

To: California Pepper Commission

RE: Research Report for 2012

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Project Title: Root-knot nematode damage to bell pepper.

Statement of the problem and background.

The Southern root-knot nematode (*Meloidogyne incognita*) has been reported to cause serious damage to peppers. The second-stage nematodes (J2) are worm-shaped, move through the soil, and enter the plant roots. In roots of a host crop, the second-stage juveniles nematodes develop into females, while the root system responds to infection with the formation of galls. The fully developed females can produce up to 400 eggs, that are contained in clusters in a gelatinous material and “glued” to the outside of the roots. From these eggs second-stage juveniles can emerge to repeat the cycle, or eggs can remain in the soil during fallow period to serve as inoculum for the next crop cycle. The duration of the nematode life cycle depends primarily on the species of root-knot nematode and on soil temperature. *Meloidogyne incognita*, the most important species infecting bell-pepper, can complete its’ life cycle in less than 4 weeks under an optimum soil temperature of 32C (90F), and become inactive when the soil temperature drops below 17C (62F). In most host crops, root-knot nematode infestation can easily be diagnosed because of obvious galling on the affected roots. Above-ground symptoms are however not specific, and can include chlorosis, wilting under sufficient soil moisture, stunting, and increased susceptibility of plants to fungal or bacterial root pathogens.

In the Coachella Valley of Southern California, approximately 5,000 acres are cropped with bell peppers, representing an estimated gross crop value of \$90,000,000. Root-knot nematodes are widespread throughout the Coachella Valley and growers report serious damage. To control nematodes, pepper growers in the Coachella Valley commonly apply fumigant nematicides such as metam-sodium (Vapam) or 1,3-dichloropropene (Telone) as a post-harvest and/or pre-plant soil treatment through the drip tubing.

There are a few studies describing the interactions between the nematodes and bell peppers, but they are primarily from Europe. Although there is a general consensus among Coachella Valley growers that root-knot nematodes can result in major crop damage if not controlled, research data on damage thresholds and tolerance limits under their growing conditions (e.g. pepper varieties grown, occurring nematode populations/species, soil types and temperature regimes) are not available.

A first goal of this project was to establish the damage potential of the nematodes to bell pepper in California, and specifically in the Coachella Valley. This will provide the basis for a risk assessment, based on pre-plant soil nematode levels as determined through soil sampling. In the next step the project will determine the efficacy of root-knot nematode resistance in bell pepper as a management strategy.

Objectives of this project are:

1. Characterize root-knot nematode populations occurring in Coachella Valley bell pepper fields
2. Establish damage thresholds and tolerance limits of bell pepper for a locally occurring root-knot nematode population.
3. Determine reproduction and damage of locally occurring nematode populations on resistant bell pepper varieties.

Field sampling

A total of 13 soil samples from pepper fields in the Coachella Valley were collected on 4/15/2012 from eight different pepper field locations. Plant symptoms at this time included minor wilting and stunting. No typical nematode symptoms (galling) were seen on the roots of these plants. The samples were taken to UC Riverside, and 100g subsamples were extracted for nematodes over a Baermann funnel. Nematodes were then counted after a 5-day extraction period under 40x magnification. In one of the 13 soil samples, one root-knot nematode (RKN) second-stage juvenile (J2) was detected. No RKN J2 were detected in the other 12 samples.

During late May 2012, further reports were received about plants with suspected nematode damage, and a second field trip was made. A field on CVARS (Coachella Valley Agricultural Research Station) with “mini-bell peppers” showed obvious strips with yellowing plants. A field in Thermal also showed yellowing plants. Soil samples were collected at various locations within these fields as before.



Figure 1. Yellowing symptoms in bell peppers at CVARS on 5/31/2012

Roots of plants collected in these fields showed obvious symptoms (galling) of RKN infestation (Fig. 2). Root and soil samples were taken to UC Riverside for nematode extraction and counting. Staining of the roots with a nematode-egg-mass specific stain (eriolglaucine) revealed a

high infestation with RKN (Fig 2).

Figure 2. Root system of field-grown bell pepper showing root galling (L), and root-knot nematode egg-masses (blue dots) on roots of field-grown bell pepper after staining with erioglaucine (R).



Nematode analysis of the soil samples and root systems showed very high levels of root-knot nematodes. A soil and root sample from another bell pepper field (code: OM) suspected of root-knot nematode infestation also had high nematode levels.

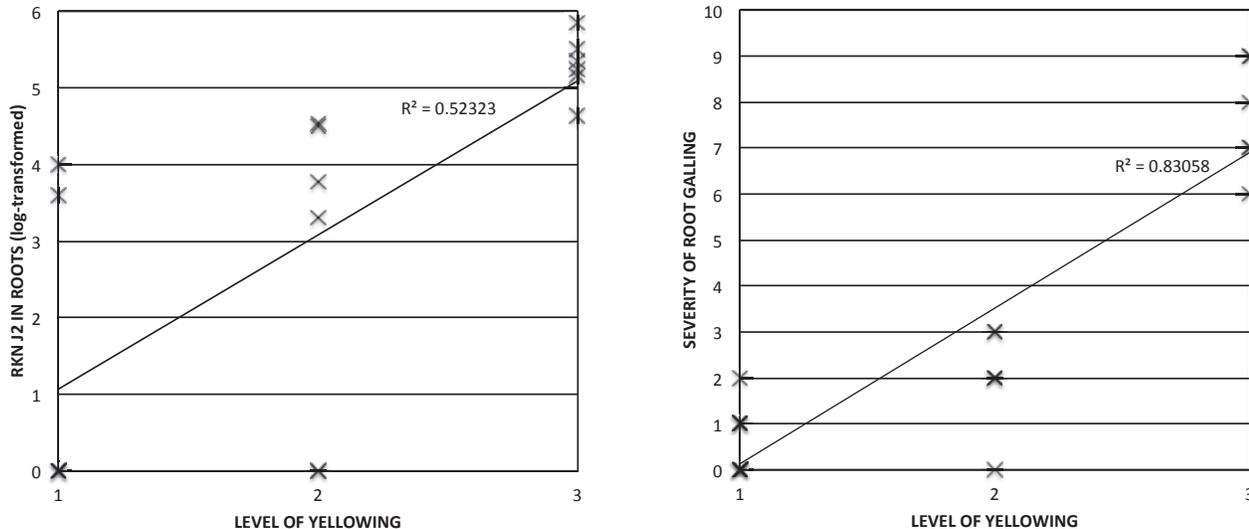
Table 1. Root-knot nematode levels in soil and roots of bell-peppers collected on 5/31/2012 in the Coachella Valley.

Field Code	Sample	RKN J2 per 100	
		RKN J2 on roots	g soil
CVARS	1	292,000	32,500
	2	92,000	4,500
	3	166,000	16,000
	4	18	4
Thermal	1	56,000	8,500
	2	56,000	1,500
OM	1	121,000	5,750
	2	41,500	3,000

On 6/15/2012 a third trip was made to visit the CVARS field, where yellowing symptoms were obvious (see Figure 1). A total of 23 bell pepper plants were removed from areas with severe yellowing (index 3), from areas with moderate yellowing (index 2), and from an area with no or very mild yellowing (index 1). Nematode infestation of the roots, severity of root-galling, and plant fresh root and shoot weight were determined, and plotted against the yellowing index. No clear correlations existed between the severity of yellowing and root or shoot weight. However a slight correlation existed between nematode levels in the roots and severity of yellowing ($R^2=0.52$), and a stronger correlation ($R^2=0.83$), between the severity of root-galling and

yellowing (Figure 3).

Figure 3. Correlation between yellowing of bell pepper plants, RKN levels in roots (Left), and severity of root-galling (Right)



In late June 2012, the peppers at CVARS were killed with a low rate of Vapam (15 gallons/acre) applied through the drip system. Soil and root samples of pepper plants were collected two weeks after the application and processed for nematode extraction. Two soil samples contained 600 and 550 RKN J2 respectively, and four root systems contained between 4,400 and 176,000 RKN J2.

Nematode identification

Adult root-knot nematode females were excised from roots of pepper plants collected from CVARS, Thermal, and OM, and perineal patterns were cut for morphological identification (5 females per field origin). All females were identified as *Meloidogyne incognita* (Southern root-knot nematode). To further identify the nematodes, a differential host range test was done using nematodes from these three fields as inoculum. Plants included in the test were: pepper, tomato, cotton, corn, tobacco, peanut, watermelon. According to the presence/absence of root-galling on roots of these plants, nematodes were identified as *M. incognita* race 3 (able to reproduce on cotton).

Effects of nematode levels and pepper cultivar on plant damage and nematode reproduction.

Root-knot nematodes originally obtained from bell pepper at CVARS were increased and maintained in a greenhouse on tomato. Approximately 8 weeks after inoculating the tomatoes, the nematodes were extracted from the tomato root systems to be used in a pot experiment.

For the experiment five different pepper cultivars were used:

Carolina Wonder	Resistant
Charleston Belle	Resistant
Crusader	Susceptible
Mini Bells*	Susceptible
Baron	Susceptible

* seed of “Mini Bells” was collected from mature fruits grown at CVARS.

Peppers were seeded in a seedling tray with potting mix, and transferred three weeks later to 1-gallon pots filled with 3 kg steam-sterilized sandy soil. For each pepper variety 20 pots were set up. On the same as day transplanting, pots were inoculated with 0, 1,500, 4,500, or 15,000 second-stage root-knot nematode juveniles. Each pepper cultivar×inoculum level had 5 replicates. The pots were randomized on greenhouse benches, watered with an automated drip system, and fertilized with slow-release Osmocote fertilizer. Fruits were harvested when mature, and their fresh weight determined. Three months after transplanting, all pepper plants were removed from the pots, root systems examined for galling, and fresh shoot and root weights were determined. Second-stage root-knot nematode juveniles were extracted from the root systems and counted.

Effects on fruit production and plant growth.

Nematodes did not significantly affect total fruit yield (g per plant) in the two resistant cultivars ‘Carolina Wonder’ and ‘Charleston Belle’. In the susceptible cultivar ‘Crusader’ the fruit yield was also similar under all four nematode levels. In the susceptible ‘Baron’, a low nematode level resulted in an increased fruit yield, but at high nematode levels the yield significantly decreased by 18% compared to the no-nematode control. The ‘MiniBells’ was the most sensitive cultivar, and in this cultivar the yield was affected at any nematode level. At the two higher nematode levels, the yield was reduced by approximately 50% compared to the no-nematode control (Table 1).

Table 1. Effect of root-knot nematode (*M. incognita*) inoculum levels on total fruit production (gram per plant) of five bell pepper cultivars.

Inoculum (J2 per pot)	Pepper cultivar ¹					
	CW	CB	CR	MB	BA	
0	100 ² a	100 a	100 a	100 a	100 b	
1,500	93 a	79 a	132 a	65 b	119 a	
4,500	61 a	65 a	146 a	46 c	89 bc	
15,000	80 a	98 a	81 a	58 bc	82 c	

¹Pepper cultivars CW=Carolina Wonder (R), CB=Charleston Belle (R), CR=Crusader (S), MB=MiniBells (S), BA=Baron (S).

²Pepper yields expressed as percentage relative to the no-nematode control, statistical analysis on non-transformed data. Different letters within the same column indicate significant differences at the 95% confidence level.

Effects of the nematode levels on the number of fruits produced per plant were very similar to effects on the total weight of the fruits harvested (data not shown). The nematode level did not affect the fresh shoot weight of the plants in any of the five cultivars. The nematode level

affected the fresh root weight only in the ‘MiniBells’ (MB), where the no-nematode control plants had significantly heavier roots than the nematode inoculated plants.

Effects on nematode symptoms and reproduction.

Statistical analysis of the root-galling data showed that its severity was significantly affected by the pepper cultivar ($P=0.0001$), and by the initial nematode level (1,500, 4,500, or 15,000 J2 per pot) ($P=0.0258$), but that there was no significant interaction between cultivar and inoculum density ($P=0.9433$ ns). This indicates that the effect of increasing nematode levels on the severity of root-galling was similar for each cultivar.

Table 2. Effect of three root-knot nematode (*M. incognita*) inoculum levels on root-galling (scale 0-10) of five bell pepper cultivars.

Inoculum (J2 per pot)	Pepper cultivar ¹					average ²
	CW	CB	CR	MB	BA	
1,500	0.0	1.4	4.2	6.8	6.6	3.8 b
4,500	0.0	2.6	6.5	7.6	7.2	4.7 ab
15,000	0.5	3.0	6.2	7.8	8.4	5.4 a
Average ³	0.1 d	2.3 c	5.6 b	7.4 a	7.4 a	

¹Pepper cultivars CW=Carolina Wonder (R), CB=Charleston Belle (R), CR=Crusader (S), MB=MiniBells (S), BA=Baron (S).

²Different letters within this column indicate significant differences at the 95% confidence level.

³Different letters within this row indicate significant differences at the 95% confidence level.

The results show that root-galling on the nematode resistant ‘Carolina Wonder’ was nearly absent, and significantly lower than on the other cultivars. Galling on the other resistant cultivar ‘Charleston Belle’ was higher, but lower than on the three susceptible cultivars. Galling on the susceptible ‘Baron’ and ‘MiniBells’ was higher than on the other susceptible cultivar ‘Crusader’. Averaged over all cultivars, the severity of root-galling was proportional to the initial nematode level (Table 2).

The number of second-stage root-knot nematodes on the pepper roots at the end of the experiment depended strongly on the pepper cultivar ($P=0.0001$), but not on the initial inoculum density ($P=0.4680$ ns). There was no significant interaction between the cultivar and the initial level of inoculation.

Table 3. Effect of three root-knot nematode (*M. incognita*) inoculum levels on the final nematode infestation levels on the roots of five bell pepper cultivars.

Inoculum (J2 per pot)	Pepper cultivar ¹					average
	CW	CB	CR	MB	BA	
1,500	150	230,500	1,700,000	4,006,000	3,317,000	1,850,730 ns
4,500	4,650	377,000	2,950,000	4,030,000	4,473,000	2,342,635 ns
15,000	15,000	1,874,500	2,800,000	6,210,000	3,877,500	3,077,917 ns
Average ²	6,000 c	827,333 b	2,450,000 a	4,748,667 a	3,889,167 a	

¹Pepper cultivars CW=Carolina Wonder (R), CB=Charleston Belle (R), CR=Crusader (S), MB=MiniBells (S), BA=Baron (S).

²Data were log(x+1)-transformed for statistical analysis. Non-transformed data are shown. Different letters within this row indicate significant differences at the 95% confidence level.

Results show that nematode reproduction was lowest on the resistant 'Carolina Wonder'. On average, final nematode numbers (Pf) were lower than inoculum levels (Pi) (reproductive factor Pf/Pi \approx 0.7). On the other resistant cultivar 'Charleston Belle', nematode levels on average increased more than a hundred-fold (Pf/Pi=121). The three susceptible varieties were excellent hosts for the nematodes, with average multiplication factors (Pf/Pi) ranging between 658 and 1327. Different initial nematode levels did not result in differences in final nematode levels. This most likely is due to the fact that the root system is restricted by the volume of the pot, and over time, all roots that are available for the nematodes to feed on, are occupied by the multiplying nematodes.

Conclusions

Conclusions from this project so far are:

- Root-knot nematodes are fairly common in pepper fields in the Coachella Valley.
- Root-knot nematode populations can increase dramatically in a short time, and reach very high levels both in soil as well as on roots.
- Chlorosis (yellowing) of bell pepper plants appears associated with root-knot nematode infestation, but these symptoms could not be reproduced under greenhouse conditions. Further research into the chlorosis is needed to understand the role of nematodes in this process.
- Killing of bell peppers post-harvest with Vapam did not eliminate nematodes in soil or roots.
- Root-knot nematodes isolated from pepper roots were identified as *Meloidogyne incognita*.
- In greenhouse pot trials, nematodes did not cause a reduction in fruit production of two resistant and one susceptible cultivar, but significantly reduced fruit yield in two other susceptible cultivars.
- Root-galling was minor on two resistant cultivars, moderately high on three susceptible cultivars.
- There were significant differences in the host status of the two resistant cultivars for *M. incognita*.
- Three susceptible cultivars were excellent hosts for *M. incognita*, with population levels increasing between 658- and 1327-fold.

Extension of Results

Results from this project were presented at

CHAPA Meeting in Palm Desert, 7/25/2012

PAPA Meeting in Indio, 9/27/2012

PAPA Meeting in Palm Desert, 12/4/2012

W2147 Regional Meeting, Riverside, 12/7/2012.