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BACTERIOPHAGES FOR BACTERIAL BLIGHT MANAGEMENT: PROSPECTS AND CHALLENGES

Annual Carrot Research Symposium, March 15, 2021

Oregon State University

Bacterial Blight in Carrot Production

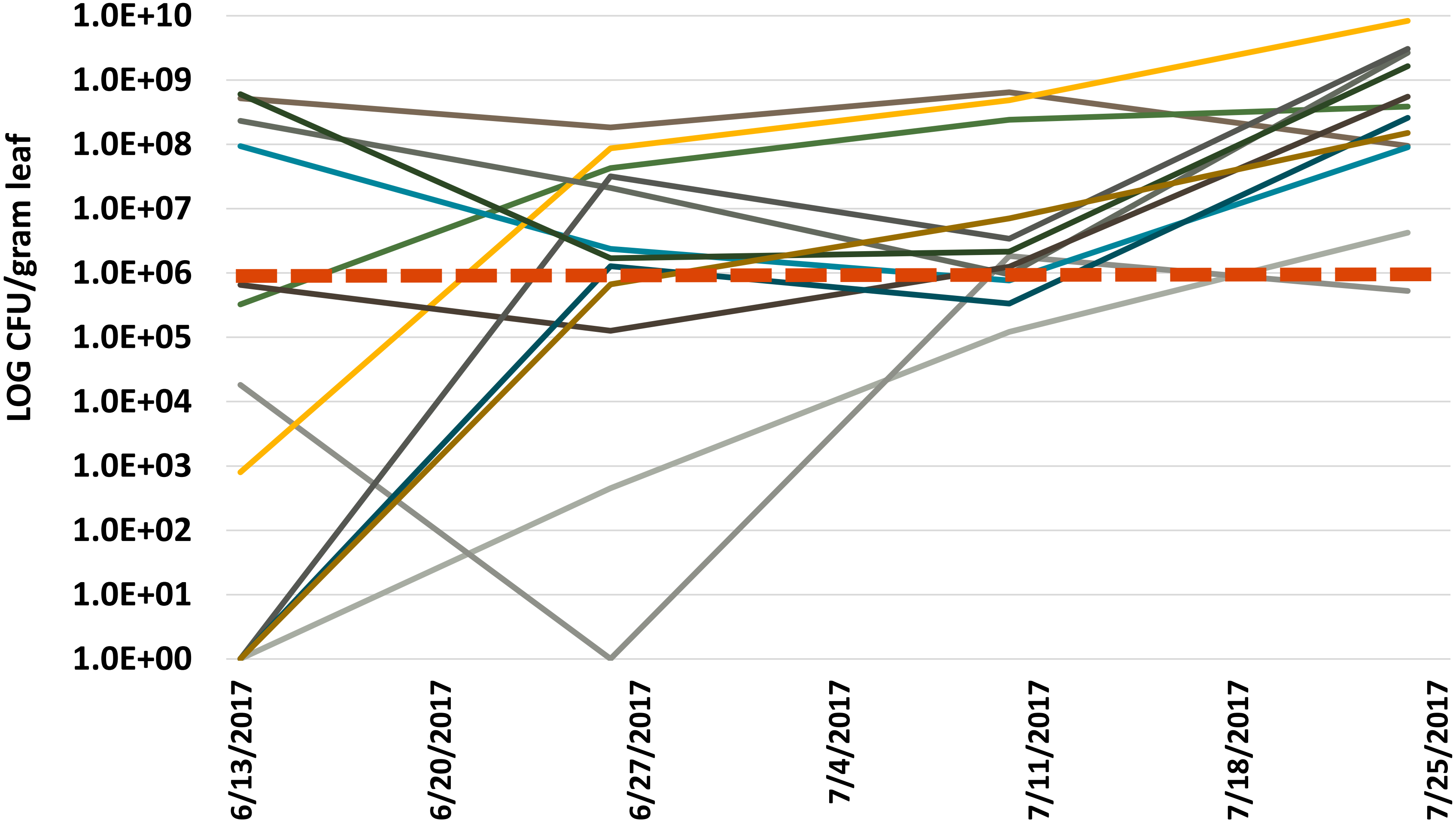
- Caused by the *Xanthomonas hortorum* pv. *carotae*
- Particularly important in carrot-producing regions with high rainfall/overhead irrigation
- For root crops, bacterial blight can:
  - Reduce yield
  - Hinder harvest
  - Affect quality
- Can survive ~1 year in field debris
- Seedborne

Impacts of *Xanthomonas* on Carrot Seed Production

- For seed companies:
  - Healthy, disease-free seed is a goal
  - Expensive and difficult hot-water treatments
  - Rejection of seed in export markets

- For seed growers:
  - Costs associated with control
  - Reduced seed yield due to blighted umbels
  - Reduced seed germination
  - Sustainability of production

Epiphytic Populations of *Xanthomonas* on Carrots



Integrated Management of Bacterial Blight in Carrot Seed Crops

<u>Cultural practices</u>

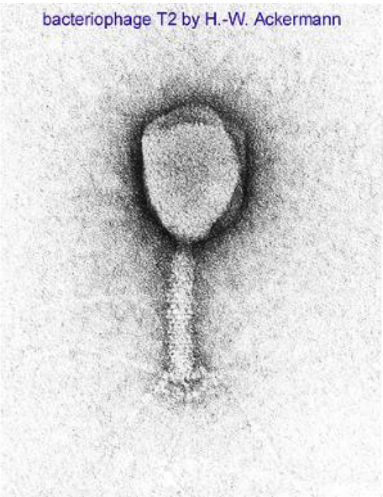
- Pathogen-free seed
- Limit overhead irrigation
  - Drip irrigation
- Bury/remove/destroy crop residue
- Crop rotation
  - 2-3 years

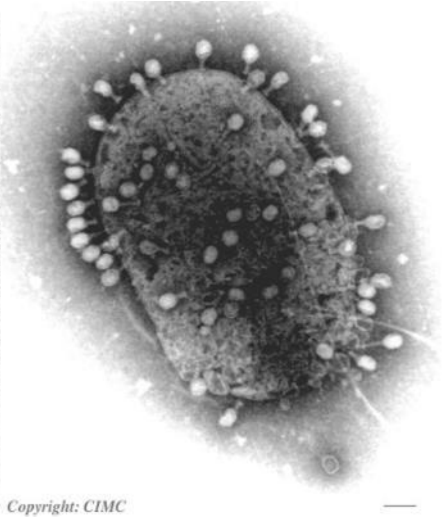
<u>Chemical</u>

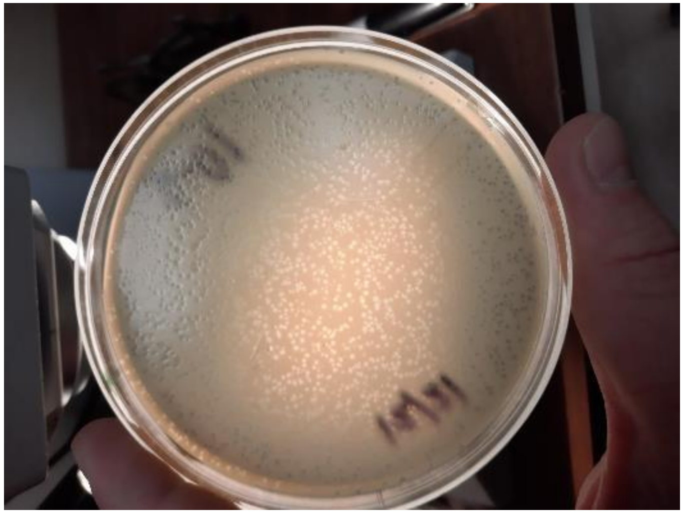
- Copper-based bactericides

Bacteriophage for *Xanthomonas* Management in Carrot and Carrot Seed Crops

- Bacteriophage (AKA phage) are viruses that infect and replicate in bacteria
  - Lytic phage lyse and kill host cells to release progeny
- The host range of most phages is relatively narrow
  - Typically limited to only a single bacterial genus, species or even strains within a species







Bacteriophages for *Xanthomonas* Management in Carrot and Carrot Seed Crops

- AgriPhage™ (OmniLytics and Certis) is a phage-based biological control product
  - Bacterial speck (*Pseudomonas syringae* pv. *tomato*)
  - Bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*)
- Labeled for organic production
  - Residue exempt
  - 4 hr. REI
- **The objective of this project was to evaluate AgriPhage for reducing epiphytic *Xhc* in carrot seedlings**

AgriPhage™

BACTERICIDE

Bactericide for use on tomatoes and peppers

Active Ingredients:

Bacteriophage active against *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato**	0.000046%
Other Ingredients:	99.999954%
Total:	100.000000%

*Contains at least 3.9 x 10^{12} plaque-forming units (PFU) per quart of product
*Contains at least 1.55 X 10^{13} plaque-forming units (PFU) per gallon of product

KEEP OUT OF REACH OF CHILDREN
CAUTION

See attached booklet for First Aid and additional Precautionary Statements.

FIRST AID
If in eyes: Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control center or doctor for treatment advice.
Hotline Number
Have the product container or label with you when calling a poison control center or doctor, or going for treatment. For non-emergency information concerning this product, call the National Pesticide Information Center (NPIC) at 1-800-858-7378, Monday through Friday, 8:00 a.m. to 12:00 p.m. Pacific Time (NPIC Web site: www.npic.orst.edu). For emergencies, call your poison control center at 1-800-222-1222.

PRECAUTIONARY STATEMENTS
Hazards to Humans and Domestic Animals
CAUTION: Causes moderate eye irritation. Avoid contact with eyes or clothing. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using the toilet.

Net Contents: 1 qt, 2.5 gal.
EPA Reg. No. 67986-1-70051
EPA Est. No. 67986-UT-001
Manufactured by:
OmniLytics™ THE PHAGE COMPANY
9075 South Sandy Parkway
Sandy, UT 84070
801-746-3600
www.omnilytics.com

Batch Code:

FOR ORGANIC PRODUCTION
Distributed by:
Certis USA LLC
9145 Guilford Road, Suite 175
Columbia, MD 21046
CERTIS

ESL 20181018
rev20190305

Personal Protective Equipment (PPE)
Applicators and other handlers must wear:
- Long-sleeved shirt
- Long pants
- Waterproof gloves
- Shoes plus socks
- Protective eyewear

Mixers/loaders and applicators must wear a minimum of a NIOSH-approved particulate filtering facepiece respirator with any N, R, or P filter; OR a NIOSH-approved elastomeric particulate respirator with any N, R, or P filter; OR a NIOSH-approved powered air-purifying respirator with an HE filter. Repeated exposures to high concentrations of microbial proteins can cause allergic sensitization.

Follow the manufacturer's instructions for cleaning/maintaining PPE. If no such instructions for washables, use detergent and hot water. Keep and wash PPE separately from other laundry.

User Safety Recommendations
User should:
Remove clothing/PPE immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

Environmental Hazards
For Terrestrial Uses: Do not apply directly to water, to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of equipment washwater or rinsate.

CHEMIGATION: Do not apply this product through any irrigation system.

Agricultural Use Requirements
Use this product only in accordance with its labeling and with the Worker Protection Standard, 40 CFR part 170. This standard contains requirements for the protection of agricultural workers on farms, forests, nurseries, and greenhouses, and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this label about personal protective equipment (PPE) and restricted-entry interval. The requirements in this box apply to uses of this product that are covered by the Worker Protection Standard.

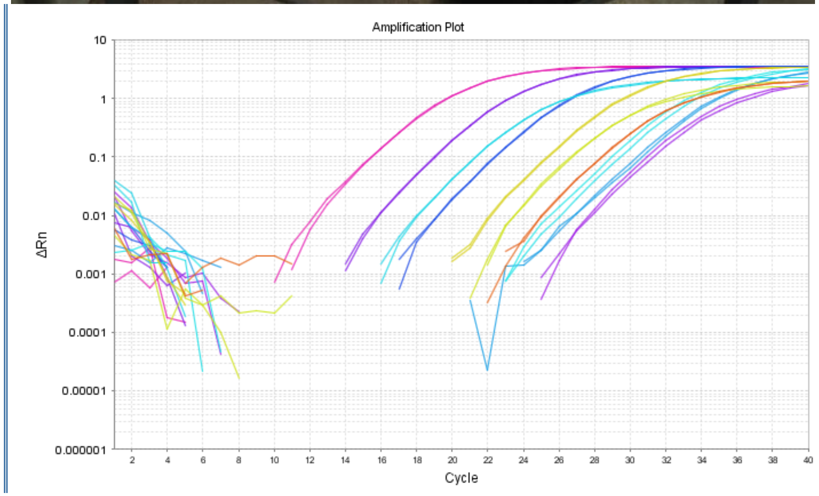
Do not enter or allow worker entry into treated areas during the restricted-entry interval (REI) of 4 hours.

Do not enter or allow workers to enter the treated greenhouse or enclosed space until the ventilation requirements in 40 CFR 170.405(b)(3) have been met and the REI of 4 hours has expired. Until then, only handlers wearing the appropriate personal protective equipment can enter the greenhouse or enclosed space.

PPE required for early entry to treated areas (that is permitted under the Worker Protection Standard and that involves contact with anything that has been treated, such as plants, soil or water) is:
- Coveralls
- Waterproof gloves
- Shoes plus socks
- Protective eyewear

Greenhouse Rate Trial

- Greenhouse flats sown to 'Napoli'
- Flats inoculated three times
    - 2 x 10^6 CFU/ml
- Treated 5 days later
    - AgriPhage 16 oz/A or 32 oz/A
    - ManKocide (2.5 lb/A)
    - Non-inoculated/non-treated (negative control)
    - Inoculated/non-treated (positive control)
- Foliage harvested after 7 d and assessed for *Xhc* populations using viability qPCR

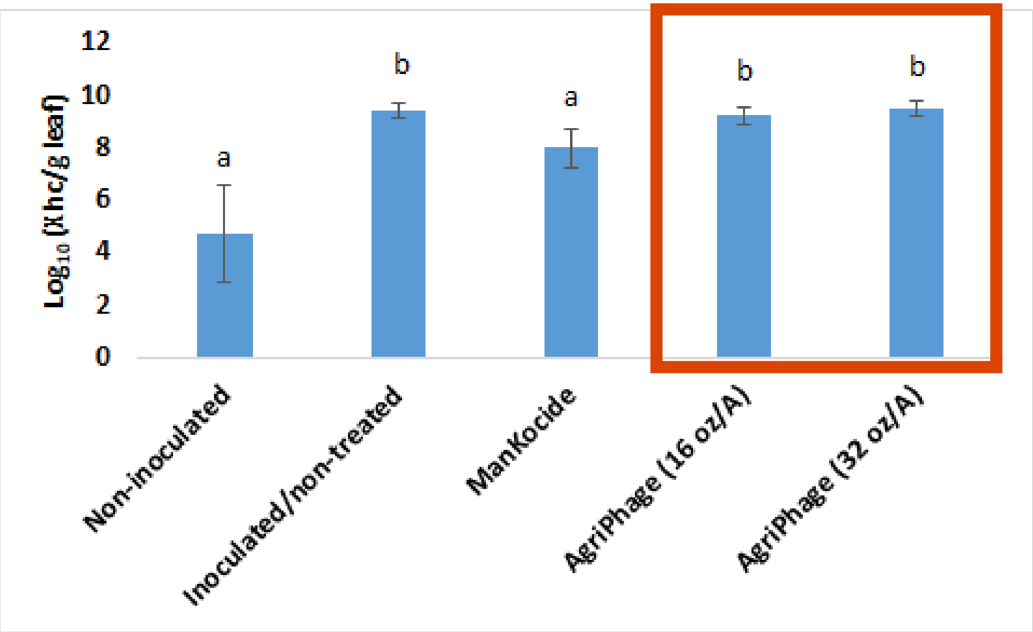


Growth Chamber Timing Trial

- 4” pots sown to ‘Napoli’
  - Polypropylene sleeves
- Flats inoculated once
  - 1 x 10^8 CFU/ml
- 6 treatments:
  - AgriPhage (16 oz/A)
    - 2 hr pre-inoculation
    - 3 d post-inoculation
    - Both timings
  - ManKocide 3 d post-inoculation
  - Negative and positive controls
- Foliage harvested after 7 d and assessed for *Xhc* populations using viability qPCR

Results

- **Greenhouse Trial:** AgriPhage at either rate was not significantly different than the inoculated/non-treated positive control



- **Growth Chamber Trials:** AgriPhage did not significantly decrease *Xhc* populations relative to the positive control at any of the three application timings

	Log_{10} (Xhc/g leaf)			
Treatment	Trial 1			Trial 2
Non-inoculated	3.26	c		3.50
Inoculated/non-treated	8.21	b		7.89
AgriPhage (pre-inoculation)	8.48	ab		7.91
AgriPhage (post-inoculation)	8.64	a		7.75
AgriPhage (pre- and post-inoculation)	8.28	ab		7.70
ManKocide	7.74	bc		7.48
P-value	0.001			0.192

Conclusions, Prospects, and Challenges

- AgriPhage did not decrease *Xhc* populations relative to the non-treated control in any of the trials regardless of rate or timing of application
  - Multiple *in vitro* trials demonstrated infectivity of *Xhc* by AgriPhage
- The phyllosphere presents a relatively inhospitable environment
  - Can adjuvants be used to improve phage dispersal, adhesion, survival and/or adsorption to bacterial hosts in crop canopies?
  - Non-target effects on other microbial epiphytes?
- Phage-based seed treatments?

Acknowledgements

- Shaelynn Downing (Undergraduate Technician)
- Scott Ockey (Certis)
- Ryan Benson and Tyler Homer (OmniLytics)

Funding and In-Kind Support:

- California Fresh Carrot Advisory Board
- United States Department of Agriculture Specialty Crops Research Initiative (grant no. 2020-51181-32154)
- Certis
- OmniLytics

Thank you

- Question 1: Bacteriophages are ______ that infect bacteria.
  - Fungi
  - Bacteria
  - Nematodes
  - **Viruses**
- Question 2: A crop rotation of _______ is recommended to reduce bacterial blight.
  - 6 months
  - 1 year
  - **2-3 years**
  - 4 or more years

Carrot cavity spot diagnostics serving CA carrot growers

Isolation and characterization of carrot cavity spot pathogens at CSU Bakersfield

Isolde Francis
March 2021

CALIFORNIA STATE UNIVERSITY BAKERSFIELD 1965 EXCELLENCE PARTNERSHIPS COMMUNITY

Carrot Cavity Spot

important disease of carrots worldwide

small brown sunken circular or elliptical lesions on the tubers
(cellulolytic activity leading to necrosis)

several *Pythium* species can cause this disease
- *P. violae*
- *P. sulcatum*
- *P. ultimum*

belonging to the oomycetes or water molds
- fungal-like organisms
- produce spores that can swim towards their host

affected tubers are rejected for the fresh as well as processing market

often overlooked/unnoticed

managed through the use of metalaxyl/mefenoxam
- resistance becomes a problem
- increased degradation in the soil

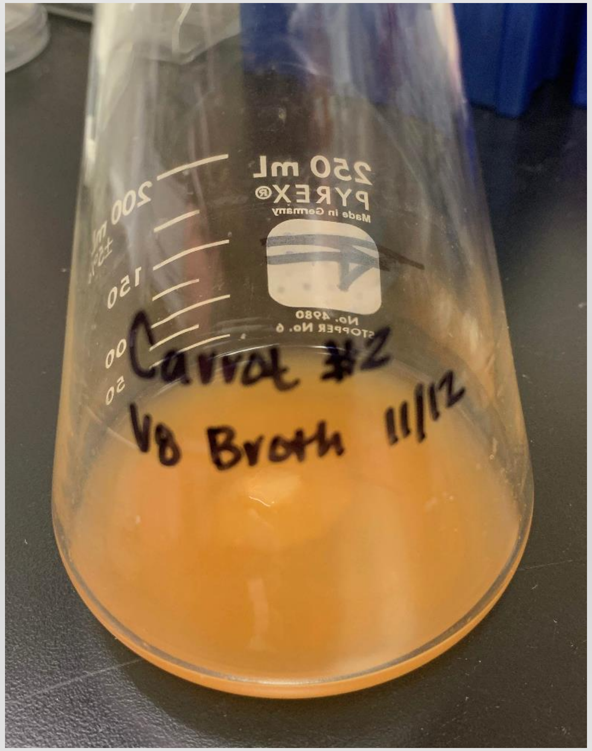
not used in organic farming

Isolation of oomycete pathogens from cavity spot lesions

- carrots were washed well in tap water
- lesions were aseptically removed and cut into 2-4 pieces
- lesion tissue was pressed into PARP agar
- incubation in the dark at room temperature (±23^{o}C)
- part of the hyphae (outer edge) was transferred to fresh PARP and later to CMA

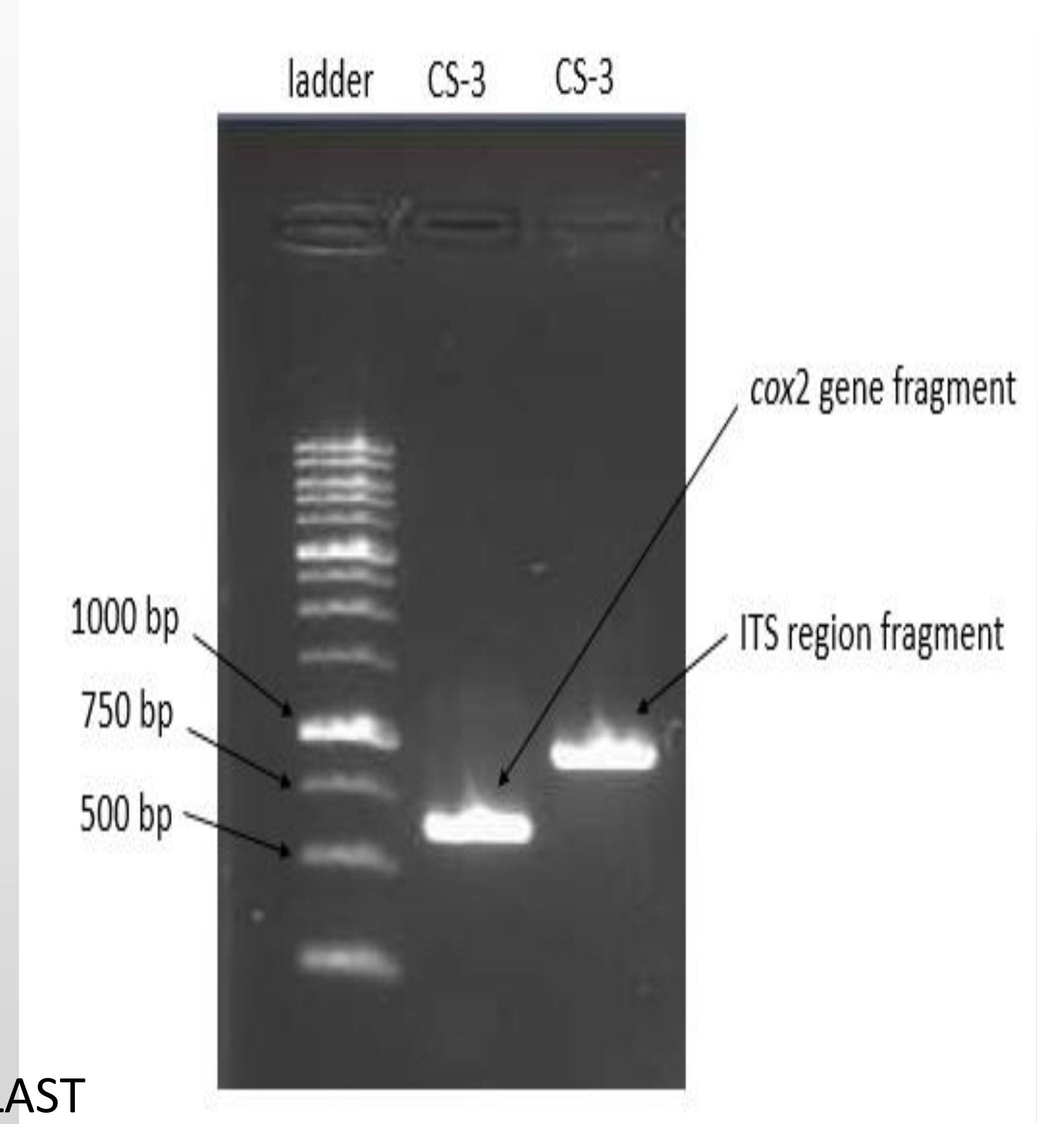
Growing isolates for genomic DNA extraction

- agar plug with active mycelium was transferred to 15 ml V8 broth
- incubation for 4 days in the dark at room temperature (±23^{o}C)
- genomic DNA extraction with the DNeasy Plant Mini Kit (Qiagen)
- measurement of DNA concentration on Nanodrop



Molecular identification of oomycete strains

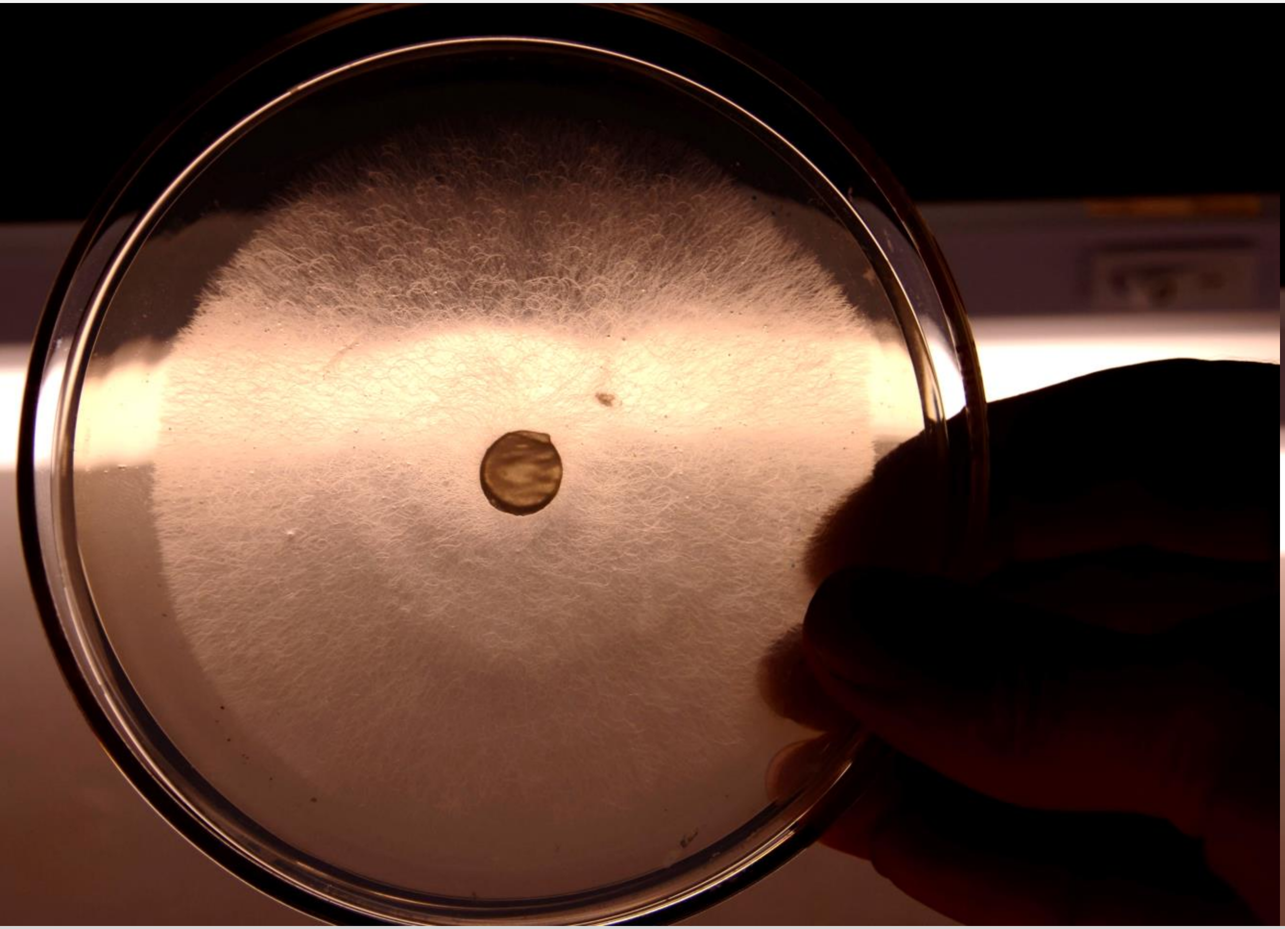
- amplification of two genetic fingerprint regions
    - *cox2* gene (Choi *et al*., 2015)
      → fragment of 628 bp
    - ITS region (Schroeder *et al.*, 2006)
      → fragment of ± 1000 bp
- verification on agarose gel
- purification
- send for sequencing (Laragen, Inc., Culver City, CA)
- sequence analysis and identification through online database BLAST



Choi, Y., Beakers, G., Glocking, S., Kruse, J., Nam, B., Nigrelli, L., Ploch, H., Shivas, R.G., Telle, S., Voglmayr, H., and Thines, M. (2015). Towards a universal barcode of oomycetes - a comparison of the *cox*1 and *cox*2 loci. *Molecular Ecology Resources* 15 (6): 1275-1288.
Schroeder, K.L., Okubara, P.A., Tambong, J.T., Lévesque, C.A., and Paulitz, T.C. (2006). Identification and quantification of pathogenic *Pythium* spp. from soils of eastern Washington using real-time polymerase chain reaction. *Phytopathology* 96:637-647.

Amplification of genetic fingerprint regions directly on the hyphae

CMA_{Difco}



CMA_{Sigma}

CMA_{Difco} is best used for hyphal tip transfer because individual hyphae are better visible

CMA_{Sigma} enables more lush growth preferred for direct amplification

Molecular identification of the isolated and received strains

Original name	Isolated from	Identified as	Working name
Cavity spot isolate 1 (CS-1)	Conventional field	*Pythium violae*	*Pv*-2
Cavity spot isolate 2 (CS-2)	Organic field	*Pythium spinosum*	*Ps*
Cavity spot isolate 3 (CS-3)	Conventional	*Pythium violae*	*Pv*-1
Pythium violae WSU	received from Dr. L. du Toit, originally isolated from CA	*Pythium violae*	*Pv*-C

used as a control for our diagnostics

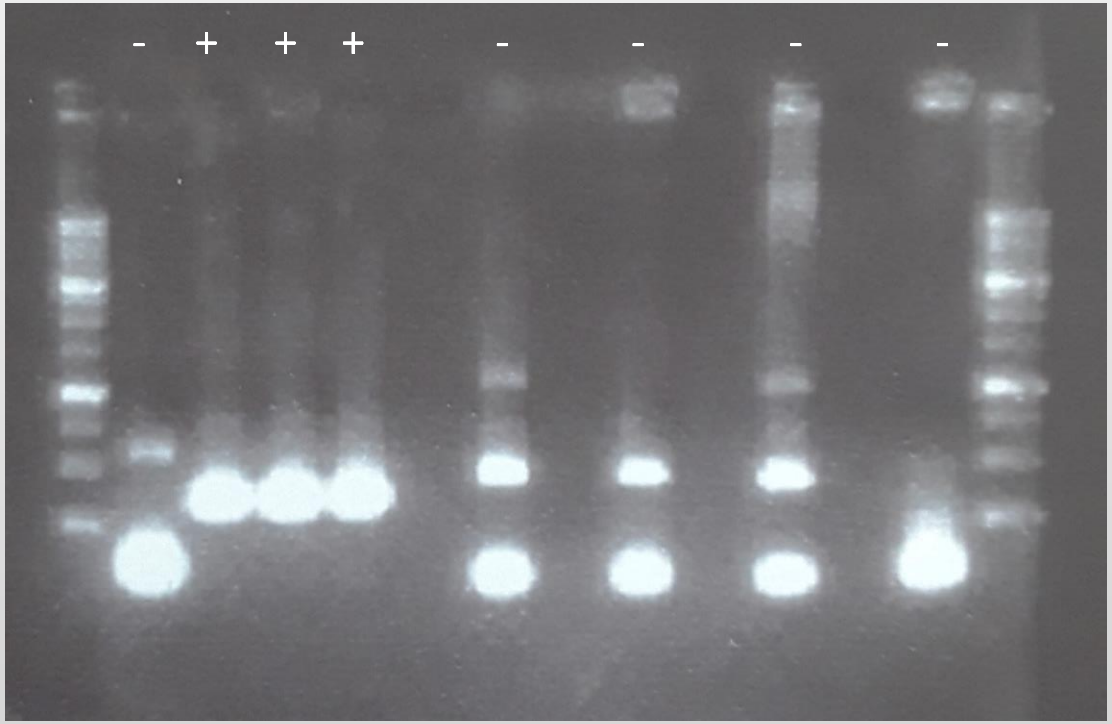
ready to accept up to 100 samples for local growers for identification (funded by CFCAB)

contact me at ifrancis@csub.edu

Amplification with *P. violae* specific primers

primers designed within the ITS region that should be specific to *P. violae* (Klemsdal *et al.*, 2008)

water *P. violae* non-*P. violae* oomycetes or fungi



the primer positions in the ITS region (underlined)

```
CS-3-Pv-1     TGTGTGTGCACAGCAATG-----------------TGTGTG----TGCGGACTGGCTGA
CS-1-Pv-2     TGTGTGTGCACAGCAATG-----------------TGTGTG----TGCGGACTGGCTGA
CS-PW-Pv-C    TGTGCGTGCACAGCAATG-----------------TGTGTG----TGCGGACTGGCTGA
CS-2-Ps       TATGTGCCTACTGCACTGCTGACTTTGCATTCATTTGTATGGTCTTGGCGGAGTGGCGGG
Ps-SL         TATGTGCCTACTGCACTGCTGACTTTGCATTCATTTGTATGGTCTTGGCGGAGTGGCGGG
              * ** *   ** *** **                 *** **    **  *** **** *

CS-3-Pv-1     GTGCAGATGTGAAGTGTCTCGCTGGCCTACTTCTCTCTTTGGGGAGTGGACAGGTATCGA
CS-1-Pv-2     GTGCAGATGTGAAGTGTCTCGCTGGCCTACTTCTCTCTTTGGGGAGTGGACAGGTATCGA
CS-PW-Pv-C    GTGCAGATGTGAAGTGTCTCGCTGGCCTGCTTCTCTCTTTGGGGAGTGGACAGGTATCGA
CS-2-Ps       TTGCAGATGTGAAGTGTCTCGCTA--TGGTTGGCATTTGTAATGAATGCACAGCTTGCGA
Ps-SL         TTGCAGATGTGAAGTGTCTCGCTA--TGGTTGGCATTTGTAATGAATGCACAGCTTGCGA
               **********************       *    * * *   ** ** **** *  ***
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Klemsdal, S.S., Herrero, M.L., Wanner, L.A., Lund, G., and Hermansen, A. (2008). PCR based identification of *Pythium* spp. causing cavity spot in carrots and sensitive detection in soil samples. *Plant Pathology* 57:877-886.

Carrot disk assay

CMA disks with active growth of *Pythium* were placed on mature freshly harvested (48h) carrots and incubated in a moist environment at 24°C in the dark
pictures taken at 5 dpi

Non-inoculated | *Pv*-C | *Pv*-2 | *Pv*-1 | *Ps*

Repeat carrot disk assay

with *P. spinosum* strain received from Dr. Cassandra Swett (UC Davis) and isolated from a cavity spot lesion from organically grown carrots in the Riverside area

/ *Pv*-C *Ps* *Ps*-SL

Soil assay

Pythium grown in V8 broth for 4 days

mixed with hand mixer

added to sand : peat moss mixture (50:50, autoclaved twice for 30 min)

transferred to tree seedling pots (cleaned with ethanol and dried)

4 carrot seeds per pot (thinned to 1 seedling per pot)

under light (16h photoperiod) at 23^{o}C

Soil assay

reinoculated at 5.5 weeks

reinoculated at 12.5 weeks

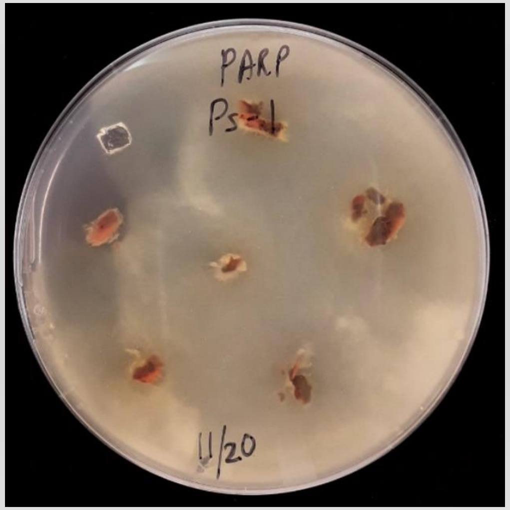
harvested at 16 weeks

Soil assay

contamination with *Fusarium*

but the different *P. violae* strains were reisolated

from the lesions as well



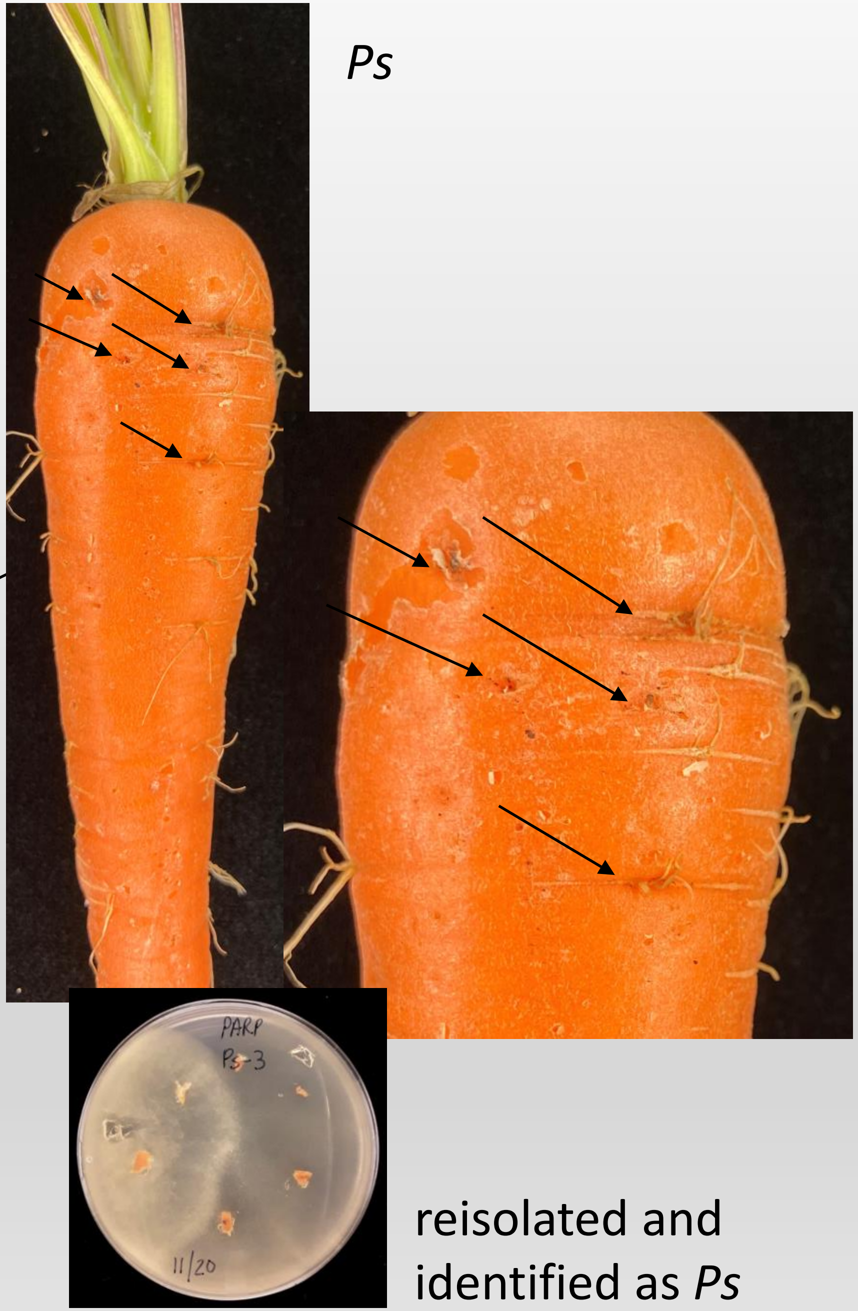
P. violae (*Pv*-C, *Pv*-1, *Pv*-2)

Soil assay

Ps

reisolated and identified as *Ps*

Ps



reisolated and identified as *Ps*

Ps-SL

reisolated and identified as *Ps*-SL

Future directions for our research

The bacterial genus *Streptomyces* is renowned for
the production of antimicrobial compounds

153 *Streptomyces* isolates
were isolates from diverse soils in the Bakersfield area

four local *Streptomyces* isolates strongly inhibited of *P. violae* and other oomycetes
some of them also inhibited *Fusarium*, *Sclerotium rolfsii*, and/or *Sclerotinia sclerotiorum*

Acknowledgements

Misbah Chaudhry
Graduate student (M.Sc.) at CSUB

Dr. Jaspreet Sidhu
Joe Nunez
UCCE Kern County

Dr. Lindsey du Toit (Washington State University)

Dr. Cassandra Swett (University of California, Davis)

Funding:

California Fresh Carrot Advisory Board

California State University Bakersfield

Student Research Scholars program
Graduate Student - Faculty Collaborative Research Program

Improved molecular diagnostics to detect and quantify root-knot nematodes

Amanda Hodson, Ph.D.
Assistant Professional Researcher
University of California Davis
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Integrated pest management

Aboveground

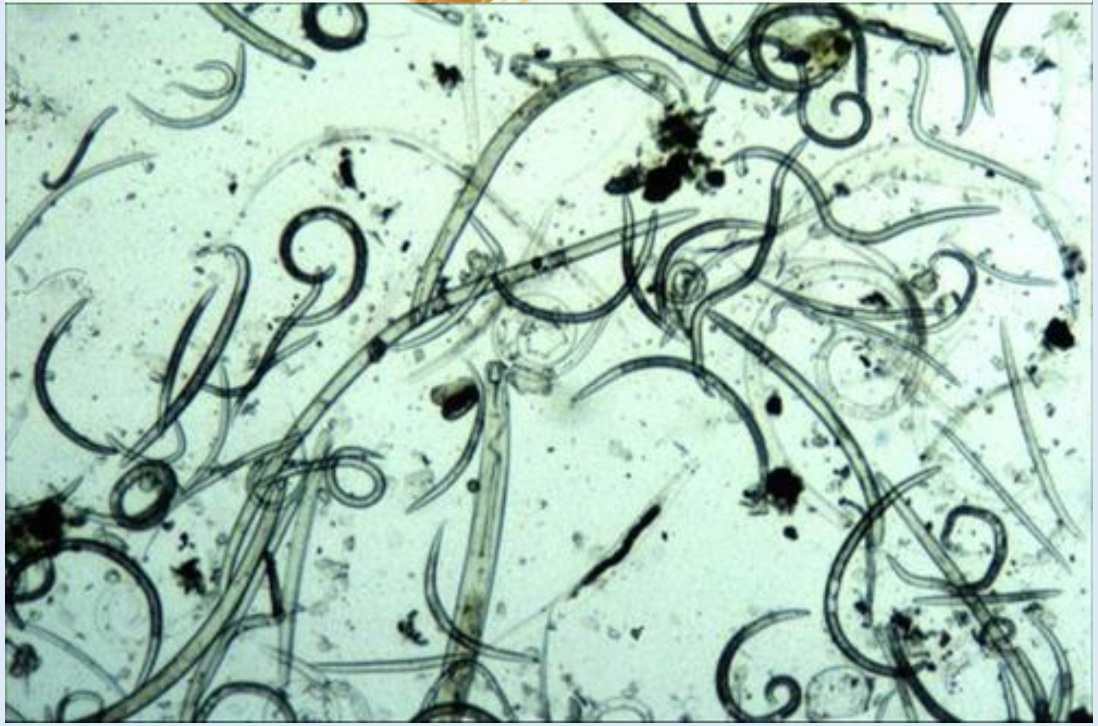
- Identification
- Monitoring
- Learn the Pest Biology
- Establish Action Threshold
- Management strategy

Belowground

- Difficult species differentiation
- Spatial heterogeneity
- Biological regulators unknown
- Damage thresholds unclear
- Management tradeoffs

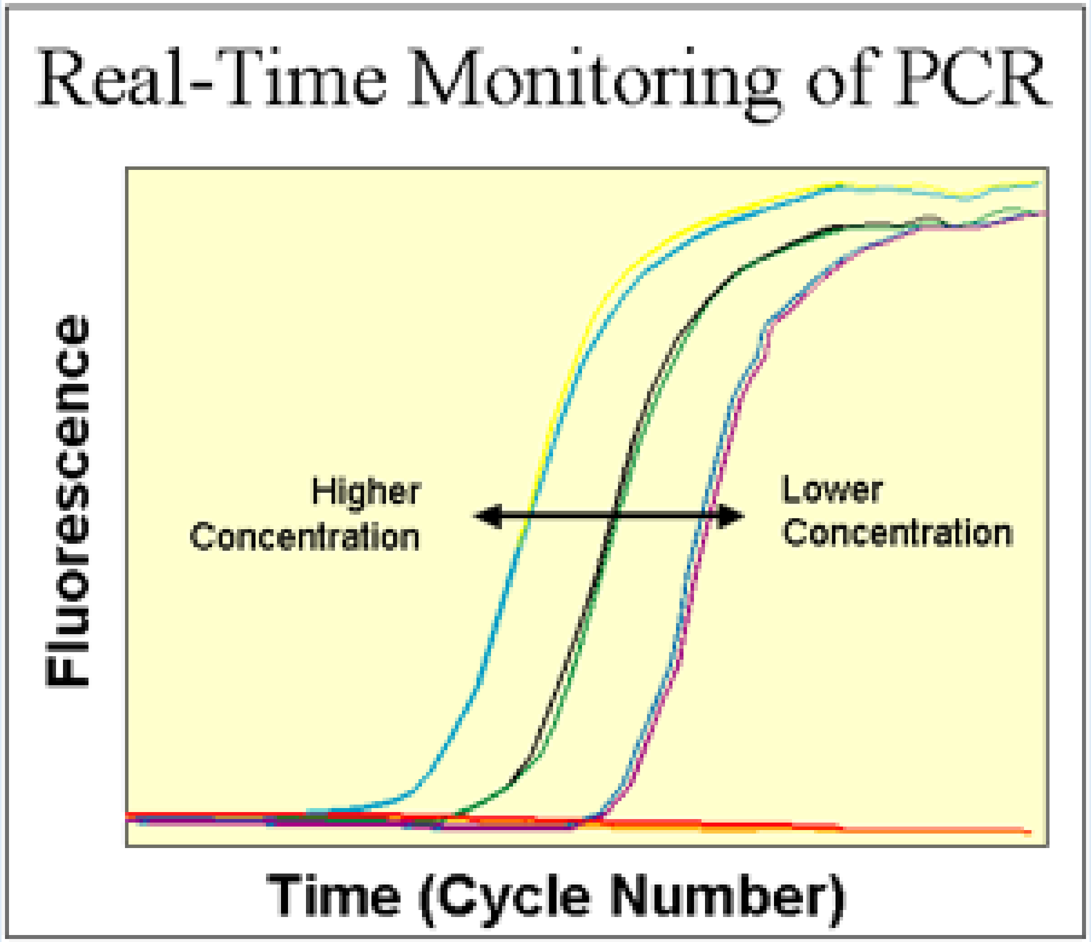
More detailed ecological knowledge needed for good integrated nematode pest management.

- In carrots, economic threshold is < 1 infective juvenile of root knot nematode.
- Current Testing:
    - Expensive
    - Not representative
    - Slow
    - Subjective
    - Difficult



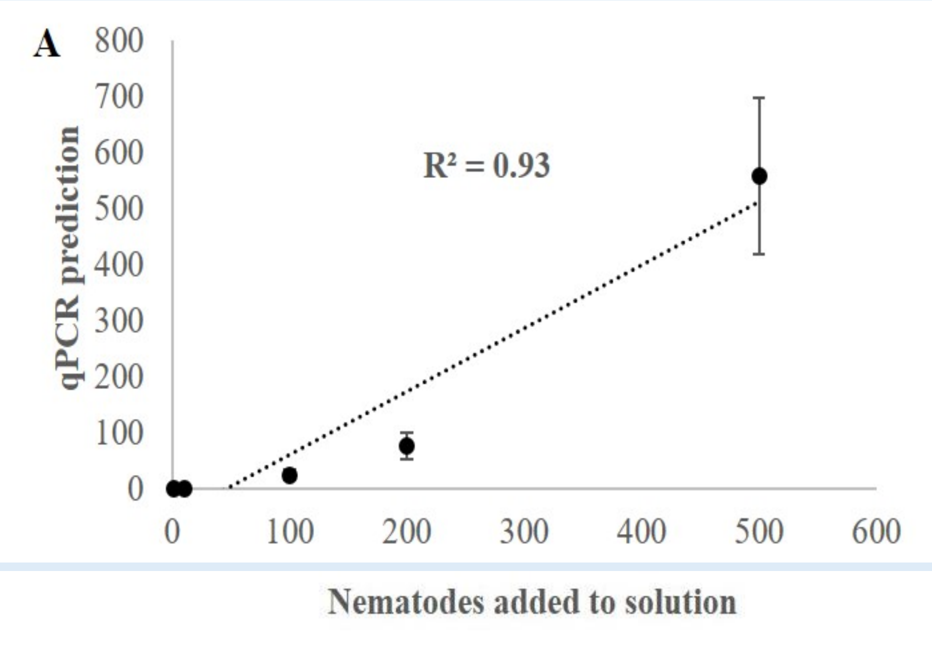
Real time PCR (qPCR)

- Simultaneously quantifies and identifies nematodes.
- Standard curve of known densities.
- Compare intensity of amplified signal to standard curve.
- Rapid and inexpensive pest identification and quantification.



Laboratory experiments

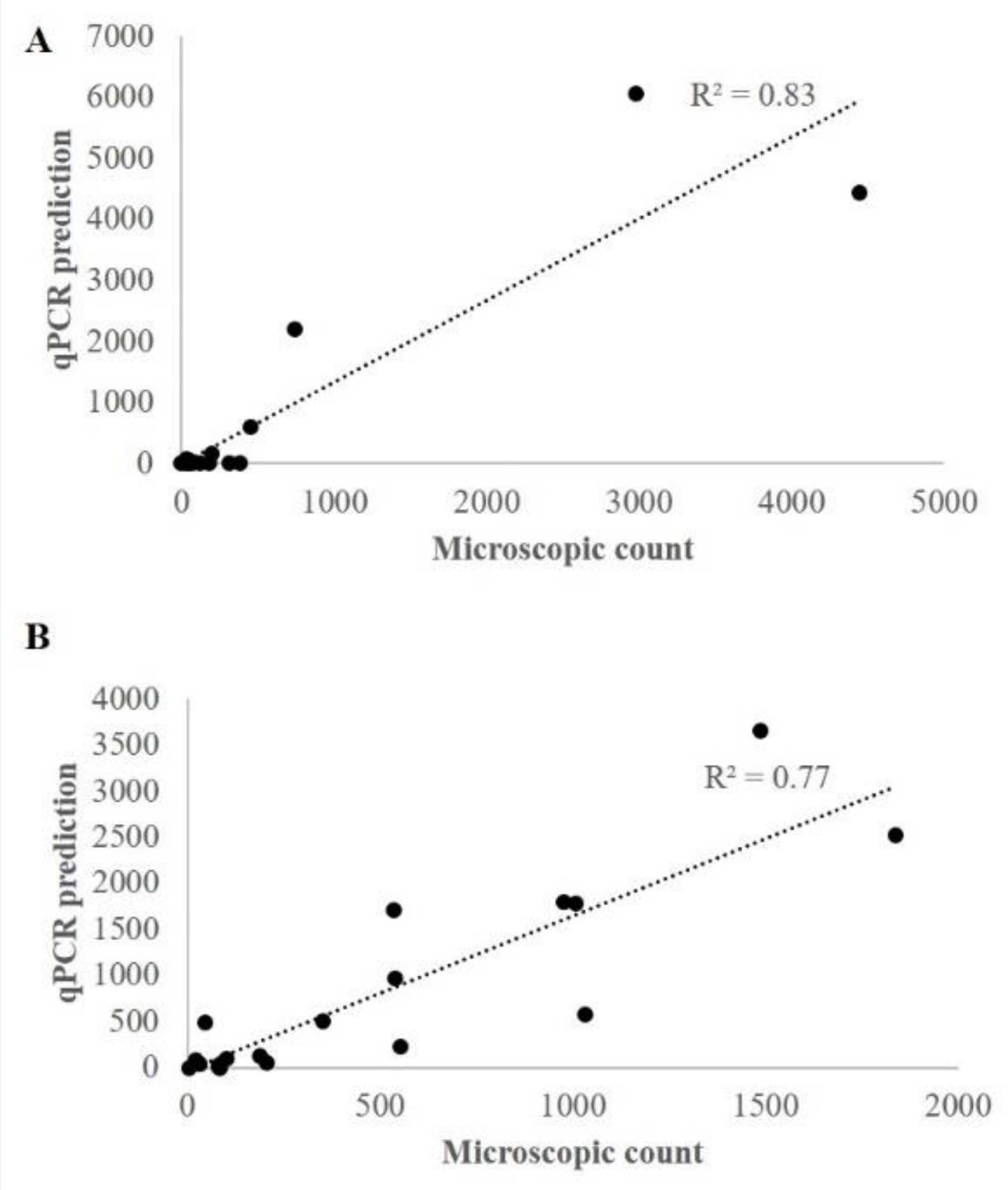
- Known quantities of nematodes in tubes
- Relationship between the numbers of nematodes inoculated into solution and resulting qPCR Ct values for *M. incognita*



Field validation: Nematode counts 100 ml soil^{-1}

Microscope count	qPCR prediction	Microscope count	qPCR prediction
50	44.7	0	2.9
36	6.7	0	4.7
40	59	8	1.8
20	0.2	12	11.4
10	7.6	6	4.1
20	0.2	16	29.8
10	7.6	4	0.2
0	0	24	35.4
0	3	34	6
0	1.4	14	17.9
449.2	368.1	18	92.1
24	42.4	4	7.8
8	0.1	8	3.5
8	33.9	6	0.1

- Relationship between numbers of root-knot nematodes in the *M. incognita* species group counted under the microscope and quantification by qPCR from A) a carrot research station trial B) a carrot grower field



Could molecular methods be less expensive?

- Estimated analysis costs/sample for my lab (including labor and chemicals)
  - $30.10 microscope
  - $33.70 molecular

Depending on the lab and labor costs, it could save time and money, especially if lacking an experienced nematologist.

Diagnostic Lab	$Cost/sample
1	33.00
2	52.50
3	125.00
4	55.00

Thank you!

Questions?

- Amanda Hodson
- akhodson@ucdavis.edu
- www.hodsonlab.org

Screening carrots for resistance to cavity spot-2020

MARY RUTH MCDONALD AND

PHIL SIMON

University of Guelph, Guelph ,Ontario, Canada

USDA-ARS, University of Wisconsin, Madison, WI

- Trials in Ontario, Canada
- High organic matter soil
- Cavity spot occurs regularly at this site
- Seeded in May, harvested in October

Several Pythium species cause cavity spot

California

P. violae

P. sulcatum

P. ultimum

P. irregulare

P. dissocticum

Ontario, Canada

P. violae

P. sulcatum

P. ultimum

P. irregulare

P. sylvaticum

P. intermedium

California Vivoda et al. 1991, Lu et al. 2012; Ontario McDonald 1994

Pythium species from cavity spot at the Muck Crops Research Station

2012: *P. sulcatum* molecular methods
2018: 85% P. sulcatum
2020: Almost all *P. sulcatum-* isolated and cultures confirmed by molecular methods

Isolations and identification continuing

Objectives

To screen carrots from the USDA-ARS breeding program for resistance to cavity spot

---

Compare these carrots to susceptible cv. ‘Atomic Red’
And resistant cv. Purple Haze
Cello carrots: Maverick, Cellobunch, Envy
Cut and peel: UpperCut, HoneySnax

Long term: Contribute to the USDA breeding program to improve genetic stocks for carrot production in California
Also look at susceptibility to leaf blights, forking (Pythium root dieback) and to rate bolting

Methods- 2013- 2020

Seeding

- 60 carrot lines, including cultivars
- Direct seeded ~ 70 seeds/m, with a push V-belt seeder on to raised beds
    - early June
- Soil 60-78% organic matter, pH 5.8- 6.5
- 4 reps/ line, each rep was 5m (2013) or 6 m = 20 ft (2014 on) in length
- No soil fungicides were applied. Standard herbicides and insecticides were applied to the plots.

Other disease of carrots

Forking may be the result of Pythium root dieback and other factors

Alternaria Leaf Blight

Methods

Harvest

- 50 carrots/rep harvested late Oct. each year and placed in cold storage until assessment.
- A separate sample is assessed for forking

Assessment

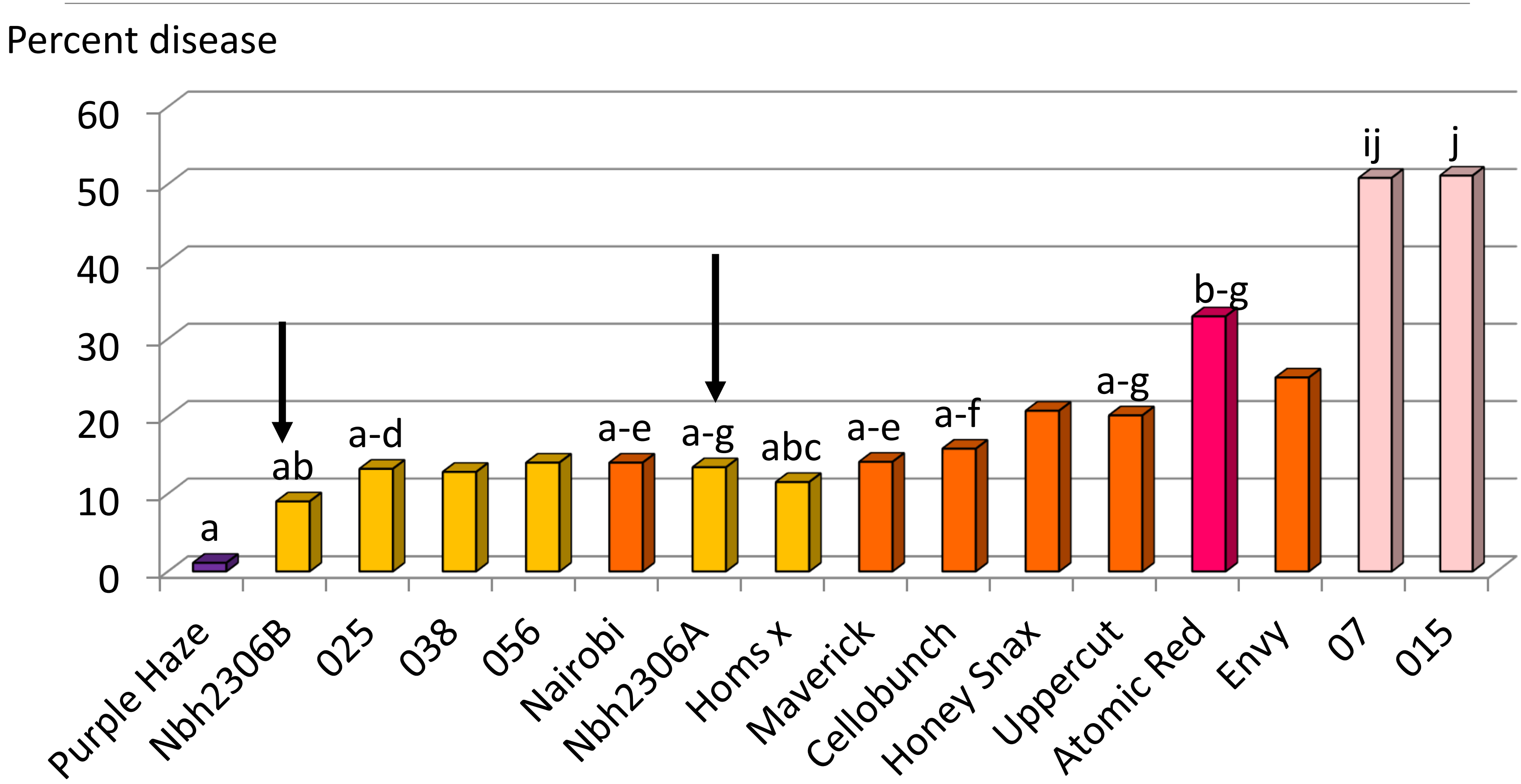
- Carrots were washed and assessed for cavity spot incidence (%) and severity based on the length of the largest lesion per carrot

  (1= <1 mm, 2= 1-2 mm, 3= 2.1- 5 mm, 4= 5-10 mm, 5= >10 mm)
- A disease severity index (DSI) was calculated
- Carrots were also assessed for carrot leaf blights (Alternaria and Cercospora), forking and bolting

Notes on 2020 trial

- Good stand overall
- Relatively high cavity spot:
  - incidence **90%,**
  - severity **51%**
- Average rainfall except above average (5.5 in) in August (mid-season)
- Carrot leaf blights moderate to high (max 3.9 on a 0-5 scale)
- Carrot forking 0 –20%

Severity of cavity spot on representative carrot lines grown at the Muck Crops Research Station, **2020**



Percent disease ranged from 0.5 to 91, Disease severity from 0.2 to 51

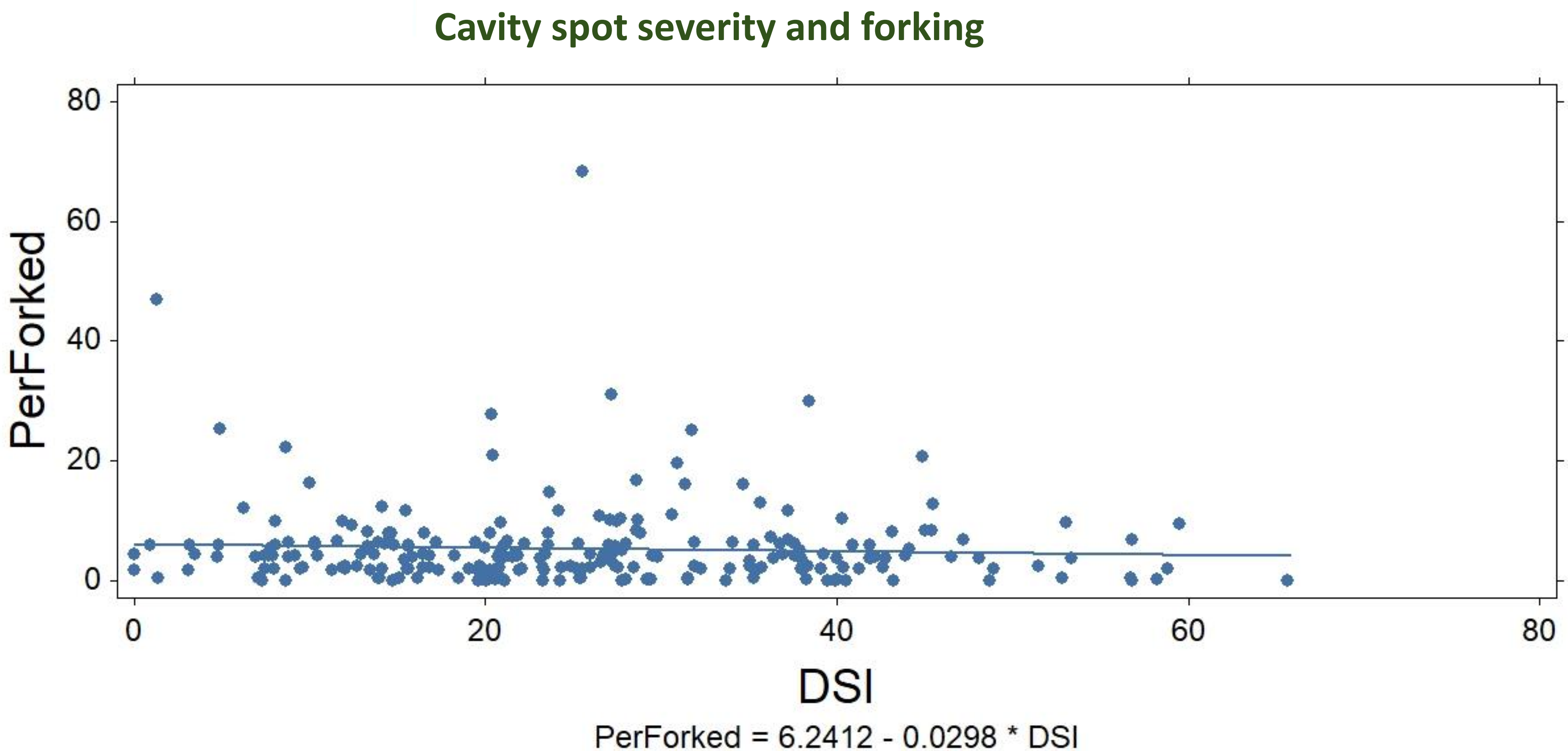

3059C
Purple Haze
Highly resistant


4034
Nbh2306B
9 severity
24% incidence
4% forking
Also nematode resistant

Pythium root dieback can cause forking of carrots

Is resistance to cavity spot also resistance to forking?

NO



Carrots with low cavity spot 2020

Line	Cavity spot (%)	Severity (0-100)	Leaf blight (0-5)	Forking (%)	Bolting (0-3)
Purple Haze	4.5 a	1 a	0.9	4	1.8
Nbh2306A	**24 ab**	**9 ab**	**1.0**	**4**	**1**
Nbh2306B	**37 a-e**	**13 a-d**	**1.0**	**9**	**0**
CS025	**31**	**13**	**1.8**	**5**	**0.1**
F5367B	**31**	**13**	**2.0**	**4**	**0.1**
CS015	90 g	51 j	3	1.5	0.3

Forking ranged from 0- 20%. Bolting ranged from 0 – 3 (over 50% seeders).

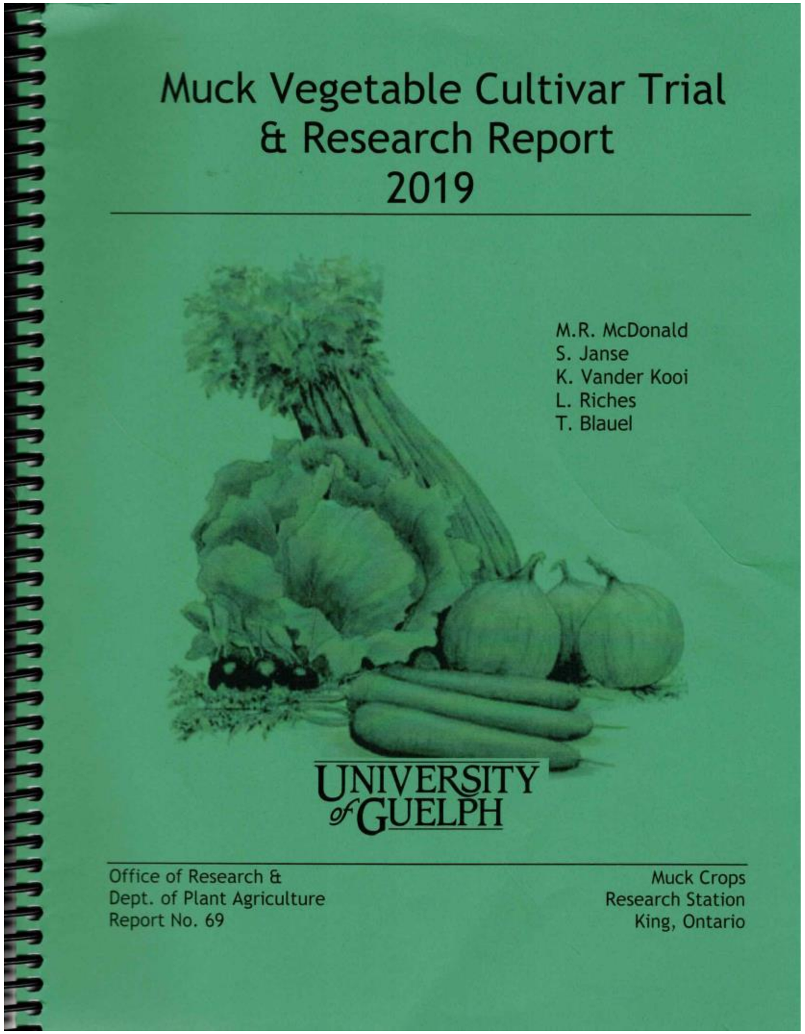
Summary

- Moderate to high disease pressure in 2020
- *Pythium sulcatum* consistently the main species causing cavity spot
- Resistance to cavity spot not related to resistance to forking overall, but some lines have low levels of both
- Information contributes to Phil Simon's breeding for cavity spot resistance

Acknowledgements

Funding provided by the California Fresh Carrot Advisory Board

Technical assistance:
Kevin Vander Kooi
Laura Riches



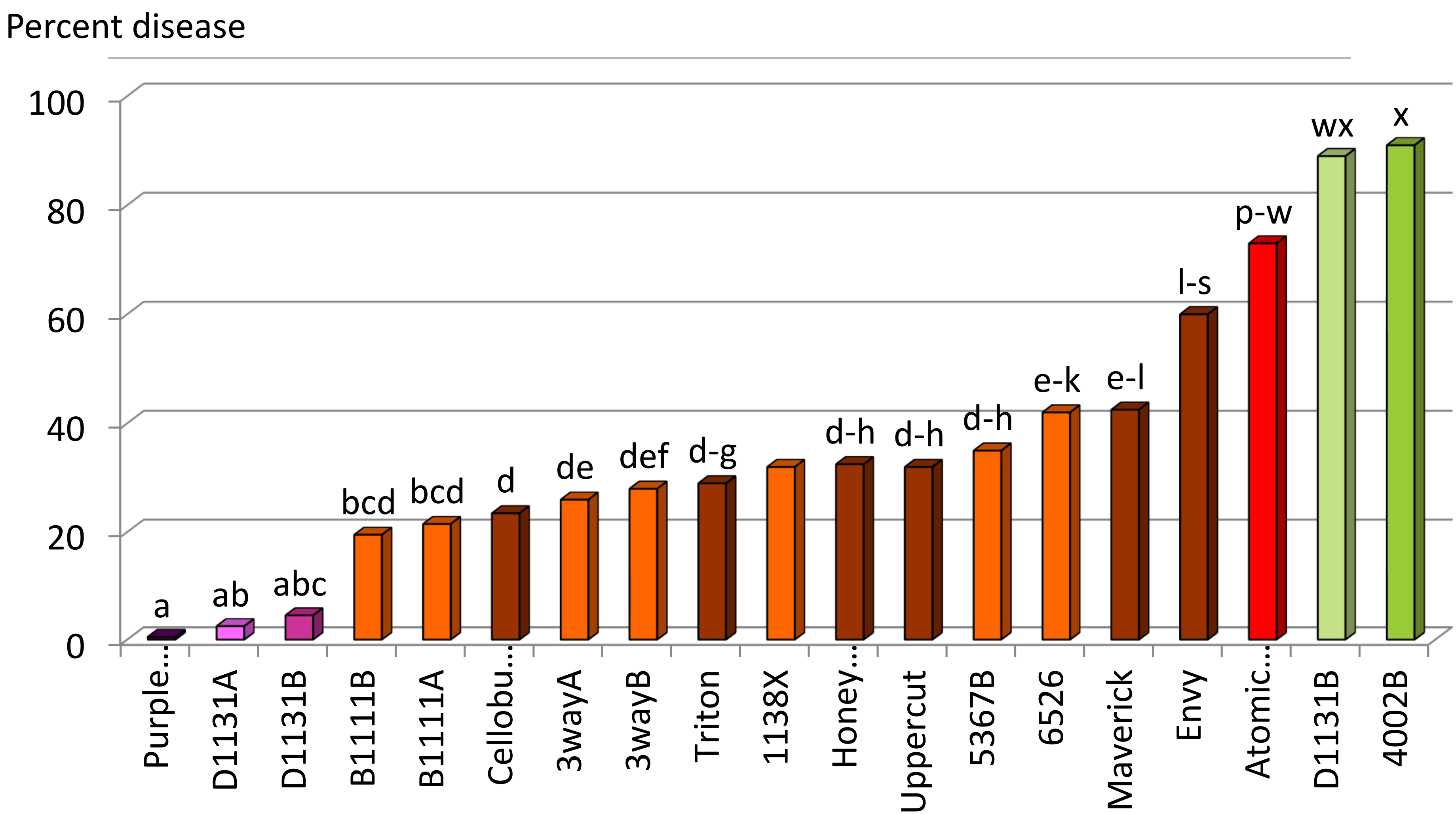
All research trials are summarized in the Annual Report

Download at the Muck Station web site:

www.uoguelph.ca/muckcrop

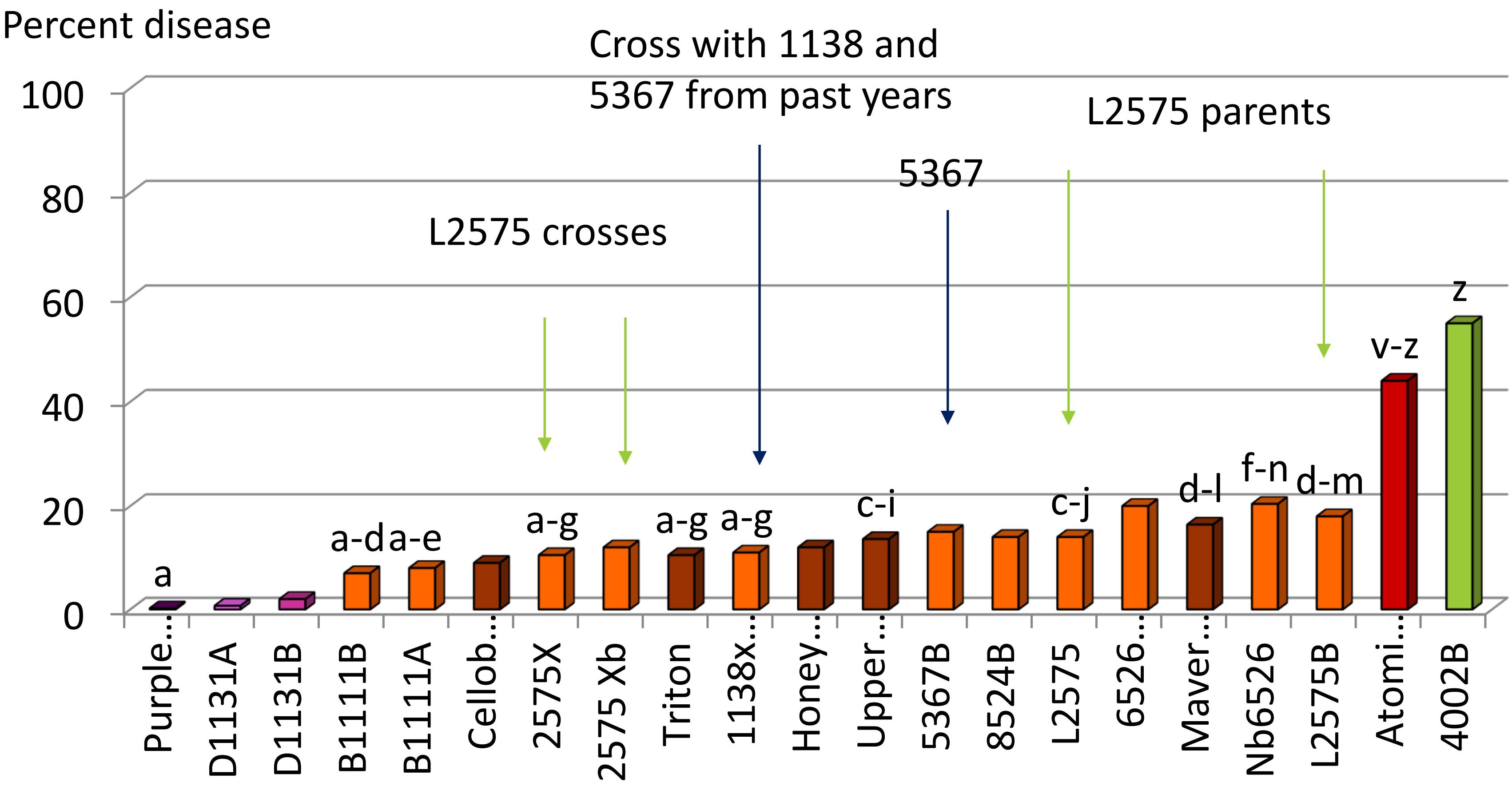

Atomic Red
57% incidence
and 33% severity
4060cc

Percent cavity spot on representative carrot lines grown at the Muck Crops Research Station, **2018**



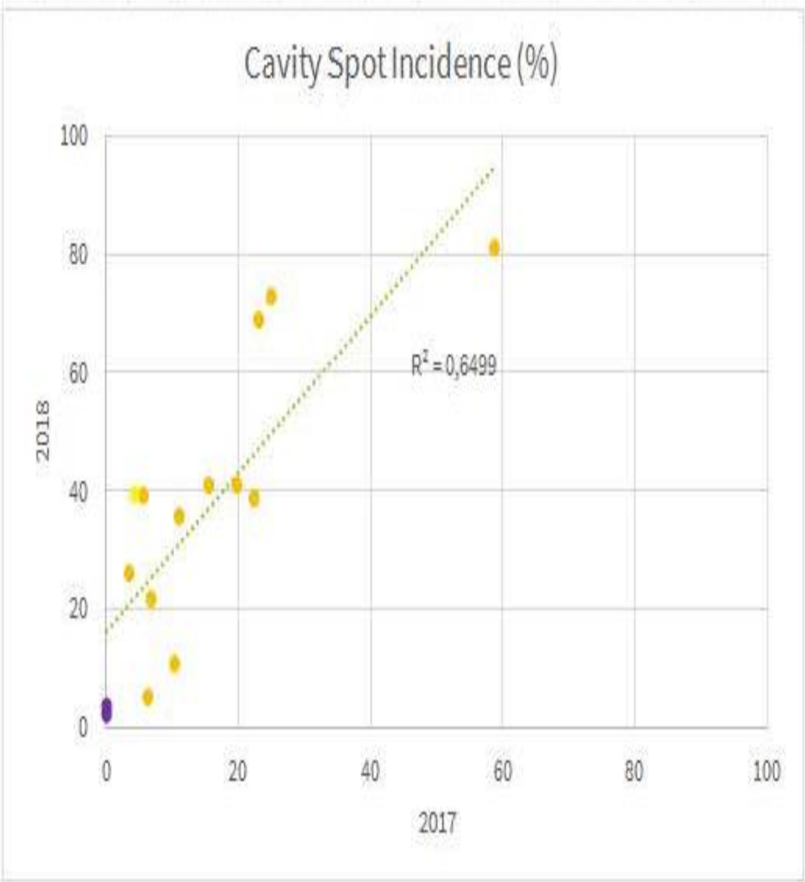
Percent disease ranged from 0.5 to 91, Disease severity from 0.2 to 55

Severity of cavity spot on representative carrot lines grown at the Muck Crops Research Station, **2018**

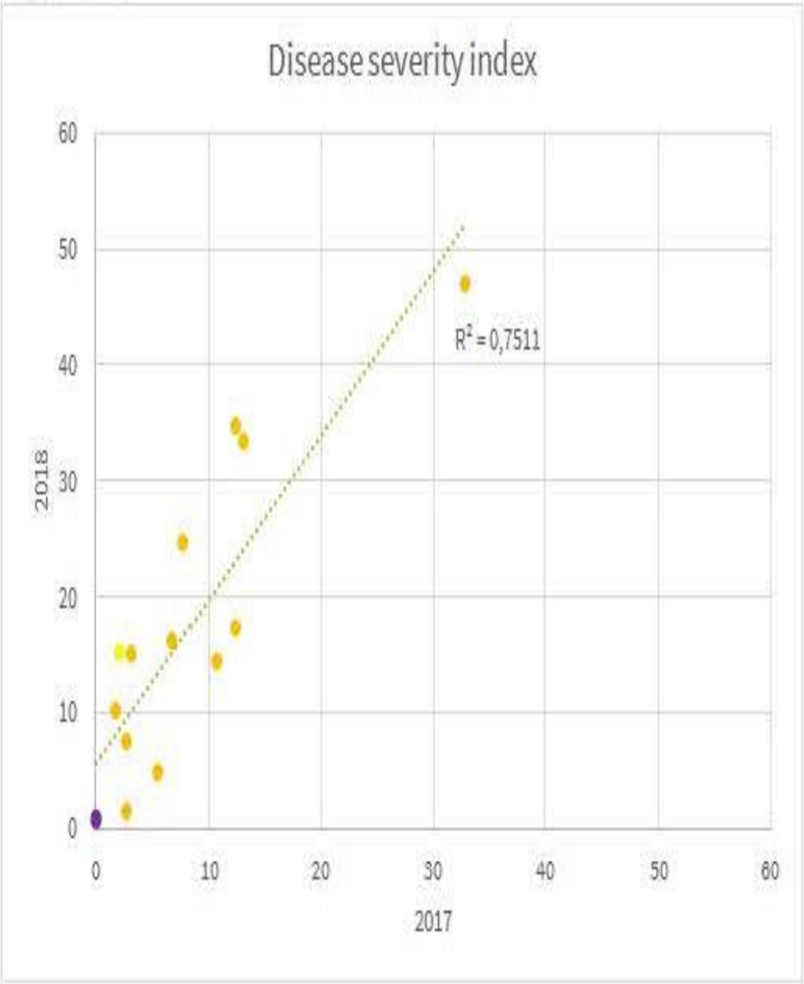


Percent disease ranged from 0.5 to 91, Disease severity from 0.2 to 55

Reproducibility of cavity spot assessments 2017 and 2018 – small trial with numbered lines



R^2= 0.6499



R^2= 0.7511

Carrot Breeding to Develop and Introduce Improved Cultivars for California Production:

Field Research, Combining Genes for Rootknot Nematode and Cavity Spot Resistance

Phil Simon, Jas Sidhu, Phil Roberts, Mary Ruth McDonald, Lindsey du Toit, Irwin Goldman, Industry Cooperators

Scope of USDA Cooperative Carrot Breeding 2020-21

- Field trials
  - In DREC, El Centro; in Kern County (Jas Sidhu et al.)
    - General breeding
  - In Tustin, Riverside (Phil Roberts et al.)
    - Nematode resistance evaluation and selection
  - In Guelph, Canada (Mary Ruth McDonald et al.) and WSU (Lindsey du Toit et al.)
    - Cavity spot resistance evaluation and crosses being made
  - Alternaria leaf blight resistance testing in Hancock, WI (Irwin Goldman et al.)
- Selected carrots sent from El Centro to Madison in March for seed production in summertime
- Selected nematode resistant carrots sent from Dr. Roberts program
- Data on cavity spot resistant carrots sent from Drs. McDonald, du Toit, and Sidhu's programs
- Lab evaluation: Molecular markers for nematode resistance; Quality factors – carotenes, sugars, flavor

UC DREC trial

- Hybrid trials with Jas Sidhu
- 99 C&P, 93 cello hybrids, 90 novel colors
- Good performance of nematode resistant inbreds in 2 of the top 15 C&P and 9 of the top 15 cello entries were USDA hybrids with nematode resistant parents

Nematode Field Trials - 2020

- In cooperation with Phil Roberts on trial plots established by him
- Tustin harvest
  - 450 entries
  - *M. incognita*
  - Identify new sources of resistance, confirm earlier sources, combine multiple sources
  - No field day due to COVID restrictions

Performance of *Mj-1* Nematode Resistance Stocks ("Nb")

- Advances in the level of nematode resistance from 'Brasilia 1252' (*Mj-1*). Both *M. javanica & M. incognita*
- Resistance levels holding up for both nematodes
- USDA inbreds with resistance used as parents in cello trial and released to seed industry
  - Primarily Br 1252 derivatives but new inbreds also include Homs
- 'Cape Market' is a new source of resistance being evaluated
- More cut and peel inbreds with nematode resistance being used in USDA experimental hybrids

Progress in Incorporating Nematode Resistance into California Carrots

Resistant & susceptible 'Brasilia'

Inbreds from orig. Br 1252 cross (L) and cello (R)

Inbred (F4) from crosses w/ C&P

Exp. Hybrids w/ C&P resistant parents

Industry Testing of Nematode Resistant Carrots

- Seed has been released to seed industry for testing and initiating incorporation of resistance in 2014
- Seed companies submitted entries into the field trials
- Strong resistance (score of 0 or 1) for several entries from seed companies

Progress in Combining Nematode Resistance Sources - 2021

	MJ	1091	WR	HM	PD	SFF	NF	CM
MJ	--	*** 0-5	*** 1-3	*** 0-2	*** 0-5	*** 0-1.5	*** 0-3	** 2
1091		--		*** 0-3	** 2-4	*** 0-2	* 1-4	
WR			--	*** 0-2	*** 3.5-4	** 2-3	* 1-3	
HM				--	*** 0-2.5	*** 0-2	** 0-2	** 2-2.5
PD					--	* 1-3	** 2-3	* 1.5-3
SFF						--	*** 0-1.5	** 1.5-2
NF							--	
Susc. Long	*** 0-1	*** 0.5-2	*** 1.5-3	*** 0-1	*** 0-2	*** 0-1	** 1-3	* 2.5
Susc. Flavor	*** 0-1	*** 0-2	*** 2-3	*** 0-1.5	*** 0-1	*** 0-1	** 2-3	** 1-3.5
Susc. Other	*** 0-1			*** 0-1	** 2-4	*** 0-1		** 2-3

Yellow highlight - Recent advances

Green highlights – Best candidates for upcoming efforts

Progress in Advancing Cavity Spot Resistant Carrots

- Trials by Mary Ruth McDonald and Lindsey du Toit to identify and advance strong resistance along with horticultural quality for the California market.
  - Resistance in diverse carrots in the breeding program
- Seed production of combined sources of identified resistance sources and search for new sources of resistance
- Similar resistance trends in both trials
  - Nbh2306 resistance of particular interest since this inbred also has strong nematode resistance
- Pyramid/combine multiple sources of resistance

Alternaria leaf blight resistance breeding

- Resistance scored in 178 breeding populations as part of CFCAB project as well as 212 OPs and wild carrots in SCRI project
- 64 sources of resistance identified in the last 5 years
- Intercrossing among these sources underway

Carrot Seed Production in Greenhouse and Field

Coming up

- Cooperative efforts for California market carrot breeding
  - New combinations of nematode resistance genes
  - Evaluate additional carrot germplasm for cavity spot resistance and advance crosses made including data and selected roots from Drs. McDonald, Sidhu, and du Toit
  - Germplasm releases - long, good flavor, nematode resistant selections
  - Alternaria resistance pyramiding
  - Heat tolerant, with Dr. Sidhu, and weed competitive carrots
  - More detailed genetic maps for all traits
  - More efficient breeding approaches

Thank you !

Updates on Kern County Trials

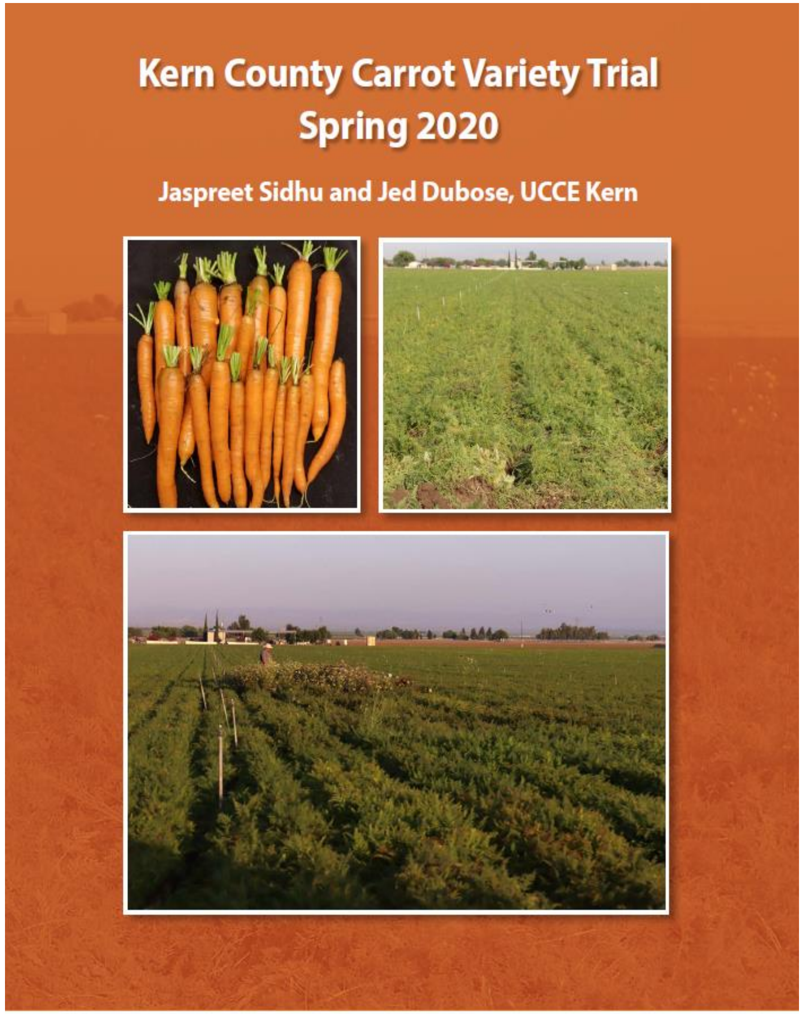
Jaspreet Sidhu

UCCE Kern

2020 Trials

- Variety trials
- Cavity spot biological screening
- Nematicide screening
- Herbicide screening

Spring variety trial



Spring Variety Trial
Planted on Jan 30, 2020
Harvested June 25, 2020
34 Cut and Peel
32 Cello
19 Colored

ID#	ID	Percent bolting incidence
69	Purple Haze	2.0
72	Redsun	10.0
76	Malbec	5.0
77	976-4	25.0
78	976-5	70.0
79	17'B103-1	40.0
80	981-5	20.0
81	983-5	85.0
82	985-5	85.0
83	17'B104-1	85.0
84	13'5364-1	50.0
85	994-6	5.0

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Fall variety trial

Planted on August 6, 2020
Harvested on December 15, 2020
36 Cut and Peel
26 Cello
22 Colored

Organic variety trial

Dead/ burned tops in the trial due to foliar diseases

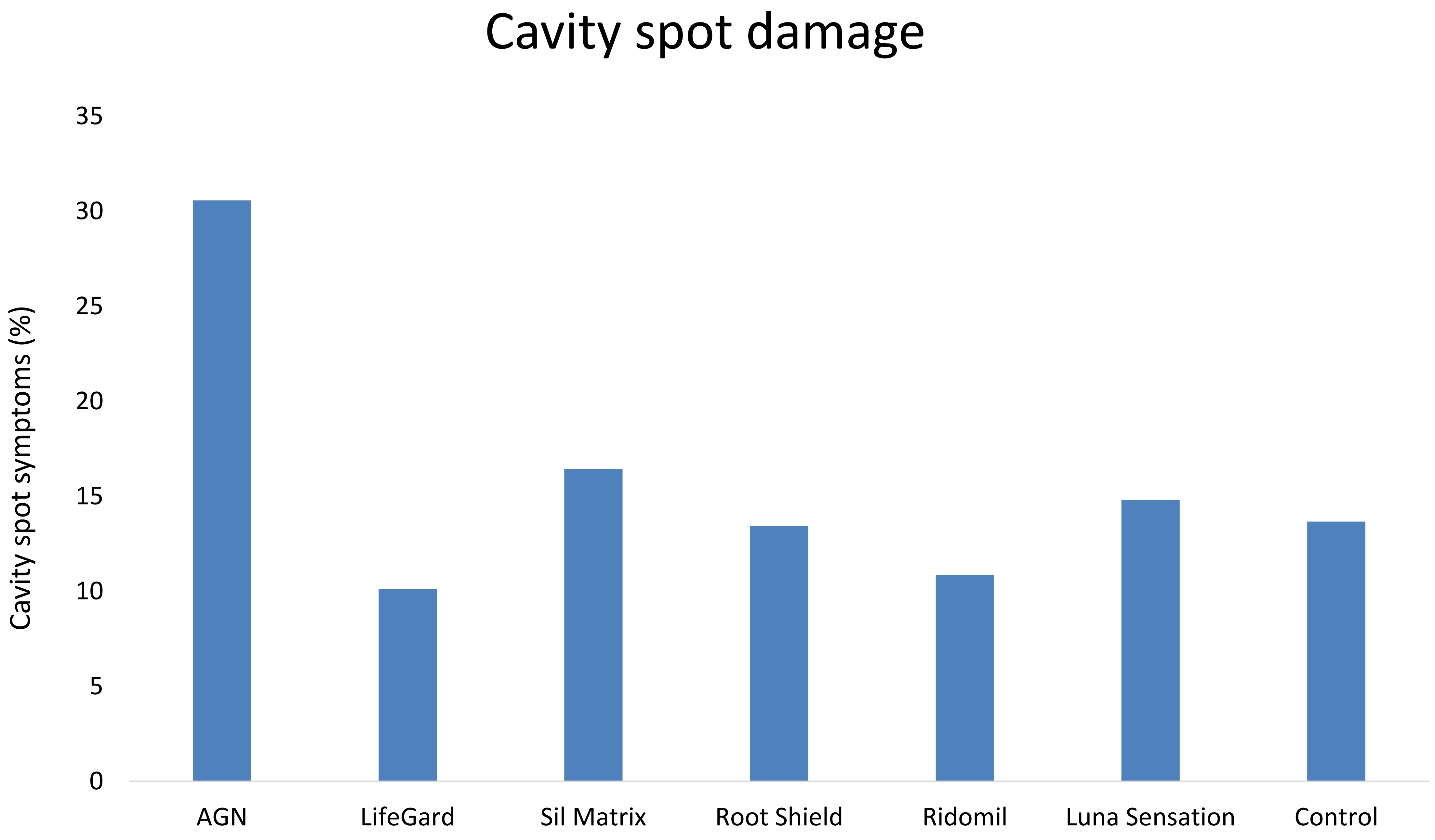
30 entries

Plant height, foliar disease severity, root count per plot, root weight, root shape, uniformity and smoothness

New growth of tops in the variety R4294

Cavity spot biologicals screening

No.	Treatment	Rate per acre	Rate per plot and applications
1	AGN	1 G/A	Every 14 days, and a week after planting
2	LifeGard	4.5 oz/ 100 GPA	At planting, every 14-18 days throughout season
3	Sil Matrix	1 Gallon/ acre	At planting, 4 and 8 weeks after planting
4	Root Shield	3-8 oz/ 100 G Water	At planting and then every 4-6 weeks.
5	Ridomil	0.5-1.3pt/acre	At planting, 30, 60 and 75 DAP
6	Luna Sensation	5oz/ acre	At planting, followed by three in season applications
7	Control		



Cavity spot damage
Cavity spot symptoms (%)
35
30
25
20
15
10
5
0
AGN
LifeGard
Sil Matrix
Root Shield
Ridomil
Luna Sensation
Control

Carrot nematicide screening trial

Challenges

- Availability of resistant cultivars
- Management relied on pre-plant fumigation
- New fumigant regulations by DPR
    - limits the amount used by a grower
    - caps on the amounts allowed in a township
    - expanded buffer zones

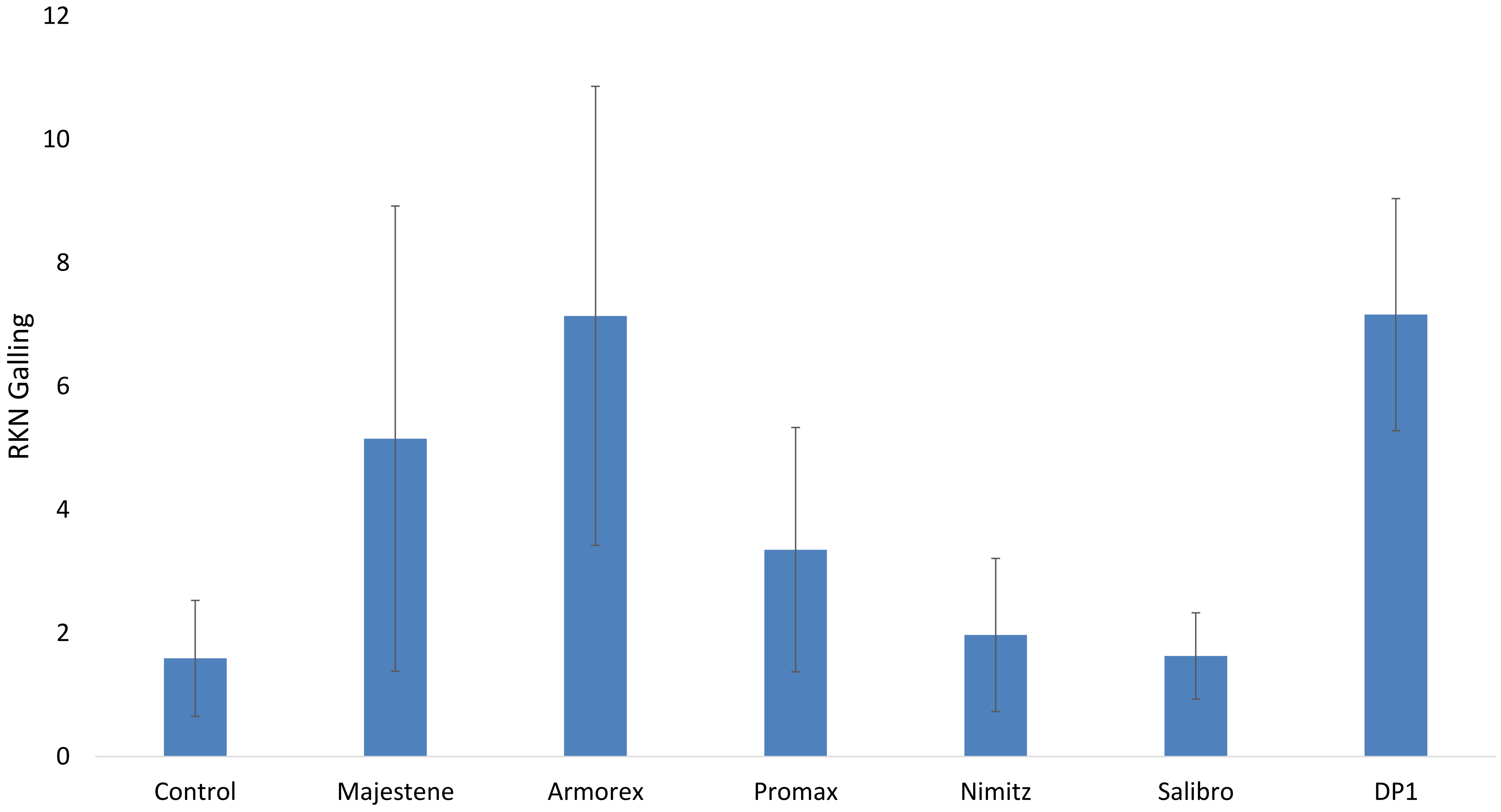
Objective

To identify any biological or conventional nematicides with a potential for use by carrot industry

Treatments

No.	Treatment	Application rates and timings
1	Control	
2	Majestene	6 qt/ A at planting, every 21-28 days interval
3	Armorex	3-5 days Pre plant, 30 days after planting followed by two more applications
4	Promax	1 gal per A. At planting, 15-21 days after 1st application, 30-40 days after 2nd application
5	Nimitz	5pt/A 14 days before planting
6	Salibro	30.7 fl oz/ A, 4 days before planting, 28 days after planting (08/20)
7.	DP1	11.4 fl oz/A At planting

RKN damage on carrot roots



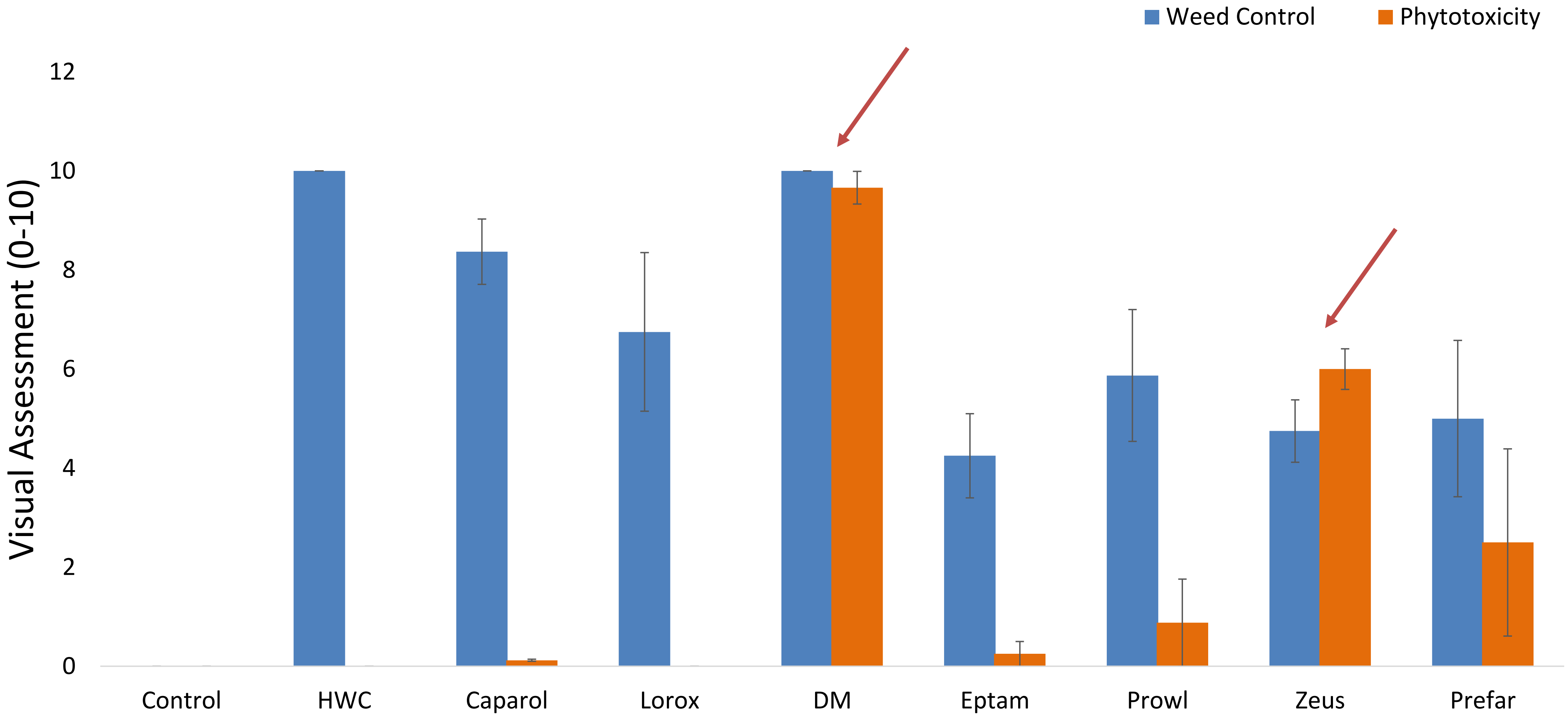
Abundance of plant parasitic nematodes in soil pre-treatment and at harvest

No.	Treatment	Pre-treatment count (J2's /200 ml soil)	At harvest count (J2's /200 ml soil)
1	Control	69.0±17.92 b	70.66±18.80 bc
2	Majestene	83.0 ±27.58 ab	86.0±20.43 ab
3	Armorex	86.0±15.45 ab	134.0±26.76 a
4	Promax	192.0±83.58 a	31.0±5.26 c
5	Nimitz	76.0±3.26 ab	79.0±10.73 bc
6	Salibro	123.0±40.41 ab	107.0±16.36 ab
7.	DP1	91.0±43.21 ab	119.0±19.55 ab
		P value=0.40	P value=0.01

Herbicide screening trial

No.	Treatment	Rate per acre		Application timing
		Preemerge	Post-emerge	
1	UTC			
2	HWC			Every two weeks
3	Caparol fb Tricor	2 pt/ A	1/3 lb /A	Pre fb post 3”all
4	Caparol fb Eptam	2pt/A	5 pt/A	Pre fb post 3”all
5	Lorox fb Lorox	1lb/ A	1.5 lb/ A	Pre fb post 3”all
6	DM fb Tricor	0.67pt/ A	1/3 lb /A	Pre fb post 3”all
7	Eptam fb Caparol	3.5pt/A	4 pt/A	Pre fb post 3”all
8	Prowl fb Eptam	2pts/A	5 pt/A	Pre fb post 3”all
9	Prowl fb Caparol	2 pt/ A	4 pt/A	Pre fb post 3”all
10	Zeus (sulfentrazone) fb Shark (Cafentrazone)	3 fl oz /A	4 fl oz/A	Pre fb post 3”all
11	Prefar fb Lorox	5 qt/ A,	1.5 lb/ A	Pre fb post 3”all

Pre-emergent weed control and phytotoxicity



Control

Hand weeded check

Caparol

Lorox

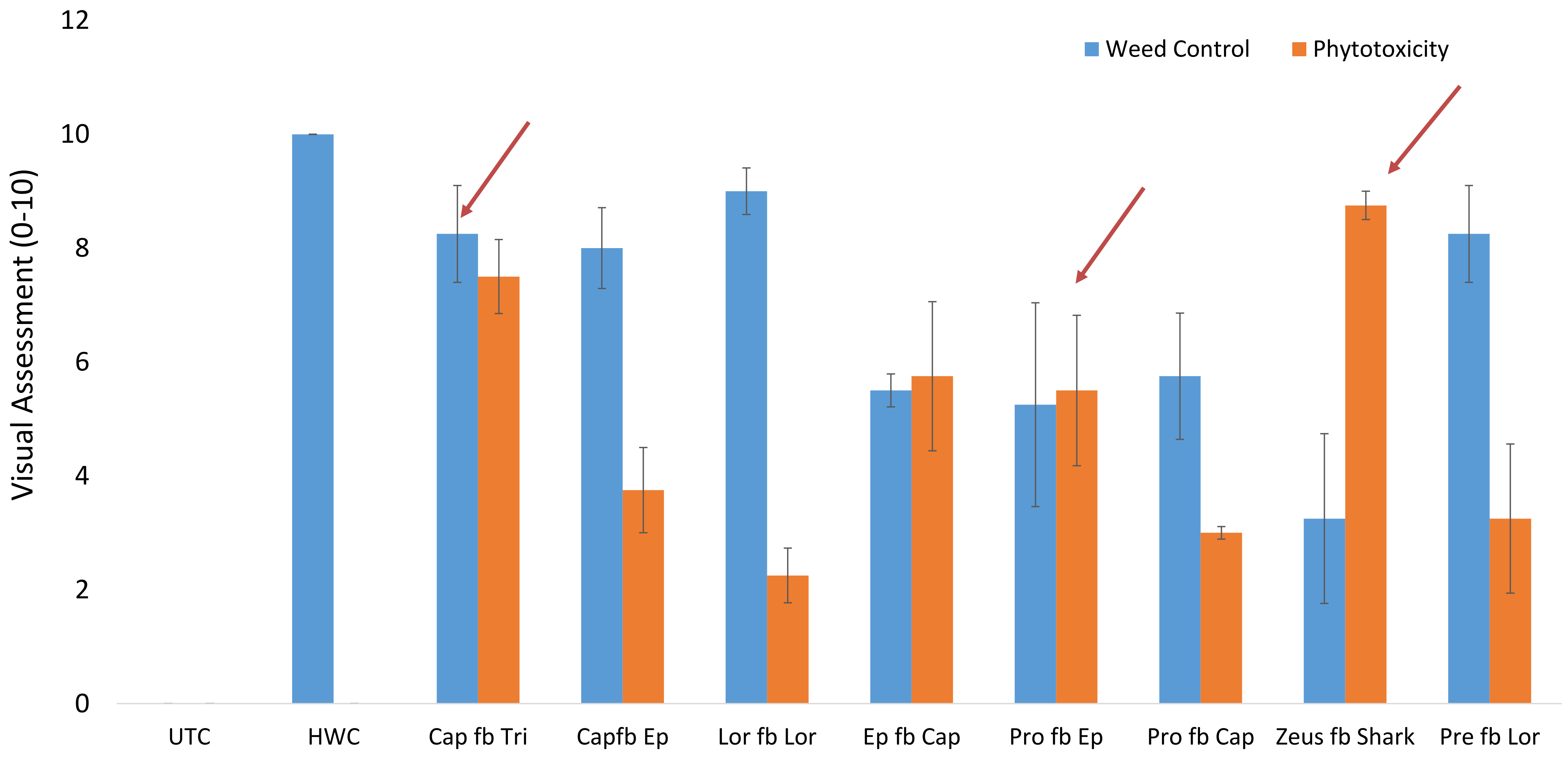
Dual Magnum

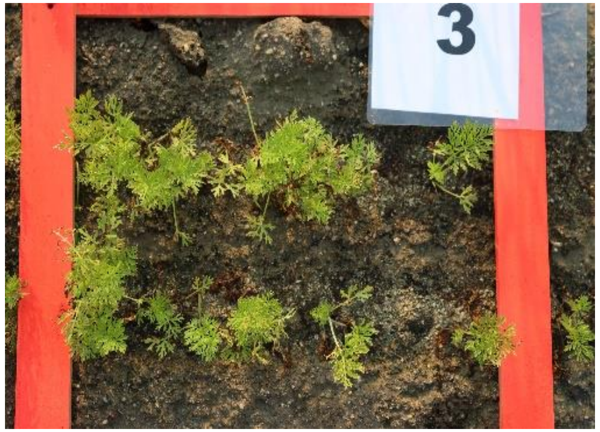
Eptam

Zeus

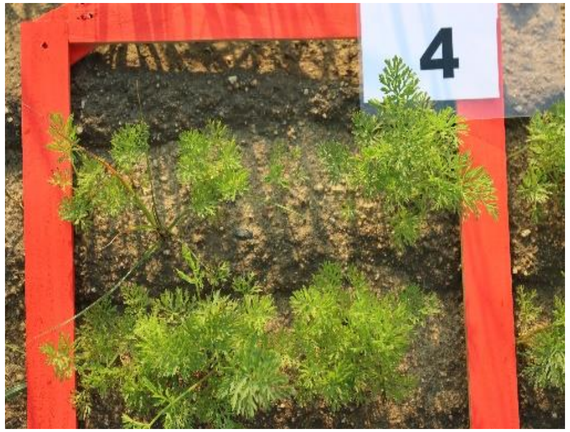
Prefar

Post-emergent weed control and phytotoxicity





Caparol fb Tricor



Caparol fb Eptam

Lorox fb Lorox

Dual Magnum fb Tricor

Eptam fb Caparol

Prowl fb Eptam

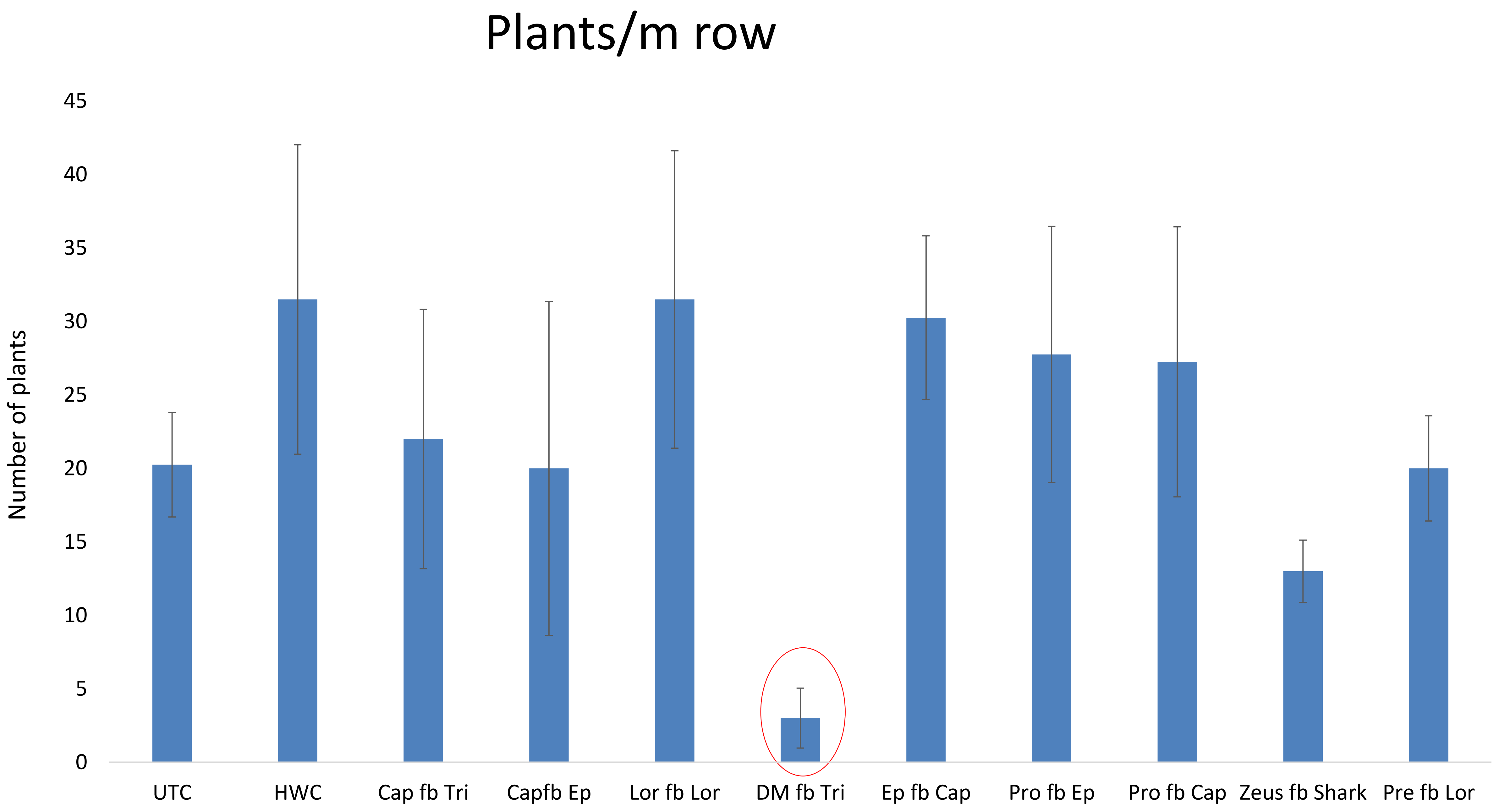
Prowl fb Caparol

Zeus fb Shark

Phytotoxicity symptoms in treatment Tricor following Caparol

Burning on leaf margins in treatment Eptam following Prowl

Leaf margin burning and stunting of plants in treatment Shark following Zeus


Plants/m row
Number of plants
45
40
35
30
25
20
15
10
5
0
UTC
HWC
Cap fb Tri
Capfb Ep
Lor fb Lor
DM fb Tri
Ep fb Cap
Pro fb Ep
Pro fb Cap
Zeus fb Shark
Pre fb Lor

Summary

- Biological LifeGard seems promising for cavity spot
- Some nematicides have potential for use by carrot industry
- Lorox and Caparol have good efficacy as pre-emerge and post emerge treatments

Moving Forward

- Establish new nurseries for Cavity spot and nematodes to get consistent and even disease pressure
- Screen additional post emergent herbicides
- Streamline use of Dual magnum

Acknowledgements

- Joe Nunez
- Amanda Hodson
- Isolde Francis
- Jed Dubose
- Jennifer Fernberg
- Cristal Hernandez

Bolthouse FARMS

CALIFORNIA FRESH CARROT ADVISORY BOARD

GRIMMWAY FARMS

BAYER

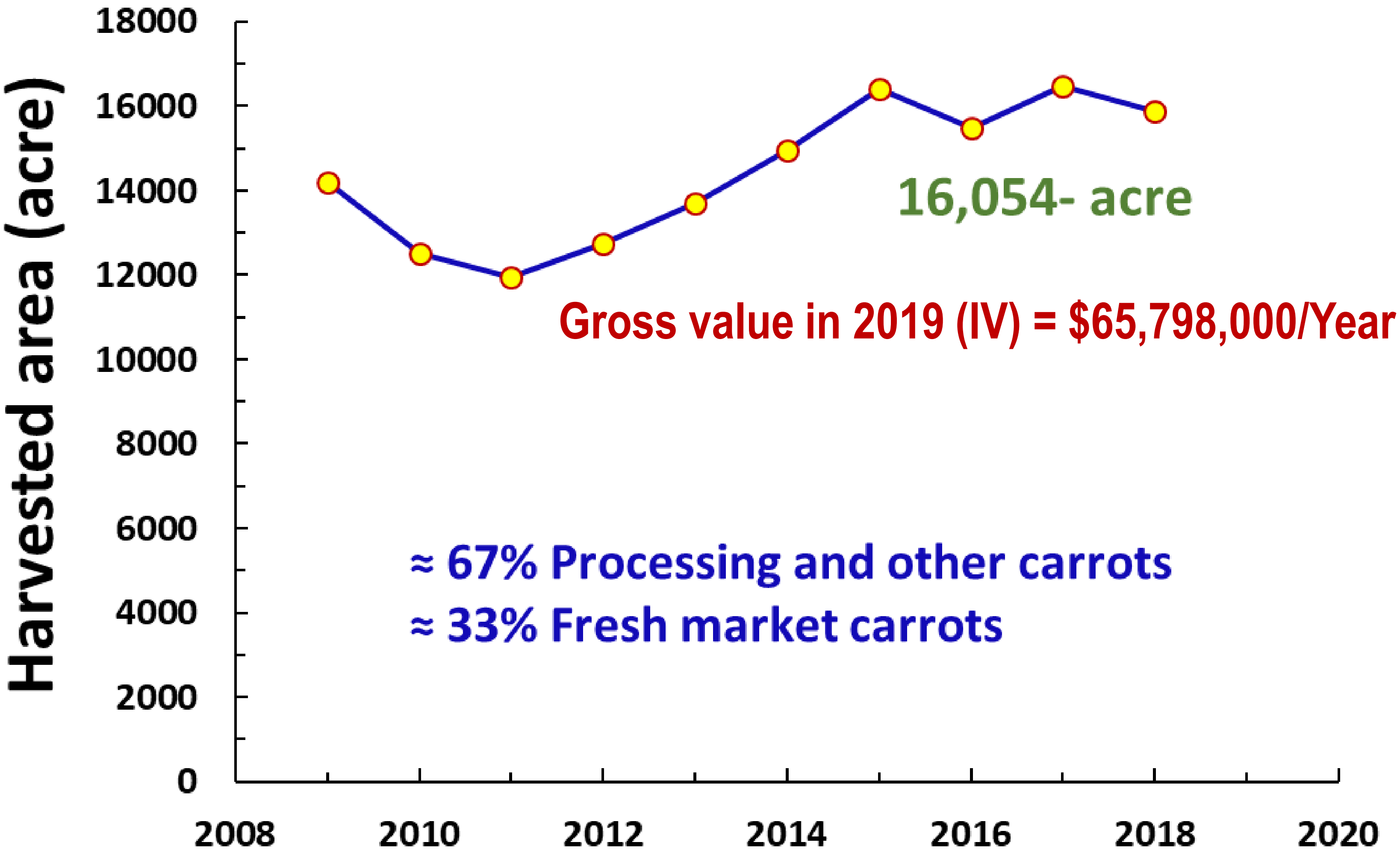
ADAMA

Irrigation and nitrogen best management practices in the low desert carrots

Ali Montazar – UCCE Imperial & Riverside

Annual Carrot Research Symposium (March 22, 2021)

Trends of carrot production acreage in the Imperial Valley (2009-2018)



Experimental Sites (2019-2021)

Site	Carrot Variety	Soil classification (0-2 ft.)	Irrigation practice
UC DREC (two trials)	Fresh market	Sandy clay loam	Sprinkler & Drip
Commercial fields	Fresh market (4 fields) Processing (6 fields)	Sandy clay loam Sandy loam Loamy sand	Sprinkler (4 fields)
			Furrow (6 fields)

Field Experiment Layout

UC DREC Trials

I1N1	I2N3	I2N3	I1N2	I1N1	DN2
I1N2	I2N1	I2N2	I1N3	I1N2	DN2
I1N3	I2N2	I2N1	I1N1	I1N3	DN2
I2N3	I1N1	I1N1	I2N3	I2N2	DN2
I2N1	I1N2	I1N3	I2N2	I2N1	DN2
I2N2	I1N3	I1N2	I2N1	I2N3	DN2

I1: 100% crop ET

I2: 120% crop ET

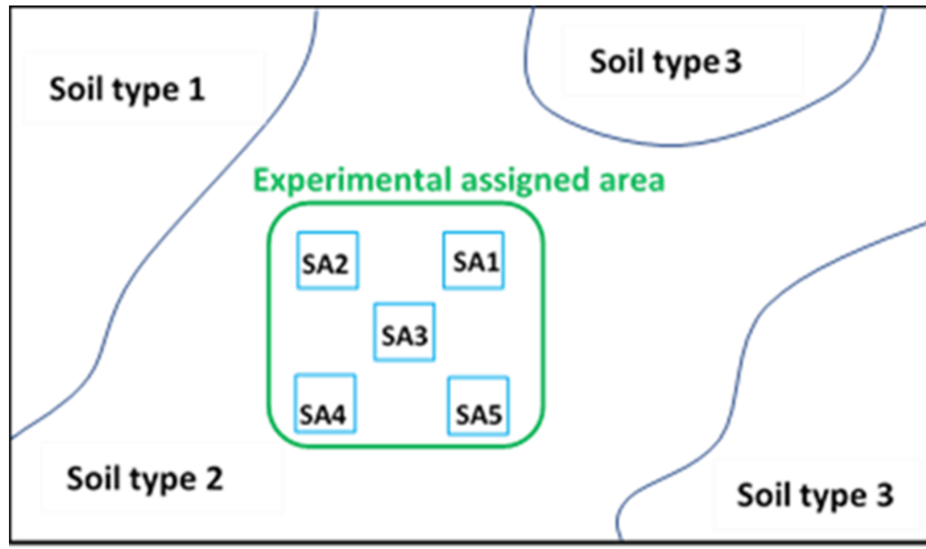
N1: 20% less than N2

N2: Commonly used by local growers

N3: 20% higher than N2

"Split-Plot in RCBD"

Commercial field/s

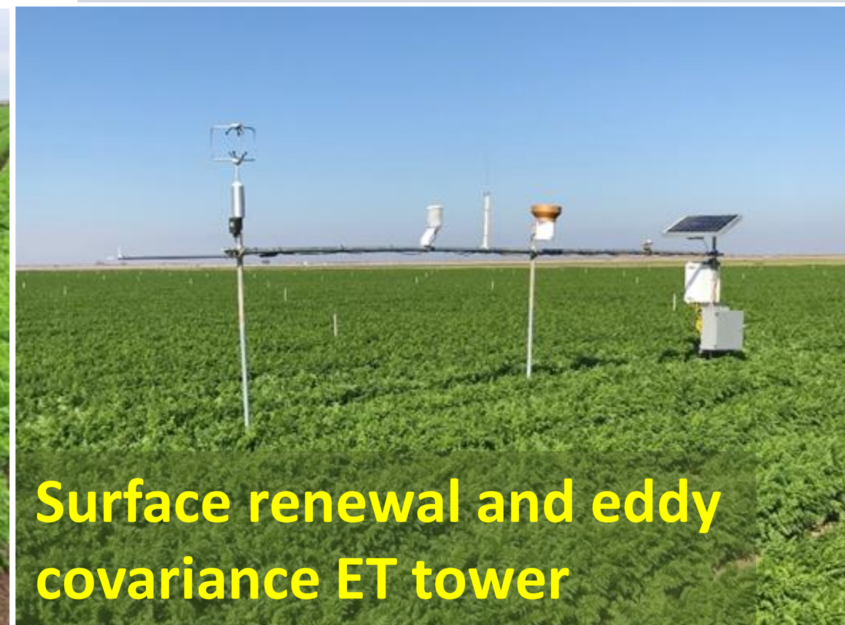


Measurements in five sub-plots (homogeneous soil) at each field under grower practice


I1N1
Research Trial

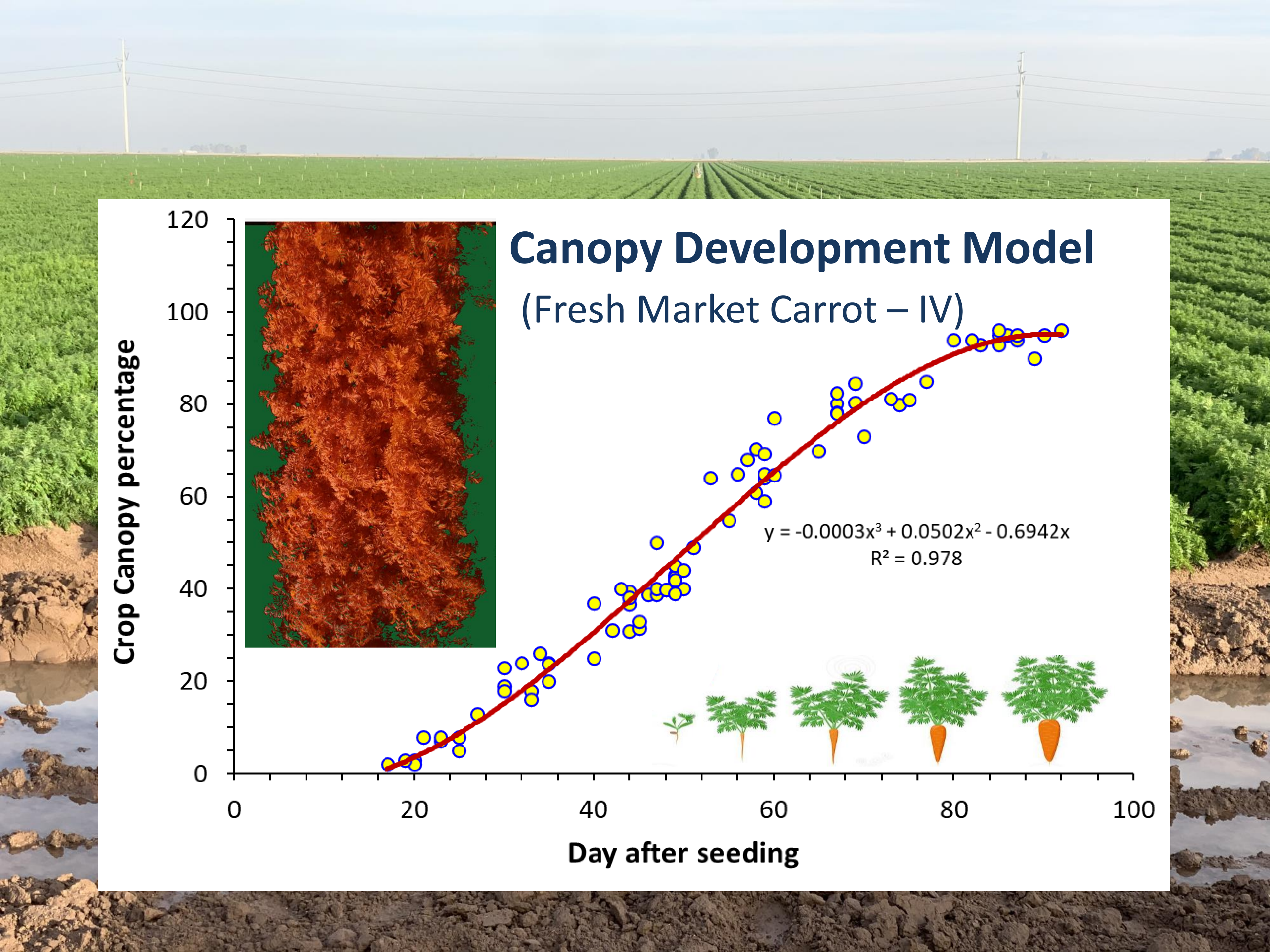

Set-up a monitoring station


Monitoring station


Surface renewal and eddy covariance ET tower

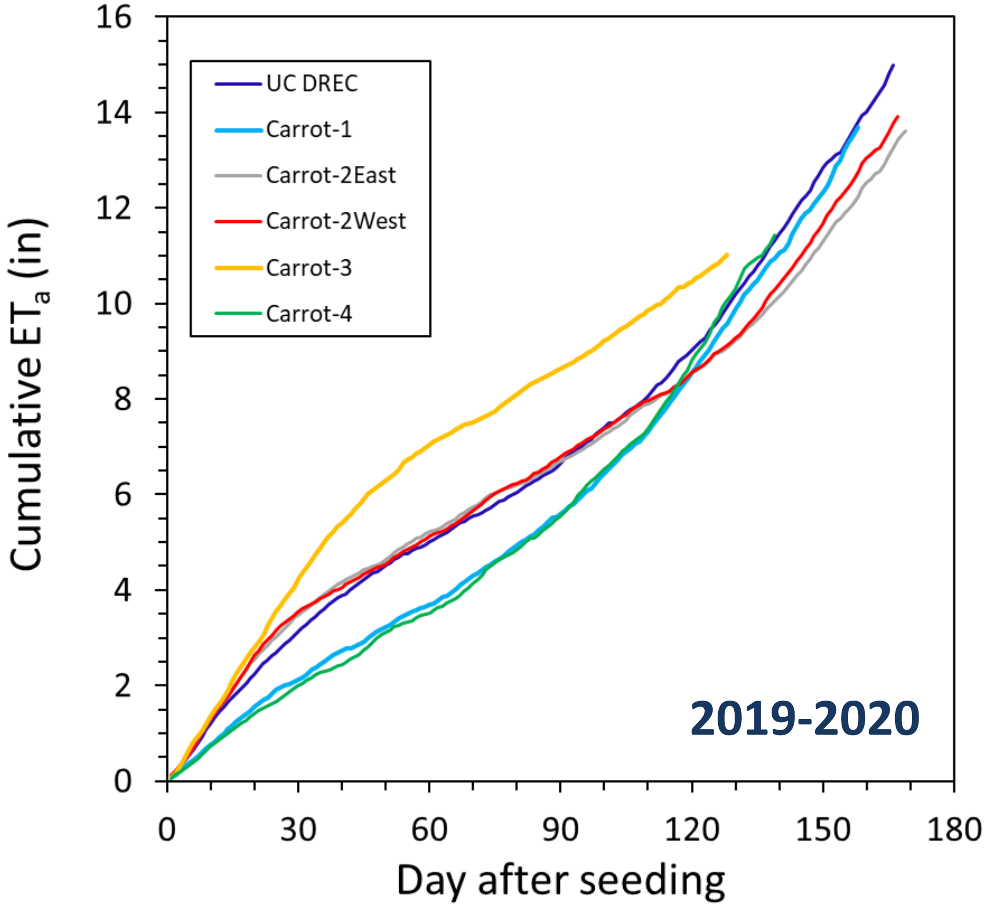

Soil & Plant Data Collection


LAB ONLY



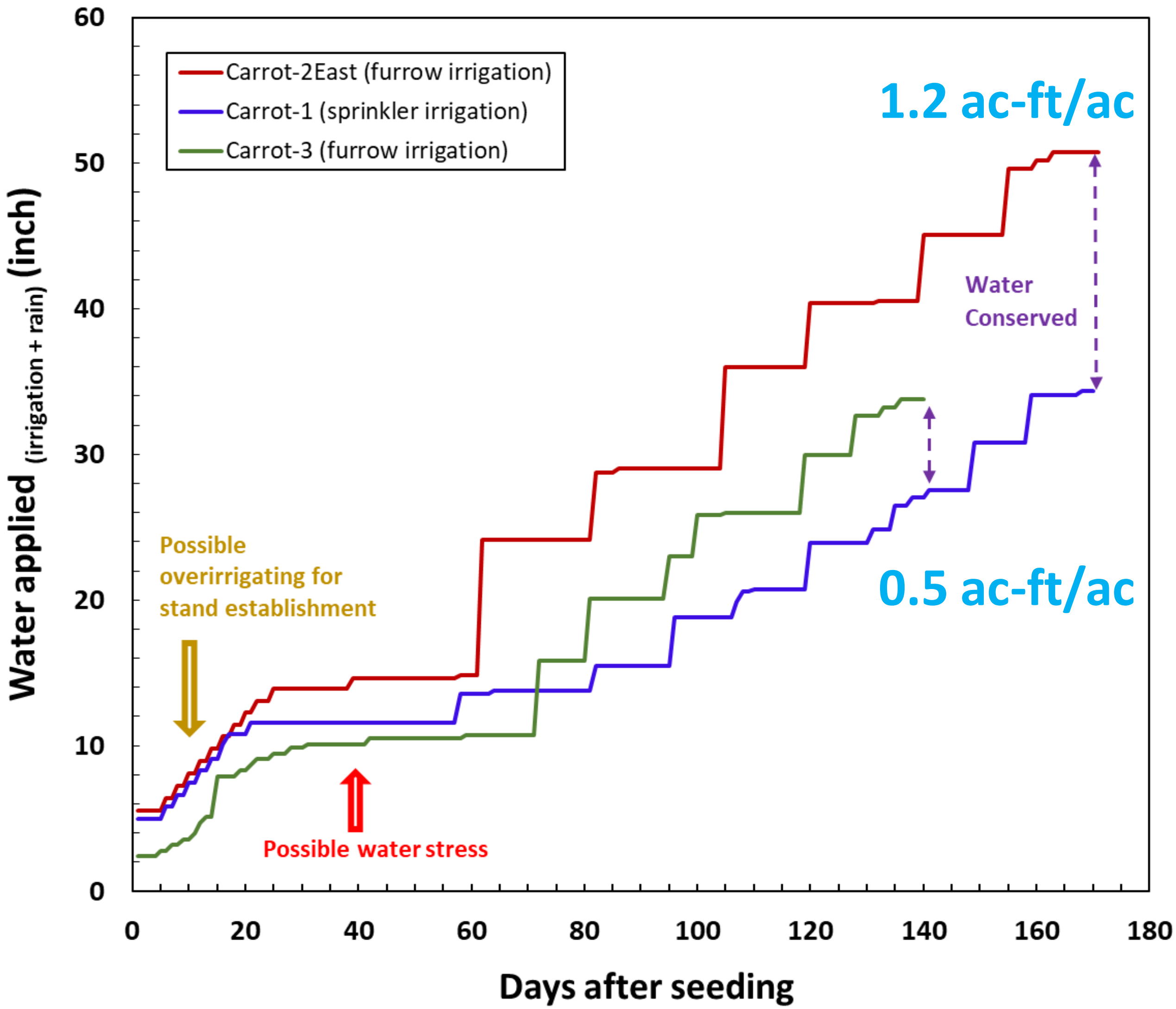
Canopy Development Model
(Fresh Market Carrot – IV)
y = -0.0003x³ + 0.0502x² - 0.6942x
R² = 0.978
Crop Canopy percentage
Day after seeding
0
20
40
60
80
100
120

Carrot Crop Water Use (actual ET)



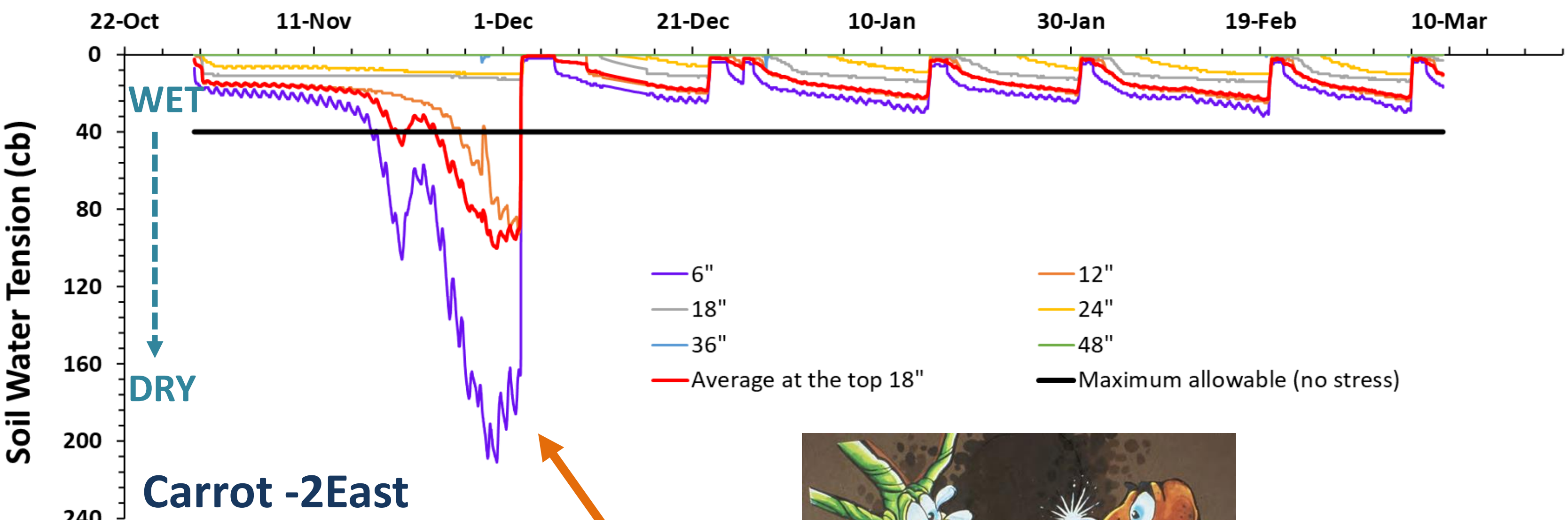
We observed variable crop water use depending upon early/late planting, variety (processing vs. fresh market), irrigation practice, soil type.

Irrigation Management in Carrots (Sprinkler vs. Furrow)



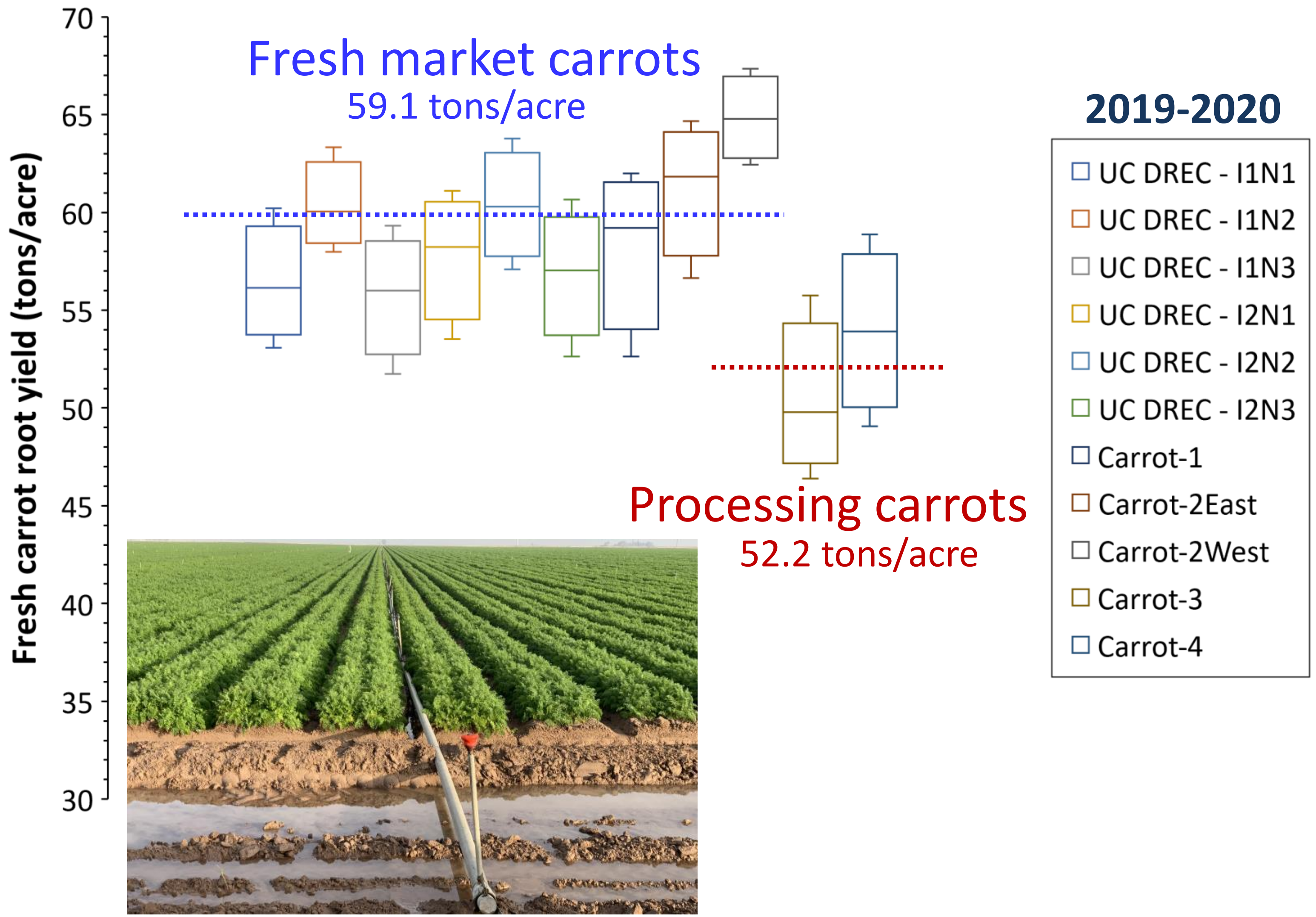
- Potential overirrigating during plant germination
- Potential water stress during cultivation practices
- Potential water conservation through irrigation practices

Soil Water Status
(furrow irrigated field)



Water stress

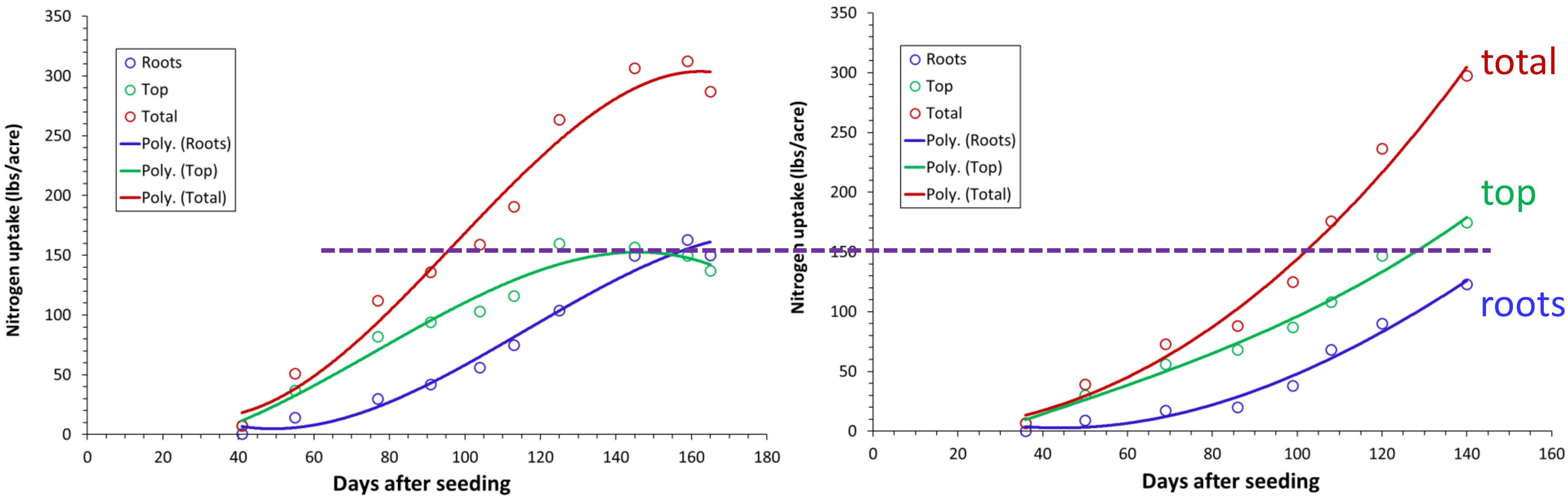
Mean total carrot yields (carrot roots)



Nitrogen Uptake Curve

Fresh market carrots
(sprinkler irrigated field)

Processing carrots
(furrow irrigated field)



286.0 lbs N/ac applied

353.0 lbs N/ac applied

Carrot field after harvest (Field Carrot-4)

Plant residues (Top) could contribute as a source of N for following season.

"45-55% Total N Uptake"

Results from the experiments 2019-2020

Mean N Removal

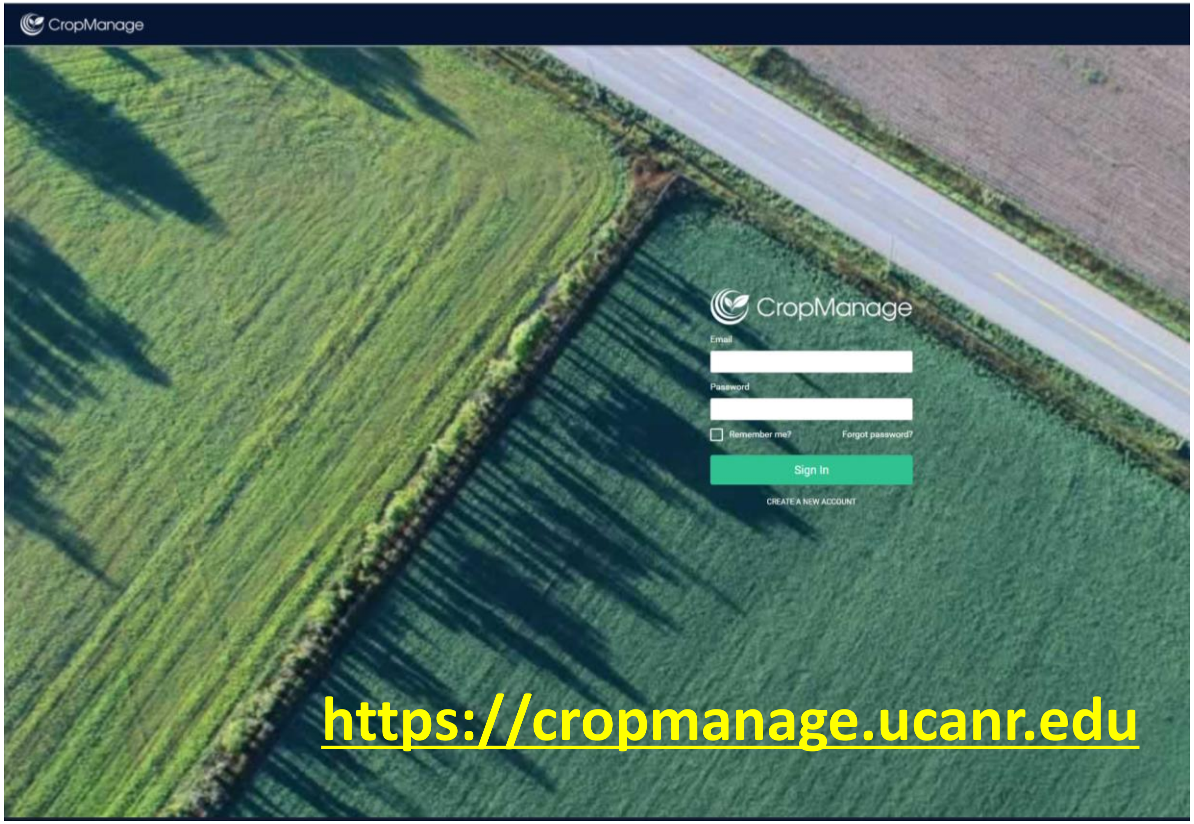
lbs. N/ton fresh weight of carrot	
1	2.6
2-West	2.4
2-East	2.3
3	2.2
4	2.3
I1-N1	2.6
I1-N2	2.5
I1-N3	2.7
I2-N1	2.5
I2-N2	2.7
I2-N3	2.7

Nitrogen Budget Analysis (lbs. N/ac)

Field/ Treatment	N units applied	Crop uptake		Total crop uptake
		Root	Top	
Carrot - 1	287	149	137	286
Carrot - 2West	347	148	136	284
Carrot - 2East	360	150	139	289
Carrot - 3	288	109	178	287
Carrot - 4	353	123	174	297
DREC - I1-N1	285	149	142	291
DREC - I1-N2	320	152	148	300
DREC - I1-N3	374	154	162	316
DREC - I2-N1	285	145	139	284
DREC - I2-N2	320	160	155	315
DREC - I2-N3	374	157	153	310

Processing carrots (Carrot - 3 and Carrot - 4)

- We received an award from CDFA-FREP to extend this project over the next two-year.
- We will develop CropManage carrot module over the next few months.



CropManage is a free online decision tool for irrigation and fertilizer management (administrated by UC ANR).

The results of the first 2-year study will be published soon.

Thank You (Q & A)

Special thanks to

- California Fresh Carrot Advisory Board & CDFA-FREP
- Cooperative Farms
- UC Collaborators: Daniel Geisseler, Michael Cahn, Jaspreet Sidhu, Joe Nunez

Contact information: Ali Montazar

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