To: California Pepper Commission

RE: Research Report for 2019

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Project Title: Efficacy of novel nematicides and plant resistance against root-knot nematodes on bell peppers.

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.

In our previous studies, also funded by the CPC, we initially found that root-knot nematodes (*M. incognita*) were relatively common in pepper fields in the Coachella Valley. Soil and root infestation levels were sometimes very high. In greenhouse pot experiments, using nematode populations isolated from Coachella pepper fields, we found that of two nematode-resistant pepper varieties tested (Charleston Belle and Carolina Wonder), the Carolina Wonder pepper was highly resistant even under high nematode inoculum levels.

2019 Greenhouse Trials.

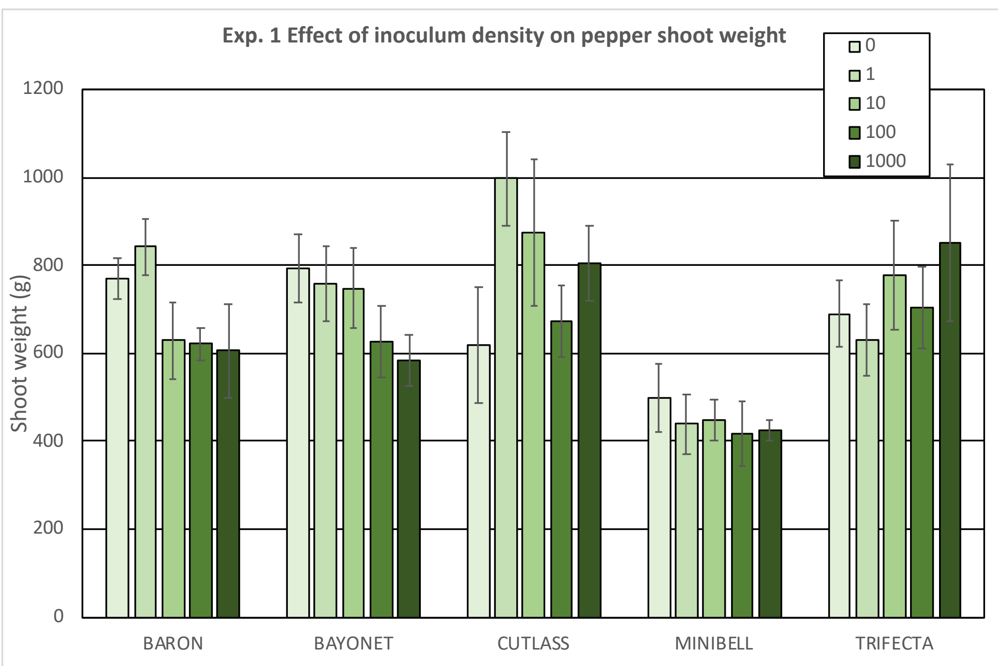
Relation between nematode inoculum and plant growth

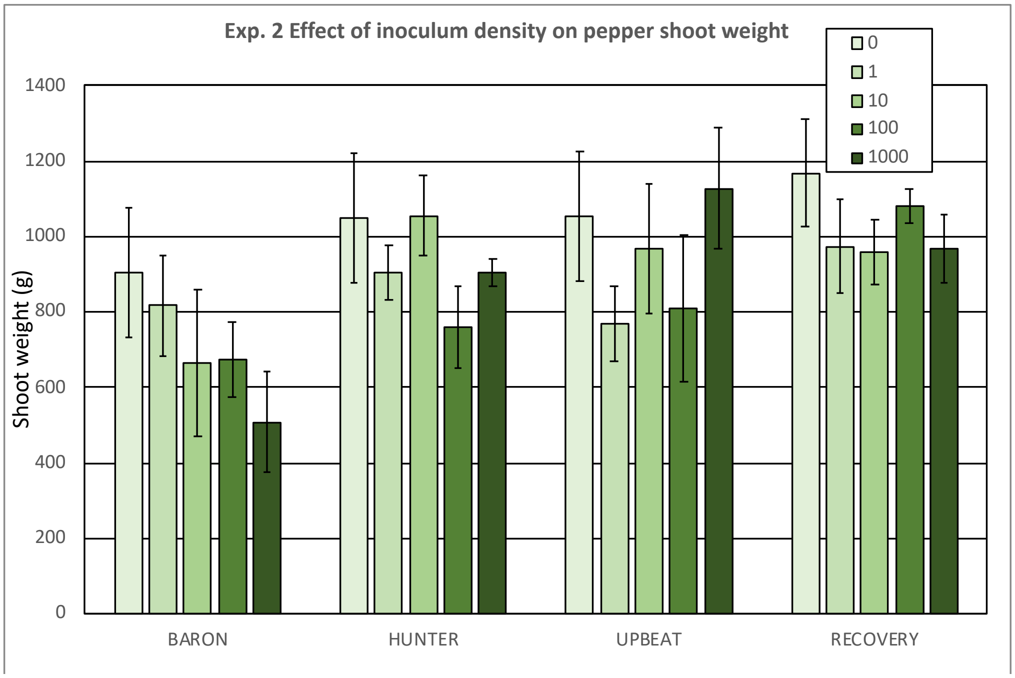
Goal of greenhouse trials was to determine at what nematode inoculum levels, pepper growth starts to suffer. For our greenhouse trials we collected seed from the following pepper cultivars: 'Bayonet', 'Cutlass', 'MiniBell', 'Trifecta', 'Hunter', 'Upbeat', and 'Recovery'. Cultivar 'Baron' was included as a "control", as we had data from this variety from earlier experiments.

All varieties were grown from seed and transplanted as transplants with 6 true-leaves into pots with 2.3 kg sandy soil. Root-knot nematode second-stage juveniles (J2, *M. incognita*) were obtained from infested tomato roots, and used as inoculum. Soil was prepared to contain 1, 10, 100, or 1,000 J2 per 100 g soil and pots were filled with this soil so that for each cultivar x infestation level there were 5 replicates. Two sets of experiments were done: In the first set the varieties 'Baron', 'Bayonet', 'Cutlass', 'MiniBell', and 'Trifecta' were tested. In the second set the varieties 'Baron', 'Hunter', 'Upbeat', and 'Recovery' were tested. Plants were grown for 3 months, and fresh shoot weight from all plants (fruit + stem + leaves) was determined.

Results:

In cultivar 'Baron' there was a trend that increasing inoculum levels resulted in lower shoot weight in both experiments. A similar trend was seen in 'Bayonet', and to a lesser extent in 'Minibell'. In the other cultivars, inoculum levels did not appear to have an effect on shoot growth. (see figures 1 and 2).





From these results it would appear that most varieties are very tolerant towards the nematodes, as clear effects of even high nematode levels were not obvious. We will repeat these experiments in 2020, and furthermore study the influence of soil temperature, and of plant age at time of exposure to the nematodes on this relationship.

Use of Nimitz as a foliar spray to control nematodes.
Other researchers previously reported that the nematicide Nimitz when as a foliar spray in pepper was able to control nematode infestation. We repeated their experiments in a greenhouse with different crops: pepper 'Baron', tomato 'Daniella', melon 'Durango' and eggplant 'Black Beauty'. Plants were sprayed with different concentrations of Nimitz and then exposed to nematode infested soil. All concentrations of Nimitz used as a foliar spray had severe phytotoxic effects. Melon was very sensitive and all plants died as a result of the spray. Tomato and eggplant were also severely damaged. Pepper was somewhat tolerant to the Nimitz spray, but still exhibited serious damage as a result of the spray. The spray did result in a reduction of nematode infestation, but at effective concentrations, the phytotoxic effects were very significant. In a follow up study, we examined if the nematicide was active in the rhizosphere when used as a foliar spray. Pepper plants were sprayed with Nimitz, and then washed from the pots, place in a vial with a water suspension containing nematodes. A high percentage of the nematodes in the suspension in which a Nimitz treated plant was placed, died within 48 hrs. These experiments showed that Nimitz moves from the leaves to the roots of plants, and "leaks" from the roots into the surrounding environment where it can still exhibit nematicidal activity. However, the phytotoxicity associated with a spray application of Nimitz would make this not a feasible treatment. In the coming year, we will study if a leaf spray with Nimitz could be used as a treatment post-harvest treatment to kill the crop and at the same time kill nematodes inside the roots.

Field Trials.

Damage threshold: At CVARS beds were cropped with nematode susceptible tomato, nematode resistant tomato, carrot, or left fallow. The purpose of this was to establish a trial site that would encompass a large range of different nematode levels. In 2020 we will "map" nematode levels in this field by intensive soil sampling, and will then grow a range of pepper varieties (same as in previous greenhouse trials) on the field. We will design the trial in such a way that all varieties are grown in some plots with high, intermediate, and low nematode infestation levels. Harvest data and nematode infestation of the plants will be collected at the end of the crop. The effect of initial nematode level on fruit yield and final nematode infestation will be determined.

Effect of novel nematicides:

<u>Field location and layout:</u> A field trial was initiated at the South Coast Research and Extension Center located in Irvine, Southern California. The field site was on root-knot nematode (*M. incognita*) infested sandy-loam (17% clay, 70% sand, 18% silt, 12% clay, 0.1% OM, pH 7.3). The field was prepared on 5/15/2019. Beds (40-inch c-c) were shaped, and 5 treatments were assigned to 25 plots (20 ft. long section of the bed, 3 ft. buffer between plots along the beds) according to a completely Latin-square design with 5 replicates per treatment (Figure 1). Soil samples were collected from all plots, and Nimitz was applied on appropriate plots on 6/12/2019. One week later (6/19/2019), Salibro was applied on appropriate plots and bell pepper transplants var. 'Baron' were planted at 16-inch spacing (15 plants/plot), as a single row in the center of each bed.

<u>Soil sampling and nematode analysis:</u> Before the first treatment (Nimitz 480 EC, 48% a.i. fluensulfone) and at harvest, soil samples were collected from all plots using a sampling tube (diameter 0.5 inches) from between 2-10 inches deep. Five cores were collected at random from each plot and mixed to form one composite sample per plot. The samples were transported to the laboratory, and nematodes were extracted from 100 cm^3 sub-samples using a Baermann-funnel technique (extraction efficiency approx. 40%). The numbers of second-stage root-knot nematode juveniles were counted under 40x magnification.

<u>Root system analysis</u>: Root systems of five plants were collected from each plot after the final harvest, transported to the lab, and visually indexed for severity of galling. The roots were then weighed, and the RKN eggs were extracted from the combined five root systems by shaking for 2 min in a 10% bleach solution, and collecting the eggs on two 500 mesh sieves. The number of eggs was counted, and the number per g root was calculated.

<u>Application of products:</u> Nimitz and Salibro 500 SC (50% a.i. fluazaindolizine) were applied by sprinkling with a watering can (2 gallons) over the top of the plots, followed by an additional 2 gallons of water. The treatments were incorporated into the plots with a rototiller to approximately 4 inches depth. Salibro was applied immediately before pepper planting.

<u>Treatments and rates:</u> There were 5 treatments: an untreated control (UTC), Salibro@low rate, Salibro@medium rate, Salibro@high rate, and Nimitz (Table 1).

Table 1. Treatments, application rates, schedule, and method.

Treatment		Active ingredient	Timing[1]	Rate per acre (US)	Liter per ha (metric)
1	UTC	untreated			
2	Salibro_L	fluazaindolizine	at planting	30.7 fl.oz.	2.25
3	Salibro_M	fluazaindolizine	at planting	46.2 fl.oz.	3.38
4	Salibro_H	fluazaindolizine	at planting	61.4 fl.oz.	4.50
5	Nimitz	fluensulfone	7 dbp	5 pt	5.84

[1]dbs: days before planting.

Trial activities, dates, and soil temperatures are shown in Table 2.

Table 2. Dates, soil temperatures, treatments[1], rates[2], and field activities.

Date	Soil temp at 6 inch (F)	Activity[2]	Treatment number[1]
5/8/2019	N/A	Seeded pepper 'Baron' in Sunshine mix in UCR greenhouse.	
5/15/2019	71	Staked out plots.	all
6/12/2019	78	Collected soil samples from all plots.	all
		Applied Nimitz @ 5 pt/A to moist soil in 2 gallons water per plot, followed by 2 gallons water per plot. Incorporated into the top 4 inches.	5
6/19/2019	72	Applied Salibro at 30.7, 46.2, and 61.4 fl.oz/A, to moist soil in 2 gallons water per plot, followed by 2 gallons water per plot. Incorporated into the top 4 inches.	2,3,4
		Planted bell pepper var. 'Baron' at 16 inch spacing; 15 plants/plot.	all
9/5/2019	82	Harvest fruits from all plants. Count and weigh fruit from each plot (first harvest).	all
9/13/2019	78	Harvest fruits from all plants. Count and weigh fruit from each plot (second harvest).	all
9/26/2019	75	Harvest fruits from all plants. Count and weigh fruit from each plot (third = final harvest). Root systems of five randomly selected plants were dug from each plot, transported to the lab to determine the severity of root galling and for extraction of RKN eggs. Collect soil samples from all plots.	all

[1]treatment numbers as shown in Table 1.
[2]Actual treatment amounts applied were calculated according to: bed surface = 20 ft. long x 1.5 ft wide = 30 sqft/plot x 5 reps = 0.0035 acre. Thus, amount per trt (5 plots) = 0.0035 x rate/acre.

Results and Discussion

The general growing conditions for the trial were good. No obvious disease problems were encountered. The initial nematode pressure was light, with an average of 7.2 second-stage root-knot nematode juveniles per 100 cm^3 soil. There were no significant differences among the treatments [log10(J2+1), treatment effect: $P = 0.11$]. At harvest, soil nematode levels had increased in all treatments, but the average final population level was much higher in the untreated control than in the other treatments (Table 3; Fig. 2). Similarly, the severity of root galling and the infestation level of the roots with RKN eggs were also significantly higher in the untreated control. All four nematicide treatments dramatically reduced the RKN infestation levels of the roots, but there were no significant differences among Nimitz or the three Salibro rates. Galling symptoms on pepper roots are usually not dramatic, and therefore difficult to rate. But in this trial obvious and significant differences existed between the untreated control and the other treatments. Although final soil nematode levels, root infestation levels, and galling was lighter as Salibro rates increased, these differences were not significant (Table 3, Figure 2-4).

Plots were visually examined every two weeks (plant size, color, general appearance), but at no time did we observe obvious differences among the treatments.

Table 3. Average (n=5, ±SE) root-knot nematode (*M. incognita*) soil and root infestation levels and root galling from a 2019 bell pepper variety 'Baron' field trial at the UC South Coast Research and Extension Center, Irvine, CA. Galling on a scale 0-10: 0%-100% of bell pepper root with galls.

	RKN J2 per 100 g soil				RKN		Galling index	
Treatment	initial		final		eggs/g root		(0-10 scale)	
UTC	9.0±1.3	a	550±86	a	10,410±2,506	a	6.1±0.5	a
Salibro_L	7.4±1.0	a	65±37	b	2,040±856	b	1.6±0.5	bc
Salibro_M	7.0±0.3	a	58±13	b	1,348±283	b	1.2±0.2	bc
Salibro_H	6.2±0.7	a	38±21	b	1,158±434	b	1.0±0.5	c
Nimitz	6.6±0.7	a	98±63	b	1,110±371	b	2.1±0.3	b
P-value	0.11		0.017		*0.0001*		*0.0001*	

Different letters in data columns indicate significant differences at 95% confidence level (LSD-test). Nematode data were log10(x+1)-transformed before statistical analysis; non-transformed data shown.

The average total pepper yield per plant ranged from 0.88 kg/plant in the low-rate of Salibro to 1.14 kg in the Nimitz treatment. However, the yields were not significantly different among the treatments (Table 4, Figure 5). The total number of fruits harvested per plant ranged from 7.3 in the untreated control to 9.2 in the Nimitz treatment, but differences among treatments were not significant (data are not shown).

Table 4. Average (n=5, ±SE) bell pepper variety 'Baron' yield from a 2019 field trial at the UC South Coast Research and Extension Center, Irvine, CA.

	Pepper fruit harvest (average kg per plant)							
Treatment	first		second		third		total	
UTC	0.39±0.06	a	0.45±0.10	a	0.10±0.02	a	0.95±0.07	a
Salibro_L	0.44±0.03	a	0.35±0.07	a	0.10±0.01	a	0.88±0.08	a
Salibro_M	0.38±0.05	a	0.44±0.09	a	0.11±0.02	a	0.92±0.12	a
Salibro_H	0.38±0.02	a	0.47±0.08	a	0.08±0.02	a	0.93±0.08	a
Nimitz	0.45±0.04	a	0.53±0.01	a	0.16±0.09	a	1.14±0.05	a
P-value	0.76		0.44		0.12		0.11	

Conclusion

In spite of large and significant differences among treatments in root infestation levels, root-galling, and root-knot nematode levels in the soil at harvest, there were no significant treatment effects on the yield of bell pepper (kg or number of fruits per plant). It is known that bell pepper, in general, is quite tolerant to root-knot nematodes. Yield loss due to nematodes is not commonly reported when peppers are grown in coastal areas in California. Still, this study clearly demonstrates that Salibro at any of the three rates and Nimitz at 5 pt/acre significantly lower the infestation of the roots with nematodes, the severity of root-galling, and the build-up of nematodes in the soil during the crop season. Although increasing the rate of Salibro appears to also increase the efficacy, this trend was not statistically significant.

Figure 1. 2019 Bell pepper variety ‘Baron’ field trial. Latin square design with five treatments and five replicates.

TRTS (5)		Rate	Application:
1	UTC		
2	SALIBRO_L	30.7 oz per acre	AT-PLANT DRENCH-TILLED
3	SALIBRO_M	46.2 oz per acre	AT-PLANT DRENCH-TILLED
4	SALIBRO_H	61.4 oz per acre	AT-PLANT DRENCH-TILLED
5	NIMITZ	5 pt per acre	PRE-PLANT DRENCH-TILLED

SCREC PEPPER TRIAL

BED 40 INCH C-C

E	3	5	4	2	1	
D	5	4	2	1	3	
C	1	3	5	4	2	
B	4	2	1	3	5	20 FT
						3FT
A	2	1	3	5	4	
	BED1	BED2	BED3	BED4	BED5	

Figure 2. Average (n=5) initial and final soil root-knot nematode levels (second-stage juveniles per 100 g soil) (kg) from a 2019 field trial at the South Coast Research and Extension Center, Irvine, CA. Soil infested with root-knot nematode (*M. incognita*). Different lettering above bars represent significant differences at the 95% confidence level (LSD-test). Nematode numbers were log10(x+1)-transformed before analysis, non-transformed data shown.

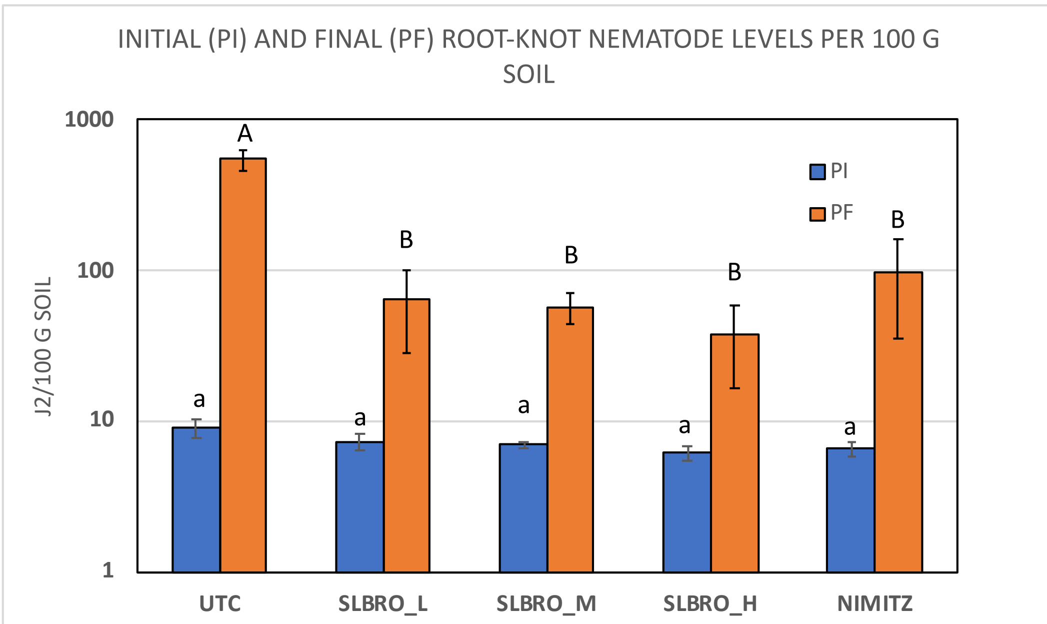


Figure 3. Average (n=5) galling index (scale 0-10; no galling-completely galled) on pepper roots from a 2019 field trial at the South Coast Research and Extension Center, Irvine, CA. Soil infested with root-knot nematode (*M. incognita*). Different lettering represents significant differences between treatments at the 95% confidence level (LSD-test).

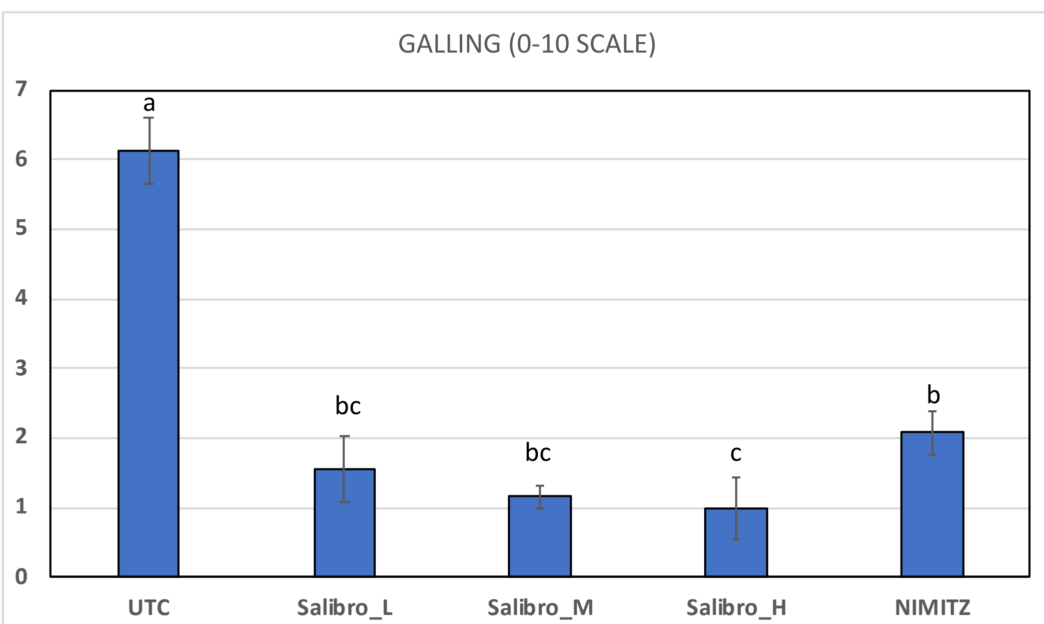


Figure 4. Average (n=5) root-knot nematode infestation (egg/g root) of bell pepper roots from a 2019 field trial at the South Coast Research and Extension Center, Irvine, CA. Soil infested with root-knot nematode (*M. incognita*). Different lettering above bars represent significant differences at the 95% confidence level (LSD-test). Nematode egg numbers were log10(x+1)-transformed before analysis, non-transformed data shown.

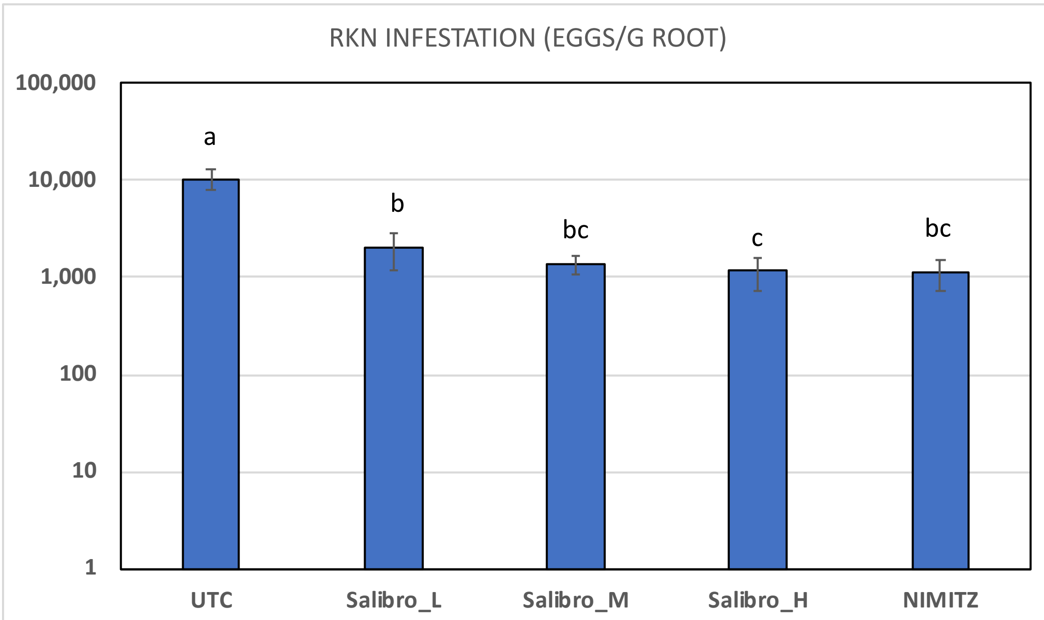


Figure 5. Average (n=5) total bell pepper yield (kg) per plant from a 2019 field trial at the South Coast Research and Extension Center, Irvine, CA. Soil infested with root-knot nematode (*M. incognita*). Differences among treatments are not significant at the 95% confidence level (LSD-test).

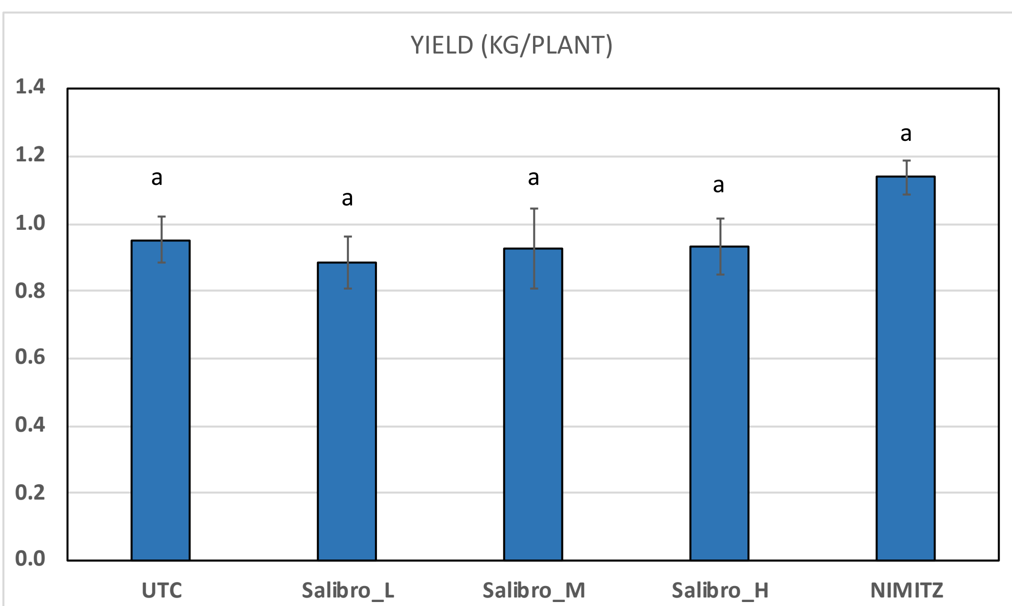


Figure 6. Soil temperatures over the trial period at 5 cm and 15 cm below the soil surface in a bell pepper crop at the UC South Coast Research and Extension Center.