*., 2014

[1] The lines 4/1 and 4/2 are the same with lines 1HVa and 1HVb (single egg mass lines from the same nematode population). In the reference Tzortzakakis *et al*., 1999 they were characterized by molecular and biochemical methods.
[2] The original population identified as *M. arenaria*, which was avirulent and the virulent *M. javanica* consisted a minor component selected by resistant tomato.
[3] The original population identified as *M. incognita*, which was avirulent and the virulent *M. javanica* consisted a minor component selected by resistant tomato.

one in Crete and the other in Macedonia, differed in their ability to reproduce on susceptible pepper cultivars (Tzortzakakis and Blok, 2007; Tzortzakakis *et al*., 2014).

In all the above studies referred, small scale surveys had been conducted in two cases: a) in Crete, in a random sampling on 37 greenhouses representative of the main vegetable growing areas, where two resistance-breaking populations of *M. javanica* were found (Tzortzakakis *et al*., 1999) and b) in Preveza Epirus, a random sampling on ten greenhouses, where the presence of four resistance-breaking populations of *M. javanica* was detected (Tzortzakakis *et al*., 2005). The remaining records came from samples which had been sent to the laboratory for identification.

The results of the current study on RKN populations collected in 2013-2014 are presented in Table 2. There was no infection in the resistant tomato cv. Silvana inoculated with the I5 and K populations, which prove that the *Mi* gene was effective under the certain experimental conditions. The population I5, identified as *M. javanica*, did not reproduce on resistant tomato and pepper. The population K, identified as *M. incognita*, reproduced on pepper at lower rate than on susceptible tomato but not on resistant tomato.

Six populations were found to reproduce on resistant tomato at a level which did not differ significantly to that obtained on the susceptible tomato. Furthermore, their ability to reproduce on resistant tomato was stable as they sustained consistent reproduction on resistant tomato for at least four successive generations. The egg masses which were randomly collected from roots of resistant tomato and pepper all contained a sufficient (>100) number of eggs. From the resistance-breaking populations, four were *M. javanica* which did not reproduce on pepper while from the two virulent *M. incognita*, the I4 reproduced on pepper, at lower rate than on tomato, while the I3 did not.

In the 20 tested RKN populations, the resistance-breaking ones were found at a quite high percentage (30%) compared with that of the survey done 18 years earlier (Tzortzakakis *et al*., 1999), in which that percentage was 5% in 37 samples collected from several areas of Crete. The population which was identified as *M. incognita* (I3), able to reproduce on resistant tomato but not on pepper, is similar to another population of *M. incognita* found earlier in another area of Crete (*Mi*C1 in Tzortzakakis and Blok, 2007). However, the *M. incognita* (I4), reproducing on both the resistant tomato and pepper, is reported for the first time in Crete and for

Table 2. Number of egg masses produced by eight populations of root-knot nematodes (*Meloidogyne* spp.) collected from greenhouses of Crete on susceptible tomato, resistant tomato and susceptible pepper.

Code	Origin	Species	No of egg masses				
			Susceptible tomato cv. ACE	Resistant tomato cv. Silvana	Susceptible pepper cv. California Wonder	SED	P value
I1	Tomato	*M. javanica*	56	44	0[1]	7.9	>0.05
I2	Tomato	*M. javanica*	45	38	0	6.6	>0.05
I3	Tomato	*M. incognita*	44	41	0	7.7	>0.05
I4	Pepper	*M. incognita*	41	44	22	6.5	<0.05
I5	Cucumber	*M. javanica*	42	0[1]	0	-	-
S	Tomato	*M. javanica*	38	32	0	5	>0.05
V	Tomato	*M. javanica*	39	36	0	6.7	>0.05
K	Pepper	*M. incognita*	45	0	29	3.8	<0.01

Origins: I1-I5 Ierapetra, S: Skourvoula Messara, V: Vori Messara, K: Kisamos Chania; Average of five replicates per treatment; [1] In case of 0 values the data were excluded from analysis; SED and P values from ANOVA.

the second time in Greece (Tzortzakakis *et al.*, 2014). It is important to notice that in Ierapetra which is the most important area of greenhouse vegetable production in Crete, four out of the eight tested RKN populations were resistance-breaking populations. These results are in contrast with the study conducted 18 years ago (Tzortzakakis *et al.*, 1999), where no resistance-breaking populations were found in this area.

Since commercial nurseries provide nematode-free tomato seedlings, a possible explanation for such a 'rise' in the percentage of resistance-breaking populations, could be a 'potential selection' by the increased cultivation of resistant to RKN tomato hybrids. However, in the fields where the pathotypes were found, there was no recent history of cultivation of resistant tomatoes. Furthermore, previous studies in pots indicated that single egg mass lines of *M. javanica* did not have the capacity of adapting to resistant tomatoes (Tzortzakakis *et al.*, 1999). Thus it is difficult to provide an explanation for the increase in the percentage of the resistance-breaking RKN populations within the last 18 years period.

In the Mediterranean area, resistance-breaking populations of *M. javanica* and *M. incognita* have been reported in several countries e.g. Cyprus, France, Italy, Morocco, Spain, Tunisia and Turkey, with the majority of them being *M. javanica* (Castagnone-Sereno *et al.*, 1994; Devran and Sogut, 2010; Eddaoudi *et al.*, 1997; Molinari and Miacola 1997; Ornat *et al.*, 2001; Philis and Vakis, 1977; Robertson *et al.*, 2006). The results presented herein, constitute additional records on the occurrence of virulent *M. javanica* and *M. incognita* populations in the area of Ierapetra, Crete.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Επικαιροποίηση της παρουσίας παθοτύπων των νηματωδών του γένους *Meloidogyne* σε ανθεκτική τομάτα στην Ελλάδα με έξι νέες καταγραφές από την Κρήτη

Ε.Α. Τζωρτζακάκης, M.-C. Vieira dos Santos και I. Conceição

Περίληψη Στην παρούσα εργασία γίνεται μία επικαιροποίηση των δημοσιευμένων πληροφοριών που αφορούν στην παρουσία νηματωδών του γένους *Meloidogyne* σε ανθεκτική τομάτα στην Ελλάδα. Την περίοδο 1994-2013, 13 πληθυσμοί (11 *M. javanica* και δύο *M. incognita*) με ικανότητα αναπαραγωγής σε ανθεκτική τομάτα καταγράφηκαν στις περιφέρειες της Κρήτης, Ηπείρου, Θράκης, Πελοποννήσου και Μακεδονίας. Έξι επιπλέον παθότυποι, τέσσερις *M. javanica* και δύο *M. incognita,* επισημάνθηκαν σε θερμοκηπιακές καλλιέργειες κηπευτικών στην Κρήτη, την περίοδο 2013-2014. Τέσσερις από τους παθότυπους, δύο *M. javanica* και δύο *M. incognita,* επισημάνθηκαν για πρώτη φορά στην περιοχή της Ιεράπετρας που αποτελεί τη σημαντικότερη περιοχή καλλιέργειας κηπευτικών σε θερμοκήπια στην Κρήτη.

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