

**CALIFORNIA FRESH CARROT ADVISORY BOARD
RESEARCH REPORT 2016/17**

- Project Title:** Evaluation of Crop Protection Products Against Root-knot Nematodes Suitable for Organic and Conventional California Fresh Carrot Production
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Introduction & Objectives

California carrot production accounts for approximately 80% of the US harvested acres for fresh market (1). About 14% carrots in California are under organic production (2, 3, 4) that represents about 11,500 acres. Root-knot nematodes (rkn, *Meloidogyne* spp.) are the primary cause of plant disease problems in California's carrot production (5, 6). In 1999 production loss was conservatively estimated at 5-8% despite the use of various soil fumigants (7). These nematodes are widespread throughout Central and Southern California and are especially damaging in lighter soil types (8). Root-knot nematodes in carrots primarily lower the quality of the harvested product due to forking and root galling. In addition, galled feeder roots are less able to sustain the demand of the plant for water and nutrients. Consequently, rkn can significantly lower the marketable yield.

Management of plant parasitic nematodes in California has typically relied on pre-planting use of soil fumigants such as Telone® II (1,3-dichloropropene) and metam-sodium or potassium metam; currently no effective post-seeding nematicide is registered. In conventional production systems, substantial portions of fields need be excluded from fumigation because of buffer zone requirements (9) or because of township restrictions to reduce emissions of volatile organic compounds (VOC) associated with fumigant use. Many so-called "soft" nematicides are promoted for organic production but none of those we have evaluated had significant effects on root-knot nematodes or the disease they cause. In fact, the "2015 Guide for Organic Carrot Production" (11), lists several nematicides and biocontrol products for use in organic carrots, but at the same time clearly states that for each of these products their efficacy is "not reviewed or no research is available".

There have been several new products under development for crop protection against plant parasitic nematodes in organic and conventional production systems. The objectives of this project were to determine the efficacy of these novel nematicides and biocontrol products on root-knot nematode population development, crop damage and marketable yield in carrot field trials at Irvine and Shafter.

We have used Emperor 58 for many years as an indicator of rkn disease because of its pronounced galling response. A question was raised by the industry if the old heirloom is still an appropriate model for more modern cultivars. Therefore we ran our 2016 trial at SCREC with two cultivars in parallel. The objective was to compare

susceptibility and treatment response of our standard test cultivar Imperator 58 with a newer cultivar Sequoia.

Materials and Methods

The soil at the UC South Coast Research and Extension Center (UC SCREC), Irvine, CA was a San Emigdio sandy loam with 18% clay, 62% sand and 20% silt, 0.2% OM, pH 7.8 at the trial site. This field site was infested with the Northern root-knot nematode *M. incognita*. For the past several years we have cropped the field during spring to fall with at least one rkn-susceptible host to keep the rkn at a high population level. During the winter months we grew rkn-susceptible wheat (cv. Yecoro Rojo) as a cover crop. The trial was designed as a randomized complete block with 5 replications. Each individual plot was 10 ft long and 2 ft wide. At the beginning of the trial (May 17, 2015) (P_i = initial population) and at harvest (August 25, 2015) (P_f = final population), from each plot six soil cores were taken to a depth of 10-12 inches, pooled, and thoroughly mixed. A 500-cm³ subsample was extracted by sieving and centrifugal flotation. Second-stage juveniles (J2) of rkn were enumerated under 40x magnification. Nimitz[®] (a.i. fluensulfone, Adama; Tab. 1, treatments #6, #7) and the mustard seed meal (*Brassica juncea* cv. Pacific Gold (fine), Farm Fuel Inc.; Tab. 1, treatments # 4 and #5) were applied on May 17, 2016. Nimitz was suspended in 2 gallons water, applied with a sprinkler can in a 1.65 ft band and rototilled into the top 4-5 inches. Additional 2 gallons water was then sprinkled on top of each plot as well as 9 days before seeding. The additional water was recommended by the manufacturer to mitigate potential phytotoxicity problems that have been encountered with certain seeded crops. The Brassica seed meal was uniformly spread onto the plots in a 1.65 ft band and rototilled 4-5 inches into the soil. All other treatments were applied June 6, 2016. Q8u80-137 5GR (a.i. fluazaindolizine) was a granular 5% formulation. MeloCon[®] WG (Certis) contained conidia of the live fungus *Paecilomyces lilacinus* (syn. *Purpureocillium lilacinum*) strain 251. Conidia were formulated and supplied as a freeze-dry powder that we stored frozen at -112°F until use. This powder (treatment #3 and #5) and the Q8u80 granules (treatment #10) were uniformly spread in a 1.65 ft band and immediately incorporated with a rototiller to approximately 4-5 inch depth, followed by 2 gal water via sprinkler can. Majestene[™] (Marrone Bio Innovations, Inc.; Tab. 1, treatment #2), a new biocontrol product based on fermentation broth of *Burkholderia rinojensis*, Q8U80 SC 500 GL (DuPont Crop Protection; Tab. 1, treatment #9), and Velum[®]One (Bayer Crop Science; Tab. 1, treatment #8) were each suspended in 2 gallons of water, applied with a sprinkler can in a 1.65 ft band and rototilled into the top 4-5 inch 2 days before planting. Each plot received additional 2 gallons of water right after the incorporation. On June 8, 2016, non-treated carrot seed cv. Imperator 58 (Lockhart Seeds Inc., Stockton, CA) and cv. Sequoia (Vilmorin North America, Salinas, CA) were seeded each in a separate row (approximately 0.62 g/10 ft), about 5 inches apart. Ridomil Gold[®]SL (a.i. mefenoxam; Syngenta Crop Protection) at 1 pt/acre was sprayed on top of the beds to mitigate potential *Pythium* damping-off and cavity spot incidence. The trial was immediately sprinkler irrigated (about 1/2 inch). The next day, Lorox DF was applied at 1 lb/ac and Prowl H2O was used at 2 pt/ac for weed control. The soil surface was kept moist until emergence. Both Majestene and Velum One treatments were repeated at the same rate 14 days after seeding (June 22, 2016); both were applied with a sprinkler can as a top drench

in 2 gallons of water per plot. The temperature at 6-inch soil depth was 69.1°F on May 17 (Nimitz and mustard applications), 72.5°F on June 6 (application all other products), 73.8°F on June 8 (seeding), and 74.5°F on June 22 (2nd application Majestene and Velum One). The trial was fertilized with 10 lb and 20lb/acre of 15-15-15 on July 13 and July 29, respectively. No other disease or pest control treatments were necessary throughout the season. Plots were monitored for stand counts and crop vigor on July 6 (0-10, worst - best). On July 25, 2016 Normalized Difference Vegetation Index (NDVI) measurements were taken. On August 2, five randomly chosen carrot seedlings per replication were carefully removed and evaluated for rkn disease symptoms (gall rating, scale 0-10). At harvest, August 25, three feet in each plot and in each of the two rows were harvested. The cultivars were separately collected in plastic bags for transport to the UCR Nematology facilities. The soil was rinsed off and the roots were rated for disease symptoms (gall rating 0-10, best-worst, Fig. 1). The total number of roots and per rating class was determined for each rep and cultivar. Total and marketable weights were noted. We considered carrots within the disease rating 0-3 as marketable. Plant vigor and disease ratings were arcsine-transformed and nematode population data were $\log_{10}(x+1)$ -transformed to normalize variances before statistical analysis. If significant, mean separation was used with Fisher's Protected LSD ($P = 0.05$) (SuperANOVA, Abacus, Berkeley, CA). The treatment list (Tab. 1) and all back-transformed data (Tab.2) are listed.

At Shafter, two trials were conducted in a root-knot nematode-infested field (*M. incognita*). In addition to some of the previously mentioned conventional products (Tab. 2), the Biological Nematicide trial (Tab. 3) contained the products Monterey Nematode Control (a.i. 8.8% saponins of *Quillaja saponaria* (Chilean soap bark tree), Monterey Lawn and Gardens, Fresno, CA), Emune (a.i. fermentation extract, Advanced Crop Nutrition, Sioux Center, IA) and Oxva (a.i. natural plant extract with a co-formulant, OMEX Agriculture Ltd, Lincoln, UK).

Tab. 1 Treatment list for both cultivars

Trt #	treatment	per acre
1	Nt control	
2	Majestene	2 gal/acre (twice)
3	MeloCon	6 lbs/acre
4	Seed meal	2 tons/acre
5	Seed meal + MelCon	#4 + #3
6	Nimitz 480 EC 5 pt	5 pt/acre
7	Nimitz 480 EC 7 pt	7 pt/acre
8	Velum One 400 SC	6.5 oz/ac (twice)
9	Q8U80 SC 500 GL	61.4 oz/ac
10	Q8U80-137 5Gr	40 lbs/ac

Results and Discussion

The general conditions for the SCREC trial were excellent. The rkn population in the trial area was fairly uniform distributed at a level at 26 J2 per 100-cm³ soil (Tab. 2). In Southern California the damage threshold for rkn in carrots is about one J2/100 cm³; at our trial level we expected an estimated yield loss of at least 20% (11).

Average stand counts for both cultivars were almost identical. The low rate of Nimitz had with both cultivars the by-way highest stand counts.

Vigor ratings one month after seeding indicated that the treatments with the biological control products MeloCon and Majestene were not different from the non-treated control. All other treatments looked much improved, with again Nimitz @ 5 pt/acre leading the field, followed by the Q8u80 treatments. This was further supported by determining the Normalized Difference Vegetation Index (NDVI). This is an indicator for "plant health"; simplified, the higher the number, the healthier the crop (Tab. 2).

About 7 weeks after seeding, we carefully excavated 5 randomly selected seedlings from each plot and both cultivars. Back at our Nematology facilities, we rinsed the roots and rated the extent of root galling. With both cultivars, MeloCon and Majestene appeared non-effective as galling were not different from the untreated control. Pre-season application of mustard meal reduced the gall ratings by 1 to 2 scoring classes but the combination with MeloCon (treatment #5) showed no additional benefit in either cultivars. The low rate and especially the 7-pt/acre rate of Nimitz reduced galling considerably. This was only topped by both formulations of Q8u8 that resulted in average gall ratings below 1 in both cultivars. On the other hand, Velum One reduced galling primarily in the top 3-4 inches of the root system, which corresponded approximately with the depth of our rototiller incorporation. We observed this in previous carrot trials. Apparently the nematicide was sufficiently distributed by mechanical incorporation into the top soil layer but did not move any further with the irrigation water.

Treatment effects on the number of harvested carrots fell into two groups; those that have little or no apparent effect and those that increase the average number of harvested carrots substantially over the non-treated control. The later group includes Nimitz and Q8U80 treatments. In contrast to the earlier gall ratings that relied on the score of only 5 root systems per replication, at the end of the season we rated all harvested carrots, which turned out to be on the average 31 for Imperator 58 and about 35 root scores for Sequoia. It should be noted that the average gall rating in the non-treated control was almost one rating class lower with Sequoia. However, overall the results with both cultivars were similar to each other and to the mid-season ratings. But we had more clearly defined result separation with Imperator 58. To better visualize these results, we combined the mid-season and harvest disease ratings in the Fig. 2a,b. The two biological products provided no obvious benefit, while the mustard seed meal significantly reduced the disease symptoms. As already seen at mid-season, the combination mustard seed meal plus MeloCon did not improve the efficacy of the seed meal alone. Velum One had similar efficacy as the mustard seed meal. With Nimitz both rates provided good reduction in disease symptoms but there was no significant benefit of the higher rate. Both Q8U80 formulations looked excellent for season-long protection against rkn (Tab. 2).

The marketable yield of both cultivars reflected the earlier described disease severity. For example, both Q8U80 formulations mitigated galling to such a degree that more than 90% of the harvested carrots were marketable compared to only 10-20% of the non-treated control (Fig. 3a,b). The high efficacy of the Q8U80 formulations was also reflected in the marketable carrot weight (Fig. 4a,b). Finally, at harvest the rkn soil populations among the treatments were highly variable. Only the Q8U80 treatments were lower than the non-treated control. Similarly, their reproduction factors (P_f/P_i) were significantly reduced compared to the non-treated control (Tab. 2).

The comparison of the two cultivars showed little difference in terms of treatment responses. Perhaps more important, it is our impression that disease scoring into rating classes was easier with Emperor 58 and consequently faster than with Sequoia. As we scored more than 1,500 carrots per cultivar, ease of quality evaluation becomes an important time factor.

The Shafter trial with the conventional nematicide confirmed the efficacy of Nimitz and Q8U80 (Tab. 3). In the second trial, none of biological products were effective in mitigating root galling by *M. incognita* (Tab. 4).

In summary, neither Majestene, MeloCon or the products applied in the Shafter biocontrol trial appeared to have any effect on the activity of the rkn. In contrast, the mustard seed meal significantly reduces rkn disease symptoms and is to date our best treatment for organic production. We hypothesize that changing the application to higher mustard seed meal rates or amendments with other additives might improve the efficacy but the higher cost might prove to be prohibitive. The same might be true for tarping the beds after application to trap biocidal gases and extend their retention time in the root zone. It should be pointed out that for both conventional and organic production pre-season cropping with resistant crops would reduce the initial population density and consequently the initial disease pressure. Among the conventional products, Nimitz and especially Q8U80 performed well compared to previous trials with non-fumigant organophosphate and carbamate nematicides that never received California registration in fresh carrot production. Q8U80 appears to be the best product under our testing conditions and is possibly even a potential challenger for Telone II. Velum One is no doubt an effective nematicide but due to its immobility in soil, it needs further research to improve its application.

There was no obvious advantage in using a more recent cultivar for efficacy testing. As an evaluation model Emperor 58 remains our cultivar of choice.

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Figures and Tables

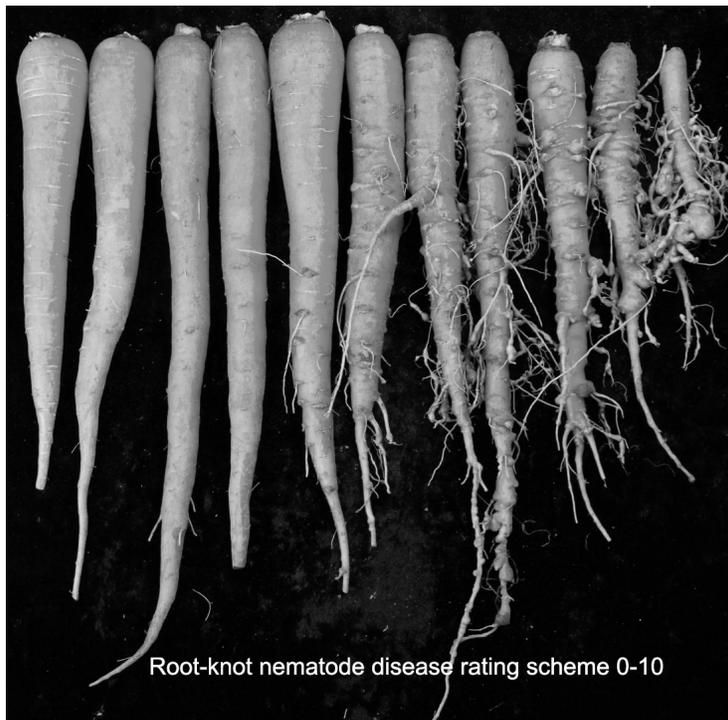


Fig. 1 Root-knot nematode (*M. incognita*) disease rating scheme (cv. Emperor 58).

Tab. 2 Summary of data collected during 2016 carrot trial at the South Coast R&E Center, Tustin, CA.

Treatments	Pre-season (P) 12/100 cm ³	cv. Imperator 58 stand/10 ft	cv. Sequoia stand/10 ft	seedling vigor @ 4 weeks	NDVI 0-1, 1 best	cv. Imperator 58 galling @ 7 wks	cv. Sequoia galling @ 7 wks
Nt control	13.8 ± 5.5	70.4 ± 7.0 abc	78.6 ± 11.8	3.0 ± 0.3 a	0.26 ± 0.02 a	5.6 ± 0.3 cde	6.2 ± 0.5 fgh
Majestene	21.8 ± 9.5	77.2 ± 12.9 abcde	82.4 ± 10.7	2.8 ± 0.4 a	0.29 ± 0.03 ab	6.6 ± 0.6 e	4.6 ± 0.7 cdef
MeloCon	17.9 ± 12.7	59.2 ± 8.1 a	66.2 ± 7.5	2.8 ± 2.8 a	0.25 ± 0.04 a	6.5 ± 0.6 de	4.8 ± 0.5 defg
Seed meal	45.3 ± 10.0	74.4 ± 7.3 abcde	92.4 ± 7.9	6.4 ± 0.2 bc	0.46 ± 0.02 c	4.6 ± 0.7 bcd	4.2 ± 0.9 cde
Seed meal+MeloCon	7.3 ± 2.8	67.2 ± 8.9 ab	76.2 ± 11.6	5.2 ± 0.4 b	0.42 ± 0.04 c	5.3 ± 0.5 cde	4.4 ± 0.5 cde
Nimitz 5 pt	24.6 ± 8.2	112.4 ± 16.9 f	105.4 ± 10.7	8.0 ± 0.9 d	0.51 ± 0.07 c	3.9 ± 1.0 bc	3.2 ± 0.8 bcd
Nimitz 7 pt	42.7 ± 18.3	92.0 ± 16.0 bcdef	92.2 ± 8.2	6.4 ± 0.4 bc	0.47 ± 0.06 c	2.9 ± 0.8 b	1.8 ± 0.4 ab
Velum One	34.6 ± 18.7	105.2 ± 16.3 ef	90.8 ± 13.4	6.6 ± 0.2 bcd	0.41 ± 0.02 bc	4.5 ± 0.8 bc	4.2 ± 0.8 cde
Q8U80 SC	21.9 ± 10.8	97.4 ± 16.1 cdef	88.6 ± 10.1	6.8 ± 1.0 cd	0.44 ± 0.07 c	0.4 ± 0.1 a	0.6 ± 0.2 a
Q8U80-5Gr	34.1 ± 14.3	102.6 ± 17.2 def	98.0 ± 7.8	7.8 ± 0.6 cd	0.50 ± 0.07 c	0.6 ± 0.2 a	0.8 ± 0.2 a

Treatments	cv. Imperator 58 total # carrots/3 ft	cv. Sequoia total # carrots/3 ft	cv. Imperator 58 carrot weight (kg)/3 ft	cv. Sequoia carrot weight (kg)/3 ft	cv. Imperator 58 galling @ harvest	cv. Sequoia galling @ harvest
Nt control	26.4 ± 3.1 a	28.4 ± 4.0	0.747 ± 0.129 bc	0.793 ± 0.113	5.4 ± 0.4 d	4.5 ± 0.3 def
Majestene	25.6 ± 1.6 a	35.2 ± 4.3	0.682 ± 0.067 abc	1.047 ± 0.118	5.8 ± 0.3 d	5.1 ± 0.2 f
MeloCon	24.4 ± 2.2 a	24.6 ± 3.9	0.655 ± 0.110 ab	0.887 ± 0.123	6.1 ± 0.5 d	5.0 ± 0.3 ef
Seed meal	27.0 ± 2.2 a	36.8 ± 5.9	0.902 ± 0.144 bc	0.970 ± 0.155	4.2 ± 0.4 c	4.2 ± 0.3 de
Seed meal+MeloCon	23.8 ± 3.8 a	28.0 ± 4.4	0.951 ± 0.077 bc	1.184 ± 147	4.3 ± 0.5 c	4.2 ± 0.3 de
Nimitz 5 pt	41.6 ± 8.5 b	39.6 ± 4.9	0.427 ± 0.164 a	1.031 ± 0.064	3.1 ± 0.5 b	3.6 ± 0.3 cd
Nimitz 7 pt	31.8 ± 6.3 ab	42.4 ± 5.6	0.880 ± 0.129 bc	1.225 ± 0.162	2.9 ± 0.2 b	2.7 ± 0.4 bc
Velum One	37.0 ± 4.1 ab	36.6 ± 5.7	0.841 ± 0.120 bc	0.846 ± 0.101	4.2 ± 0.5 c	4.3 ± 0.4 def
Q8U80 SC	40.4 ± 4.8 b	37.2 ± 4.5	0.967 ± 0.129 c	0.892 ± 0.210	1.0 ± 0.2 a	1.3 ± 0.3 a
Q8U80-5Gr	36.4 ± 6.2 ab	36.6 ± 1.3	0.900 ± 0.100 bc	1.263 ± 0.116	1.1 ± 0.4 a	1.8 ± 0.3 ab

Treatments	cv. Imperator 58 #market.carrots/3 ft	cv. Sequoia #market.carrots/3 ft	cv. Imperator 58 market.weight (kg)/3 ft	cv. Sequoia market.weight (kg)/3 ft	M. incognita @ harvest (Pf)	M. incognita Pf/Pi
Nt control	3.2 ± 1.5 a	5.8 ± 3.2 a	0.070 ± 0.031 ab	0.106 ± 0.048 a	573.8 ± 97.0 c	120.1 ± 52.9 c
Majestene	0.6 ± 0.4 a	2.8 ± 0.9 a	0.012 ± 0.008 a	0.093 ± 0.036 a	417.4 ± 42.7 c	49.4 ± 20.7 bc
MeloCon	2.6 ± 2.4 a	2.8 ± 1.1 a	0.061 ± 0.059 ab	0.074 ± 0.038 a	281.0 ± 83.7 bc	321.4 ± 193.6 c
Seed meal	8.8 ± 2.9 a	11.2 ± 5.0 ab	0.263 ± 0.087 abcd	0.203 ± 0.090 ab	729.0 ± 192.4 c	18.0 ± 3.7 abc
Seed meal+MeloCon	8.6 ± 4.3 a	8.6 ± 3.8 ab	0.321 ± 0.182 bcd	0.303 ± 0.174 ab	568.6 ± 171.8 c	351.6 ± 288.4 c
Nimitz 5 pt	22.4 ± 7.6 bc	19.4 ± 4.6 bc	0.414 ± 0.072 cd	0.482 ± 0.079 bc	506.2 ± 271.4 bc	72.8 ± 59.0 abc
Nimitz 7 pt	20.6 ± 4.7 b	28.8 ± 6.0 cd	0.503 ± 0.066 d	0.805 ± 0.168 cd	422.8 ± 105.4 c	146.9 ± 122.2 abc
Velum One	8.8 ± 3.7 a	11.0 ± 4.1 ab	0.201 ± 0.115 abc	0.243 ± 0.075 ab	695.2 ± 214.2 c	31.7 ± 7.7 abc
Q8U80 SC	38.4 ± 4.3 d	35.2 ± 4.8 d	0.915 ± 0.119 e	0.796 ± 0.193 cd	141.2 ± 75.1 ab	13.2 ± 5.3 ab
Q8U80-5Gr	33.0 ± 3.7 cd	31.2 ± 3.3 d	0.805 ± 0.070 e	1.004 ± 0.080 d	143.6 ± 65.8 a	5.2 ± 1.9 a

Pi initial population density
Pf final population density
Pf/Pi reproduction factor
stand mean number of seedlings per plot (10 ft), 5 reps
vigor mean health rating of 5 reps on scale 0-10 (10 best)
NDVI Normalized Difference Vegetation Index (scale 0-1), mean of 5 plots
galling mean score of carrots from 3 ft row (5 reps), rating scale 0-10 (0 best, 10 worst)
marketable carrots scored 0 to 3
non-marketable carrots scored 4-10
 nematode data were log10 + 1 transformed and ANOVA analyzed, different letters in columns (original data) indicate significant effects (P=0.05)

Tab. 3 Shafter Carrot Conventional Nematicide Trial 2016

Treatment	Average Root Gall Rating*
1. Control	3.2
2. Velum One 6.5 fl oz /A pre and 21 DAP post	3.8
3. Nimitz 3 1/2 pints/A	2.7
4. Nimitz 5 pts/A	3.9
5. Q8U80 at 30.7 fl oz /A & 2 post at 15.4 fl oz/ A 2.2	
Probability=	0.2849
%CV=	43.35
LSD P=0.05	Not Significant

1st application for all treatments on 4/21/16

2nd application of Velum One and Q8U80 on 5/30/16

Variety: Sequoia

*Nematode rating scale: 1=no nematode galling, 10=100% of roots galled.

Tab. 4 Shafter Carrot Biological Nematicide Trial 2016

Treatment	Average Root Gall Rating*
1. Control	3.5
2. Nematode Control 1 gal/A	4.9
3. Majestene @ 1.5 gal/A	4.8
4. EMUNE @ 2 gal/A	3.7
5. EMUNE Plus @ 2 gal/A	3.8
6. OXVA @ 0.5 gal/A	4.3
7. OXVA @ 1 gal/A	4.7
Probability=	0.6959
%CV=	34.98
LSD P=0.05	Not Significant

Planted on 4/28/16

1st application on 4/21/16, 2nd on 5/19/16 and 3rd on 6/15/16

Variety: Sequoia

*Nematode rating scale: 1=no nematode galling, 10=100% of roots galled.

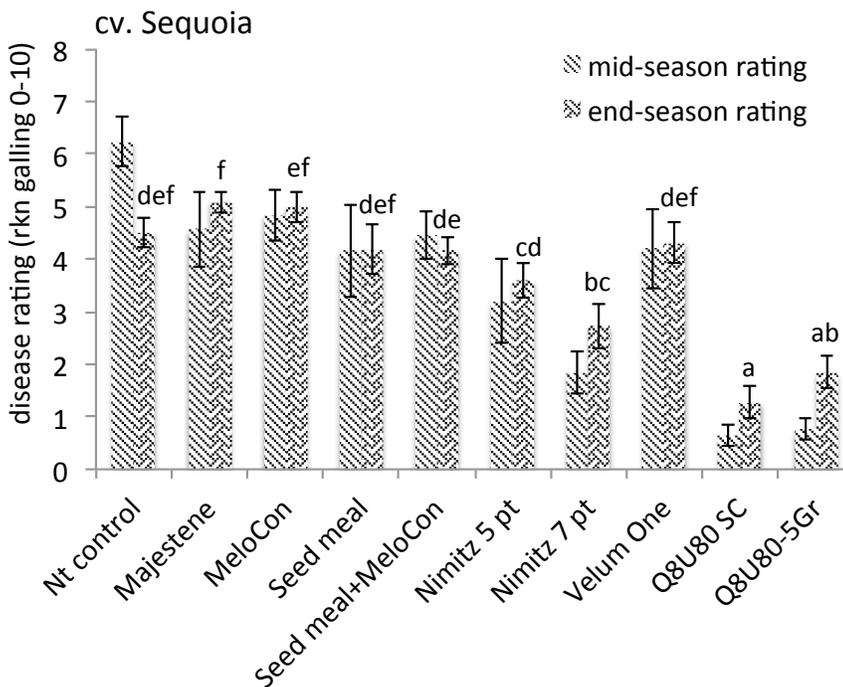
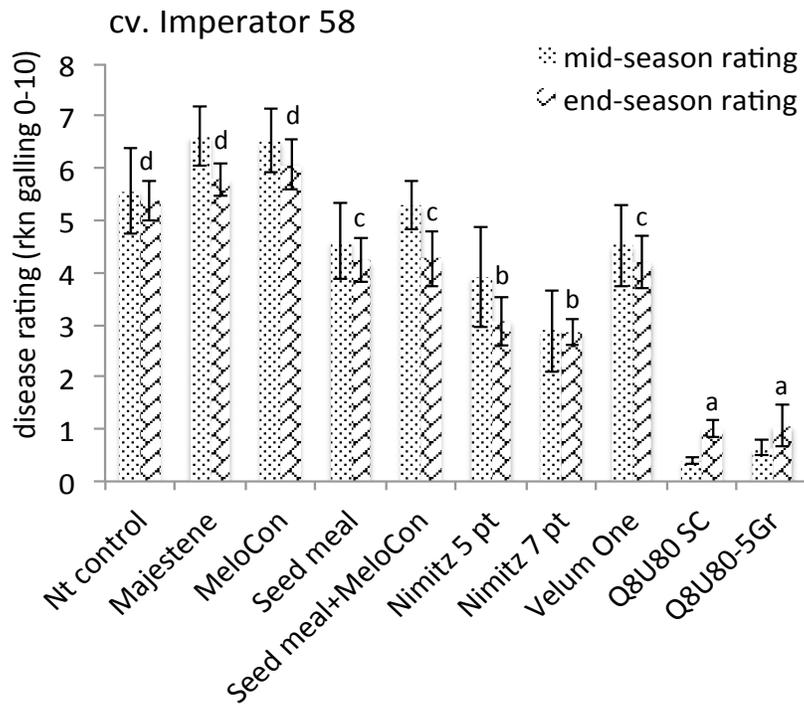


Fig. 2a,b Effect of soil treatments on carrot root galling. Root-knot nematode disease ratings 7 weeks after seeding and at harvest for cv Emperor 58 and Sequoia (SCREC 2016). Data represent the mean of 5 replicates, bars indicate standard error. Means with the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$).

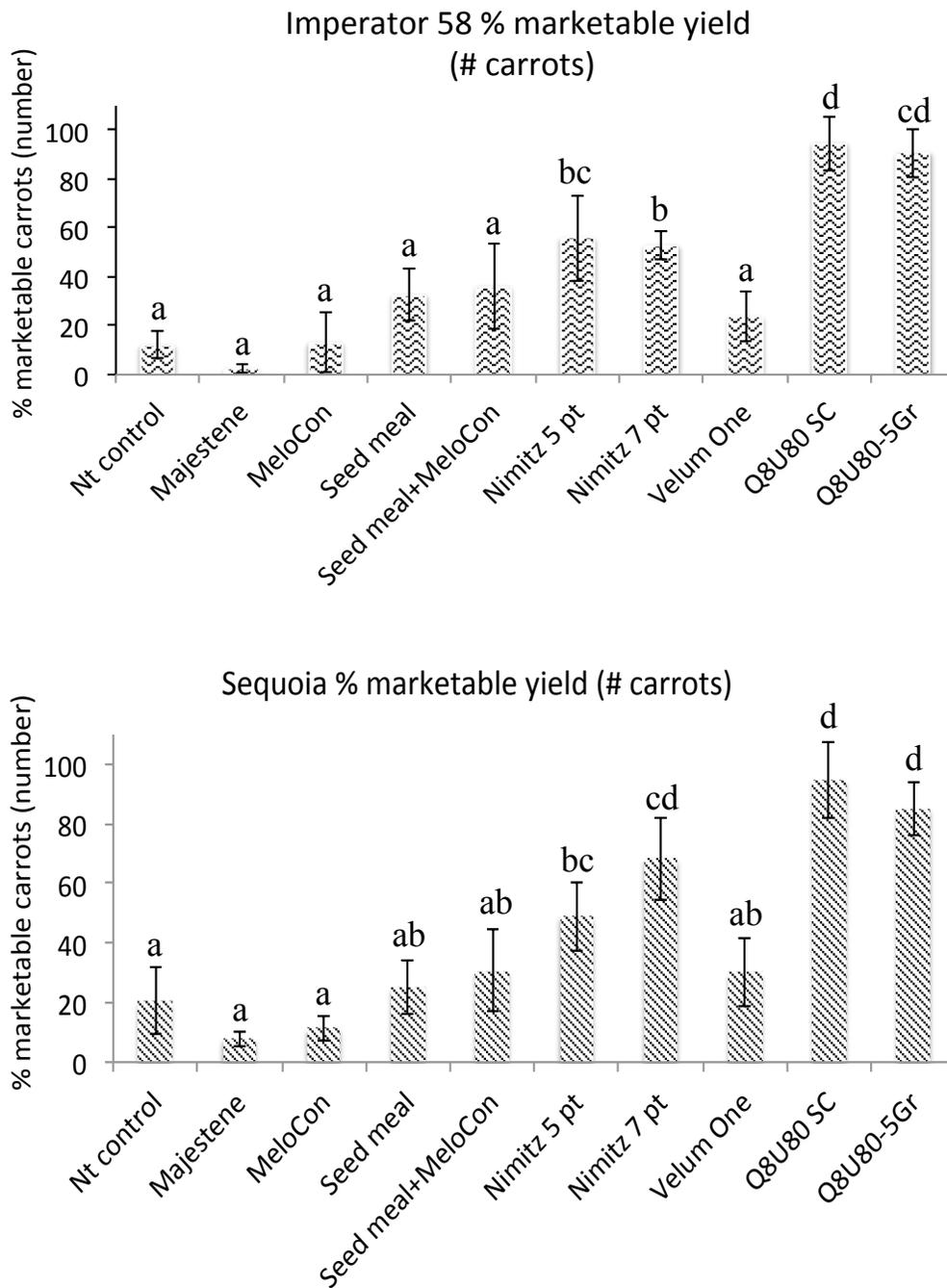


Fig. 3a,b Effect of soil treatments on percent marketable yield (number of carrots). Data represent the mean of 5 replicates, bars indicate standard error. Means with the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$).

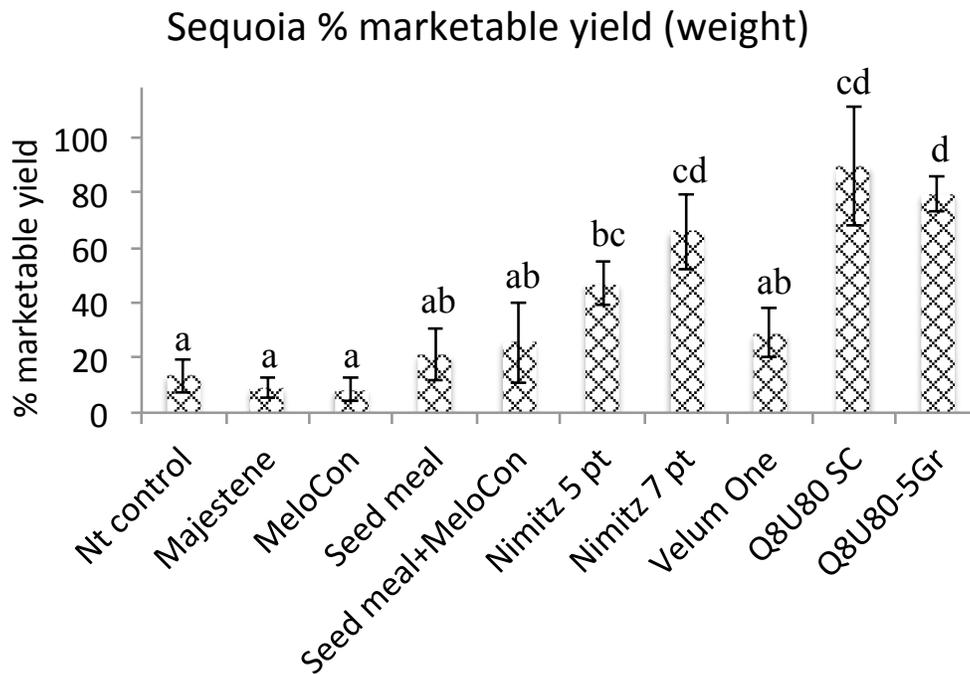
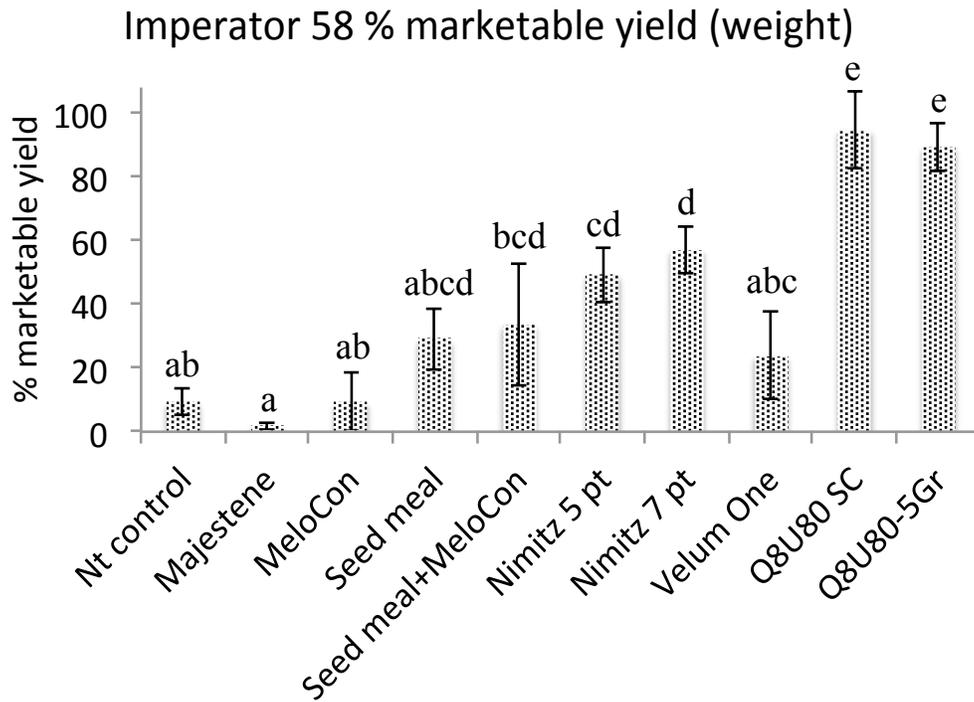


Fig. 4a,b Effect of soil treatments on percent marketable yield (number of carrots). Data represent the mean of 5 replicates, bars indicate standard error. Means with the same letter are not significantly different (Fisher's protected LSD, $P \leq 0.05$).