Summary of CRDF funded Research Projects: 2009 - present

Swadeshmukul Santra

University of Central Florida

Presented to

Citrus Research and Development Foundation
February 22, 2017

Acknowledgements

CRDF Citrus Research and Development Foundation, Inc.

USDA

NIFA

University of Central Florida

BRANDT®

TRADEMARK NITROGEN

Gowan® The Go To Company

UF UNIVERSITY of FLORIDA

AUBURN UNIVERSITY

MATERIALS INNOVATION FOR SUSTAINABLE AGRICULTURE MISA

OAK RIDGE National Laboratory

Contents

1) Description of the materials
2) Evidence of efficacy
3) Commercial readiness (using ag grade chemicals and the like, regulatory)
4) Patent family reference
5) Commercial Sponsored Research and Licensing status
6) Reference to CRDF support (grant titles, period and amount)

New Bactericide/Fungicide product concepts

Mixed-Valence Copper-Silica Nanogel

Mixed-valence copper-silica nanogel (MV-CuSiNG) is a composite biocide material in which Cu active is embedded within the silica gel matrix. MV-CuSiNG is sprayable non-phytotoxic liquid formulation that uses EPA approves chemicals (active and inerts) and meets all the industry requirements for commercial viability. MV-CuSiNG are shown to be effective against citrus canker, melanoses and scab even at lower metallic Cu rate in comparison to industry standards.

Core-Shell Copper-Silica Nanoparticle

Core-shell copper-silica nanoparticle (MV-CuSiNP) is a composite biocide material in which Cu active is embedded in the shell region of a core-shell silica particle where the core is pure silica. The objective is to further reduce Cu per application. MV-CuSiNP is sprayable non-phytotoxic liquid formulation that uses EPA approves chemicals (active and inerts) and meets all the industry requirements for commercial viability. MV-CuSiNG are shown to be effective against citrus canker, melanoses and scab even at lower metallic Cu rate in comparison to industry standards.

New Bactericide/Fungicide product concepts

Fixed-Quat: A Cu Alternative

Fixed-Quat is a composite biocide material in which Quaternary Ammonium Compound (active) is integrated with silica gel matrix. Fixed-Quat is sprayable non-phytotoxic liquid formulation that uses EPA approves chemicals (active and inerts) and meets all the industry requirements for commercial viability. Fixed-Quat are shown to be effective against citrus canker, melanoses and scab in comparison to industry standards.

Copper-Fixed Quat Composite

Copper-Fixed-Quat (Cu-Fixed-Quat) is a composite biocide material in which Cu and Quat actives are blended with silica gel at the molecular level. The objective is to further reduce metallic Cu per application. Cu-Fixed-Quat is sprayable non-phytotoxic liquid formulation that uses EPA approves chemicals (active and inerts) and meets all the industry requirements for commercial viability. Cu-Fixed-Quat are shown to be effective against citrus canker, melanoses and scab even at lower metallic Cu rate in comparison to industry standards.

New Bactericide/Fungicide product concepts

T-SOL

T-SOL is a Zn-chelate. It is sprayable non-phytotoxic liquid formulation that uses EPA approves chemicals and meets all the industry requirements for commercial viability. T-SOL is shown to be effective against citrus canker, melanoses and scab in comparison to industry standards.

Zinkicide™

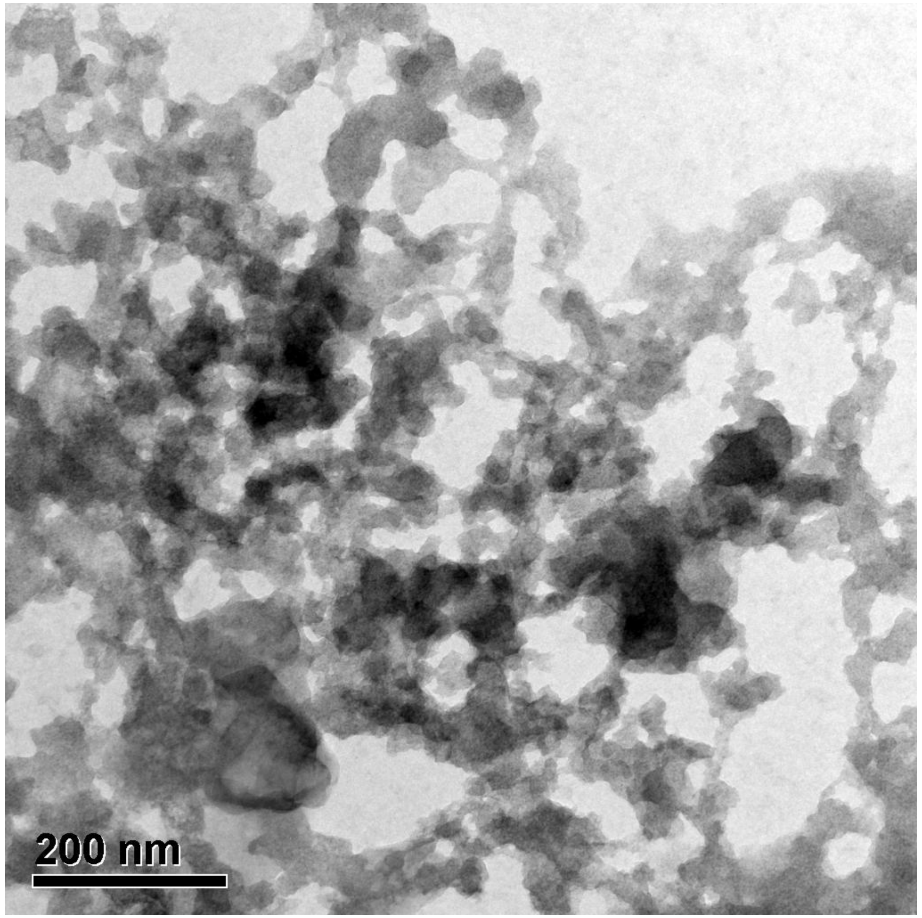
ZinkicideTM is a ZnO nanoparticles based biocide material. It is sprayable non-phytotoxic liquid formulation that uses EPA approves chemicals (active and inerts) and meets all the industry requirements for commercial viability. Zinkicide is shown to be effective against citrus canker, melanoses and scab even at lower metallic Cu rate in comparison to industry standards.

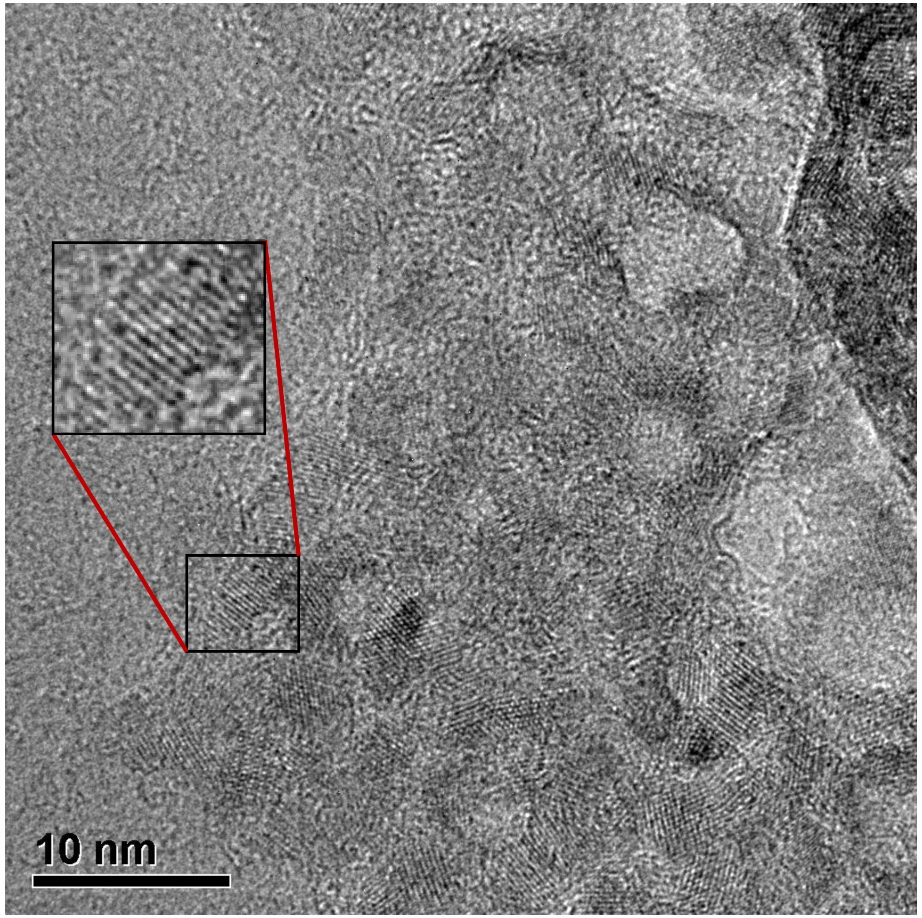
Zinkicide™ : Transition from reagent grade to agri-grade

Zinkicide™ SG-6 | **TMN 110**

Zinkicide™ SG-4 | **TMN 113**

HRTEM of SG6 (2015 version)

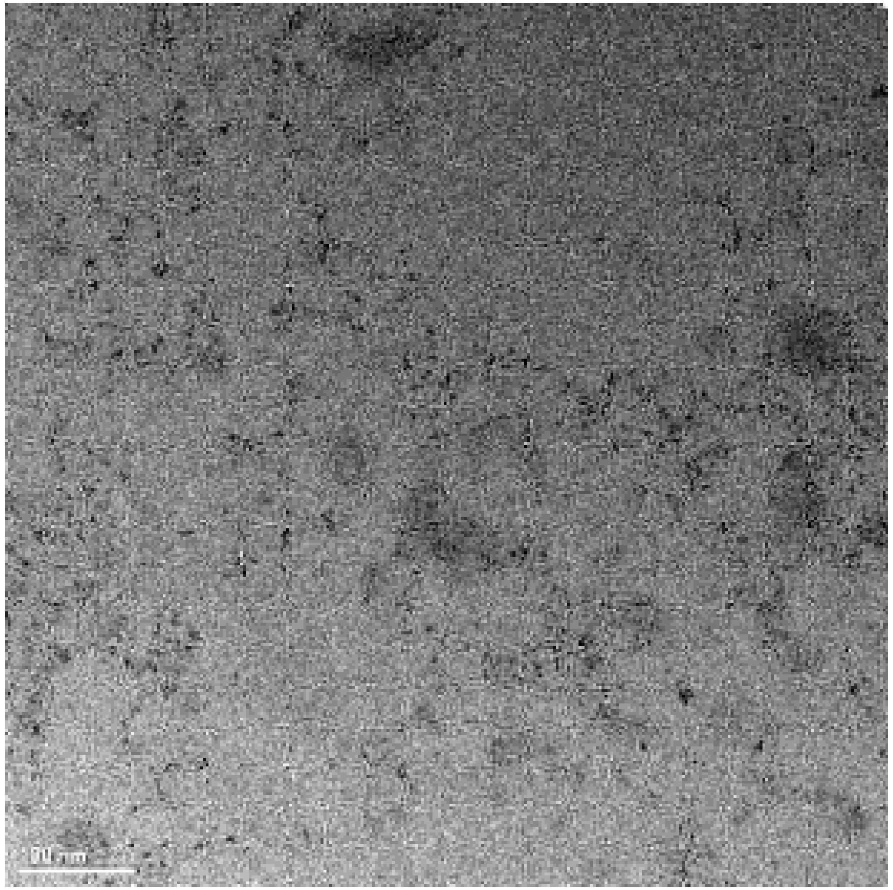




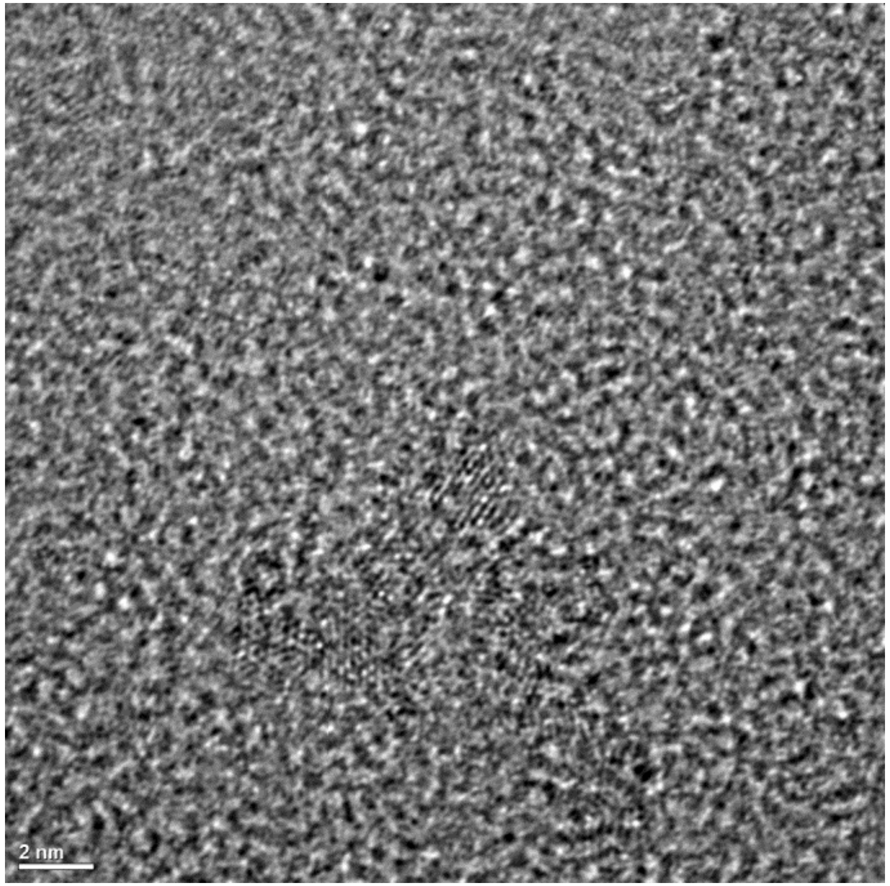
Gel-like morphology showing aggregates of particles

Aggregates of <10 nm size crystalline particles

HRTEM of TMN 110 (2016-2017 version)



Fairly dispersed particles



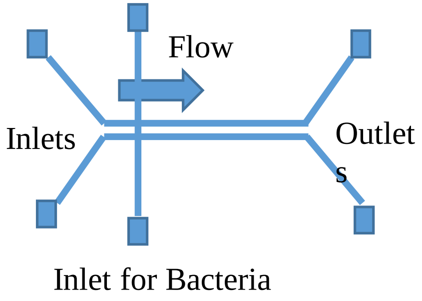
~2 nm size crystalline particles

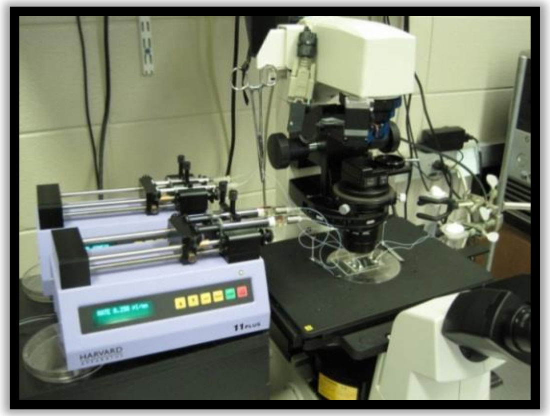
Minimal Inhibitory Concentration (MIC)

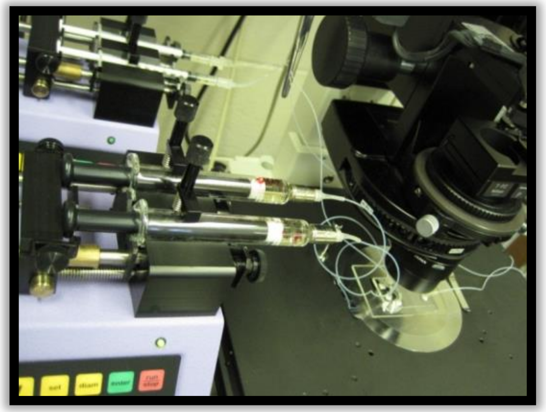
Conducted using a broth microdilution protocol

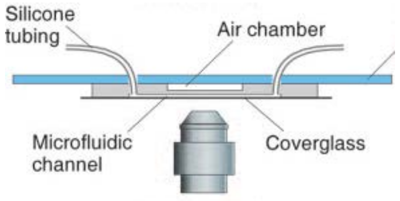
Materials	*E. coli* *(ATCC 10536)* (μg/mL)	*X. alfalfae* *(ATCC 49120)* (μg/mL)	*P. syringae* *(ATCC 19310)* *(μg/mL)*
Batch 110 – As synthesized	16-31	31-62	31-62
Batch 113- As synthesized	250	31-62	31-62
Batch 110 - Dialyzed and lyophilized	125	31-62	31-62
Batch 113 - Dialyzed and lyophilized	125-250	31-62	31-62
Batch 110 - Rotary evaporated	16-31	31-62	31-62
Batch 113 - Rotary evaporated	250	31-62	31-62
Kocide 3000	250-500	250-500	250
Nordox 30/30	125-250 (Cu), 125-250 (Zn)	125 (Cu), 125 (Zn)	125 (Cu), 125 (Zn)

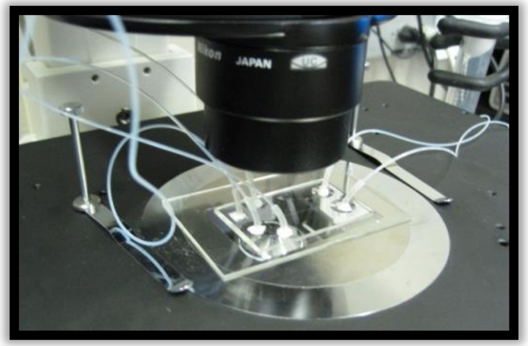
Microfluidic chambers: ‘artificial’ vascular system: Zinkicide efficacy study







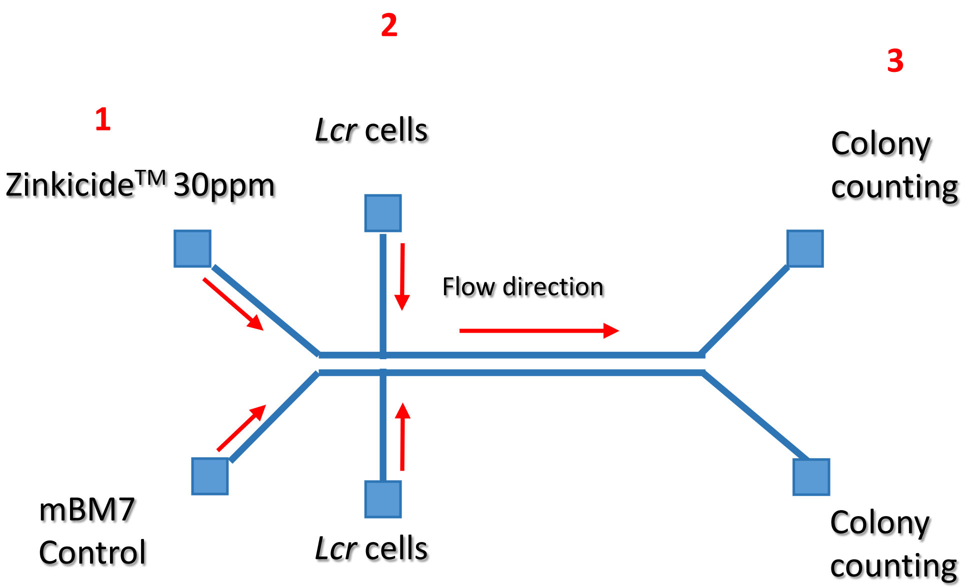




Credit: Leonardo De La Fuente, Auburn University

Evaluation of the preventive antimicrobial activity of Zinkicide™ against *L. crescens* in microfluidic chambers

Materials and Methods



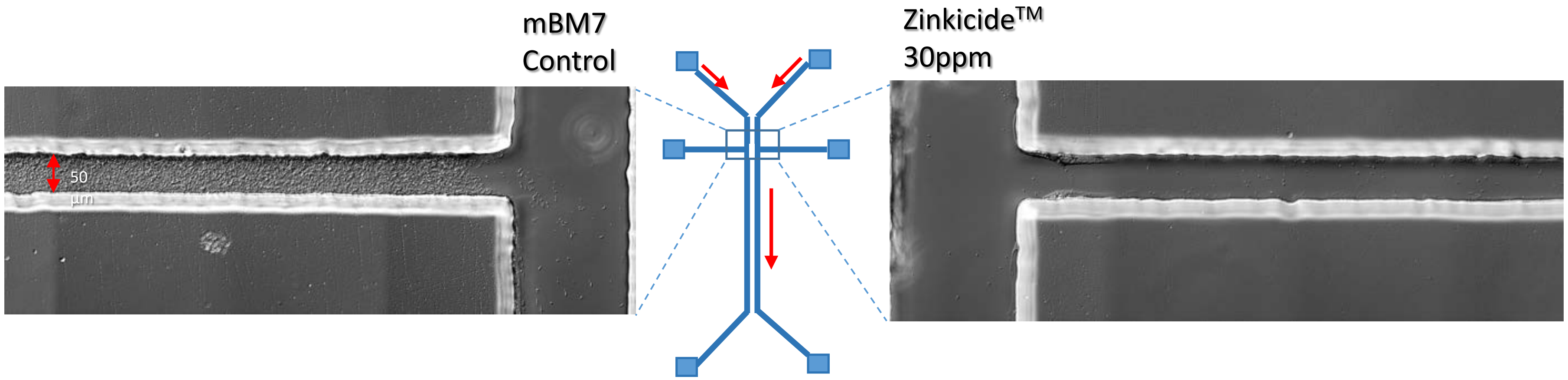
24 hrs post innoculation, the waste of the collecting syringe was aseptically removed to eliminate residual inoculated cells.

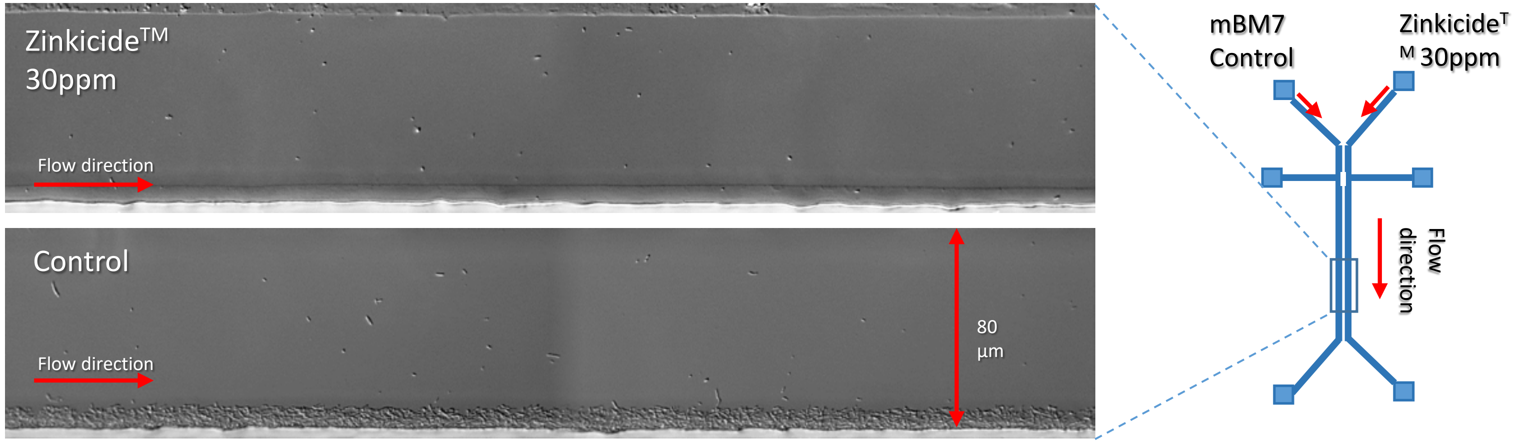
Time lapse videos were captured in order to detect cell division.

After seven days of treatment, the effluent of the microfluidic chamber was collected, $OD_{600\ nm}$ measures were performed, and 100µl of each effluent was streaked in mBM7 agar plates by triplicate.

Credit: Leonardo De La Fuente, Auburn University

Evaluation of the preventive antimicrobial activity of Zinkicide™ against L. crescens in microfluidic chambers 7 days post innoculation





Credit: Leonardo De La Fuente, Auburn University

Microfluidic chamber: *X. citri* pv. *citri* + TMN Zinkicide™

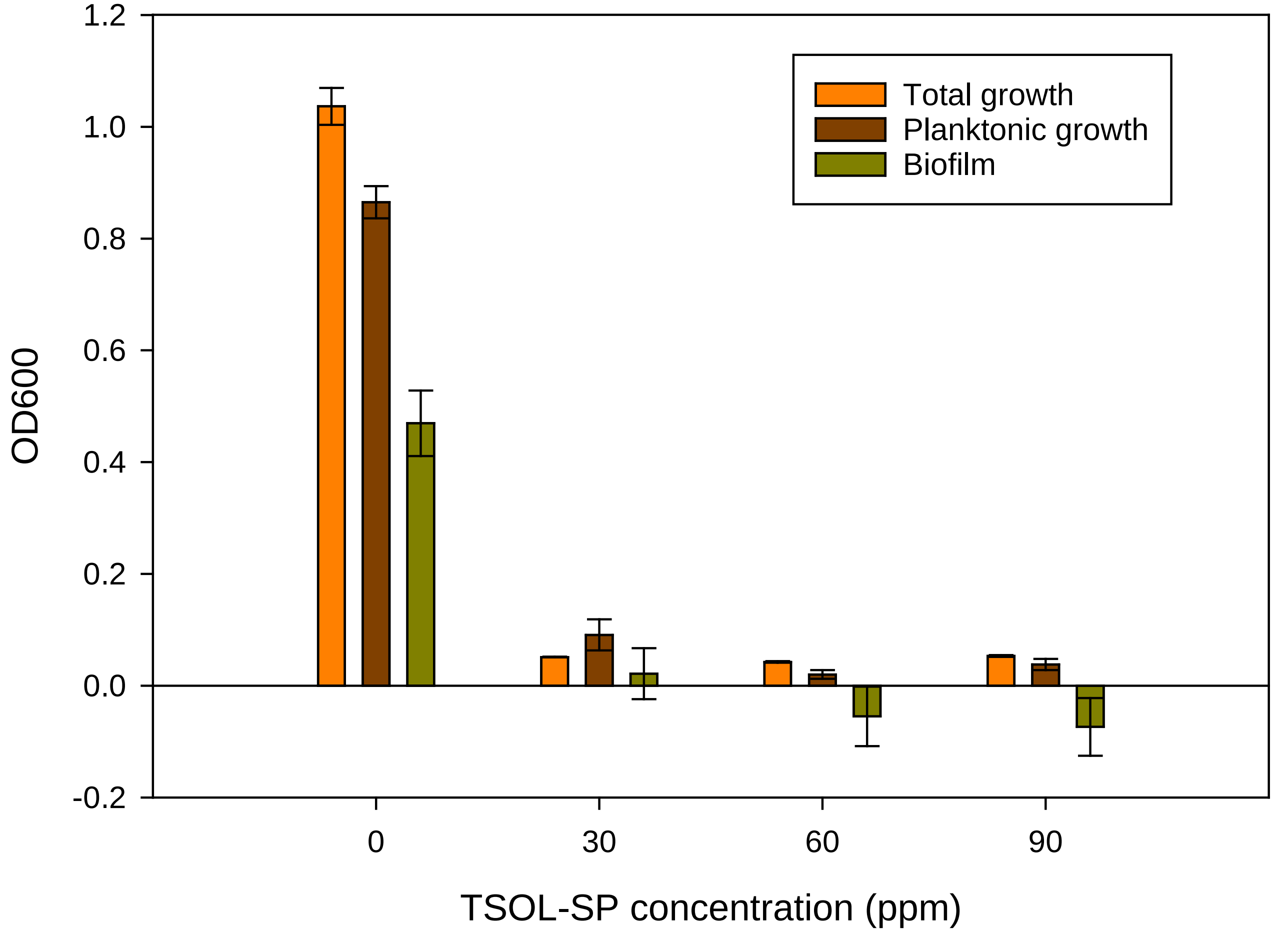
TMN
Zinkicide

SB Medium

20 µm

Credit: Leonardo De La Fuente, Auburn University

TSOL-SP (*X. citri*)



N=6, Error bars show SE, this experiment was repeated twice

Credit: Leonardo De La Fuente, Auburn University

X. citri
Control media before chemical treatment

Credit: Leonardo De La Fuente, Auburn University

X. citri

UP: Chemical treatment

TSOL-SP

DOWN: Control media

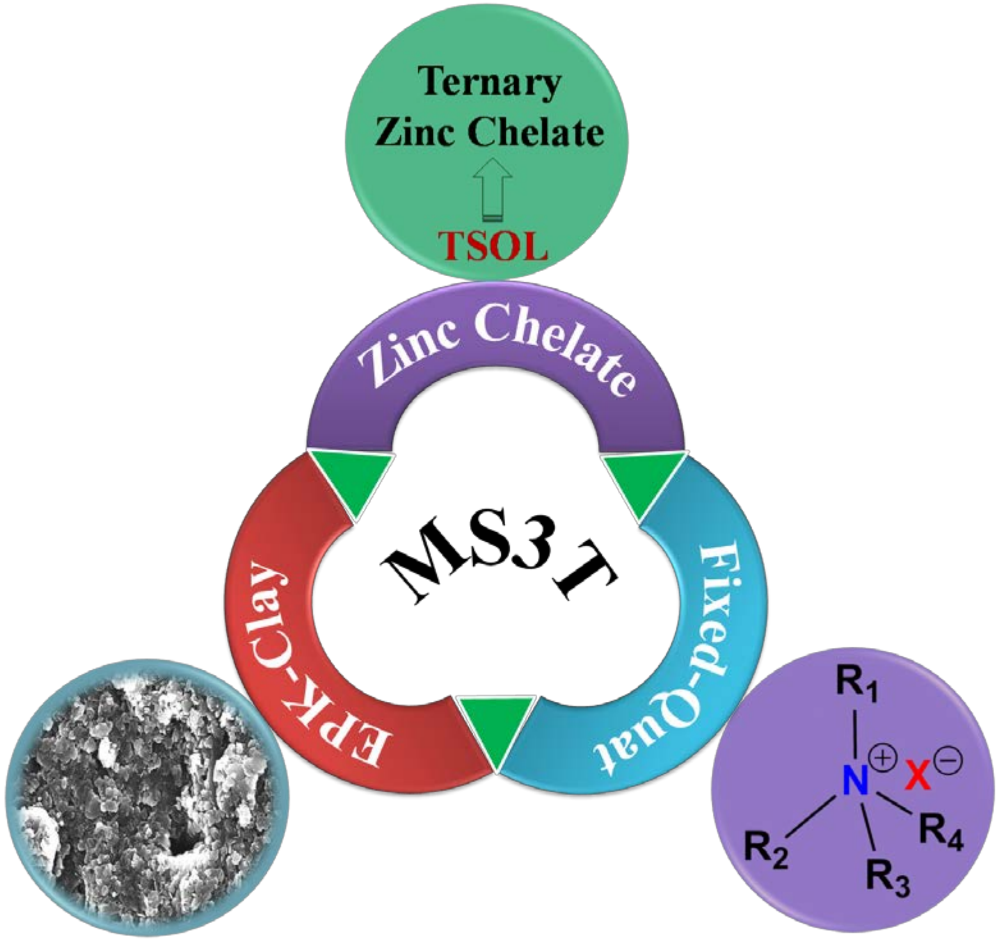
Credit: Leonardo De La Fuente, Auburn University

Phytotoxicity conducted on *Sour Orange*
Plants were treated and results recorded after 72 hrs

Treatment (ppm)		Result
TMN 110	300	-
	500	-
	800	-
TMN 111	300	-
	500	-
	800	-
TMN 112	300	-
	500	-
	800	-
TMN 113	300	-
	500	-
	800	-
Result scaling : (-) No observed phytotoxicity, (+) low phytotoxicity, (++) moderate phytotoxicity		

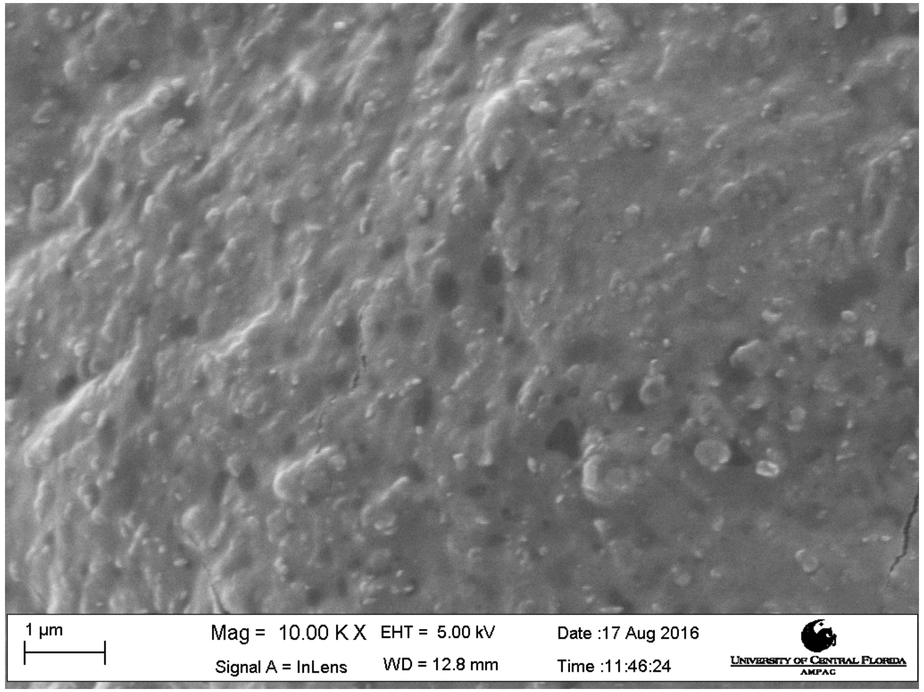
MS3T formulation concept

- MS3T is a composite material which contains two active ingredients (A.I.), a Zn-chelate (T-SOL) and Quaternary ammonium compound (Fixed-Quat)
- A.I. are stabilized and delivered using natural clay (EPK clay; inactive ingredient)
- Clay also serves as ACP repellent
- T-SOL is micronutrient based, designed for systemic activity.
- Fixed-Quat is non-phytotoxic and designed to control surface/sub-surface restricted bacterial and fungal diseases

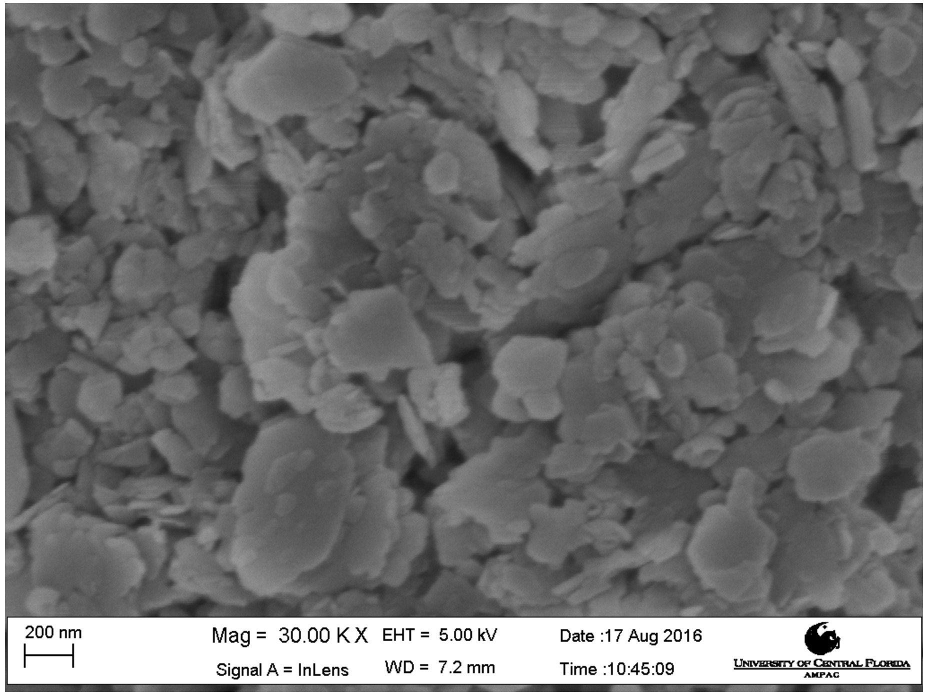


SEM of MS3T and EPK-clay

MS3T



EPK-clay



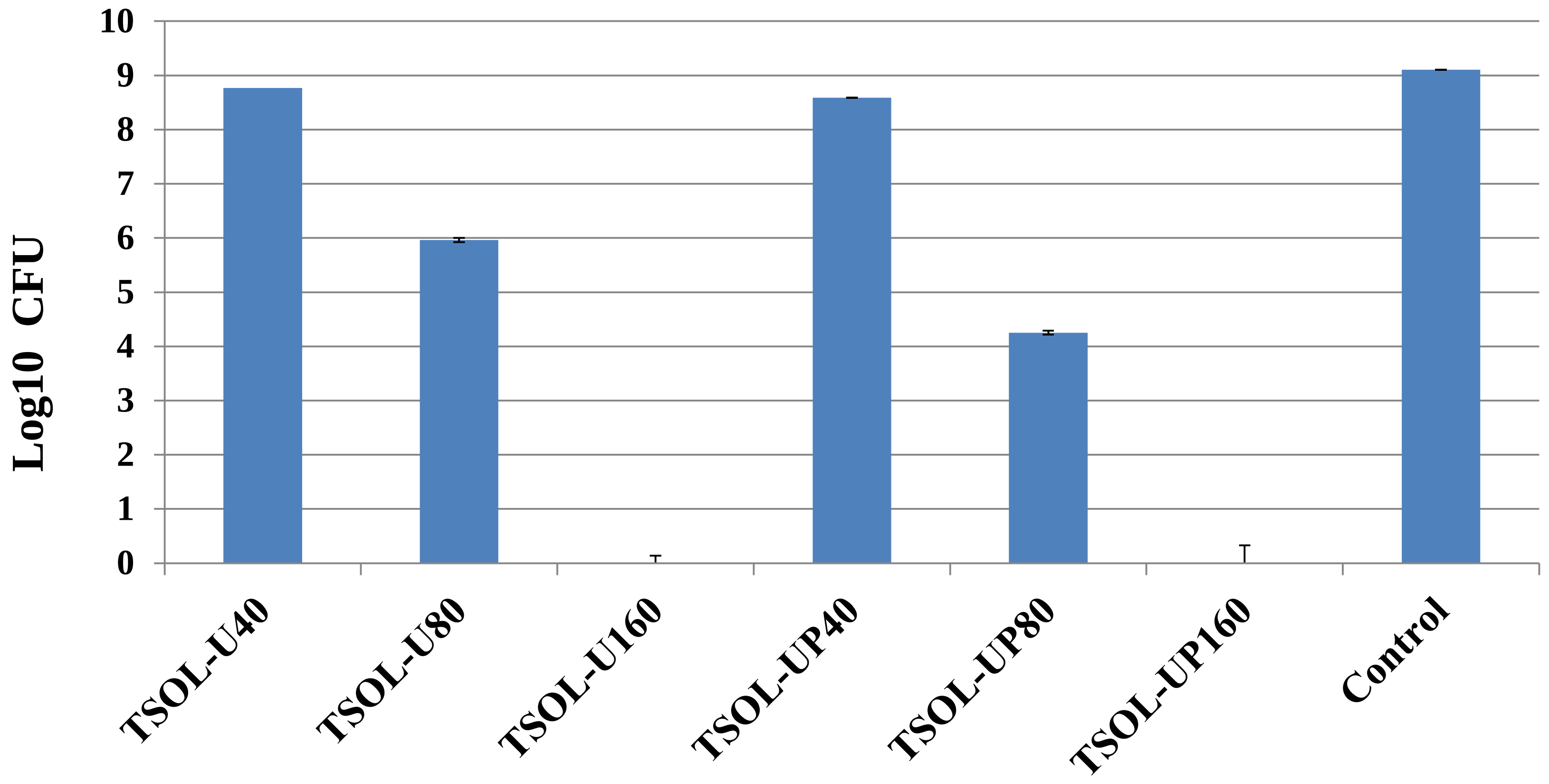
- MS3T is film-forming
- Fixed-Quat and T-SOL contribute to colloidal stability of MS3T formulation

MS3T Minimum Inhibitory Concentration (MIC)

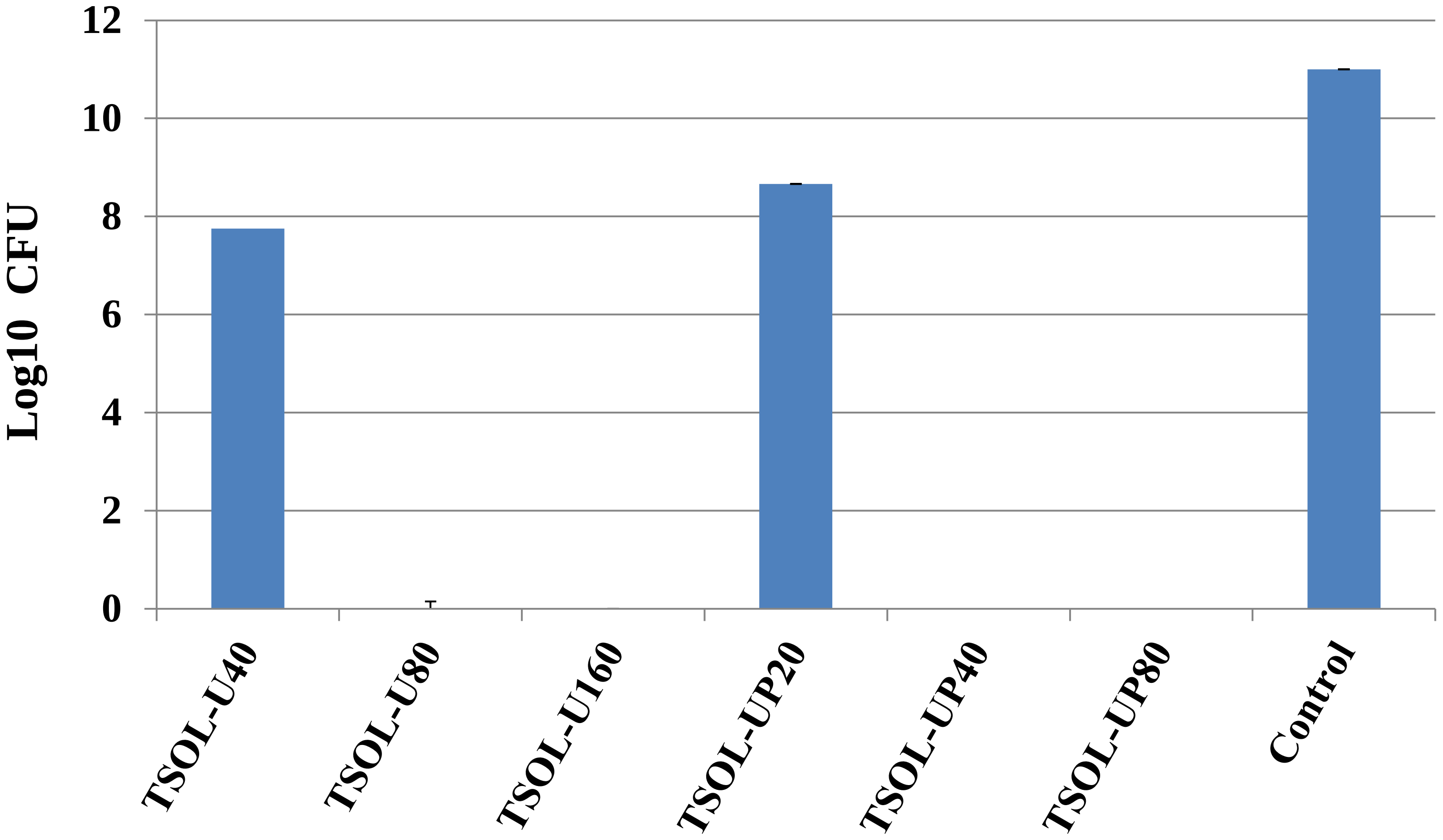
Material	Month 1			Month 2			Month 3		
	X. alfalfae (ppm)	*E. coli (ppm)*	*P. syringae (ppm)*	*X. alfalfae (ppm)*	*E. coli (ppm)*	*P. syringae (ppm)*	*X. alfalfae (ppm)*	*E. coli (ppm)*	*P. syringae (ppm)*
MS3T	**< 20 (zinc) < 5 (DDAC)**	**< 20 (zinc) < 5 (DDAC)**	**< 20 (zinc) < 5 (DDAC)**	**< 20 (zinc) < 5 (DDAC)**	**< 20 (zinc) < 5 (DDAC)**	**< 20 (zinc) < 5 (DDAC)**	**< 20 (zinc) < 5 (DDAC)**	**< 20 (zinc) < 5 (DDAC)**	**< 20 (zinc) < 5 (DDAC)**
Clay+T-SOL	**80 (zinc)**	**170 (zinc)**	**-**	**170 (zinc)**	**340 (zinc)**	**80 (zinc)**	**80 (zinc)**	**170 (zinc)**	**80 (zinc)**
Clay+DDAC	**5 (DDAC)**	**5 (DDAC)**	**-**	**5 (DDAC)**	**10 (DDAC)**	**10 (DDAC)**	**5 (DDAC)**	**10 (DDAC)**	**10 (DDAC)**
TSOL-U	**80 (zinc)**	**80 (zinc)**	**170 (zinc)**	**40 (zinc)**	**170 (zinc)**	**80 (zinc)**	**80 (zinc)**	**340 (zinc)**	**80 (zinc)**
TSOL-UP	**40 (zinc)**	**80 (zinc)**	**170 (zinc)**	**40 (zinc)**	**170 (zinc)**	**80 (zinc)**	**40 (zinc)**	**340 (zinc)**	**80 (zinc)**

- Antimicrobial efficacy of A.I. in MS3T is not compromised in presence of clay.
- Improved efficacy of TSOL is observed in MS3T
- MS3T antimicrobial efficacy is maintained for at least 3 months.
- Formulation contains all agri-grade chemicals except DDAC.

Colony Forming Unit (CFU) or Viability assay, E. coli



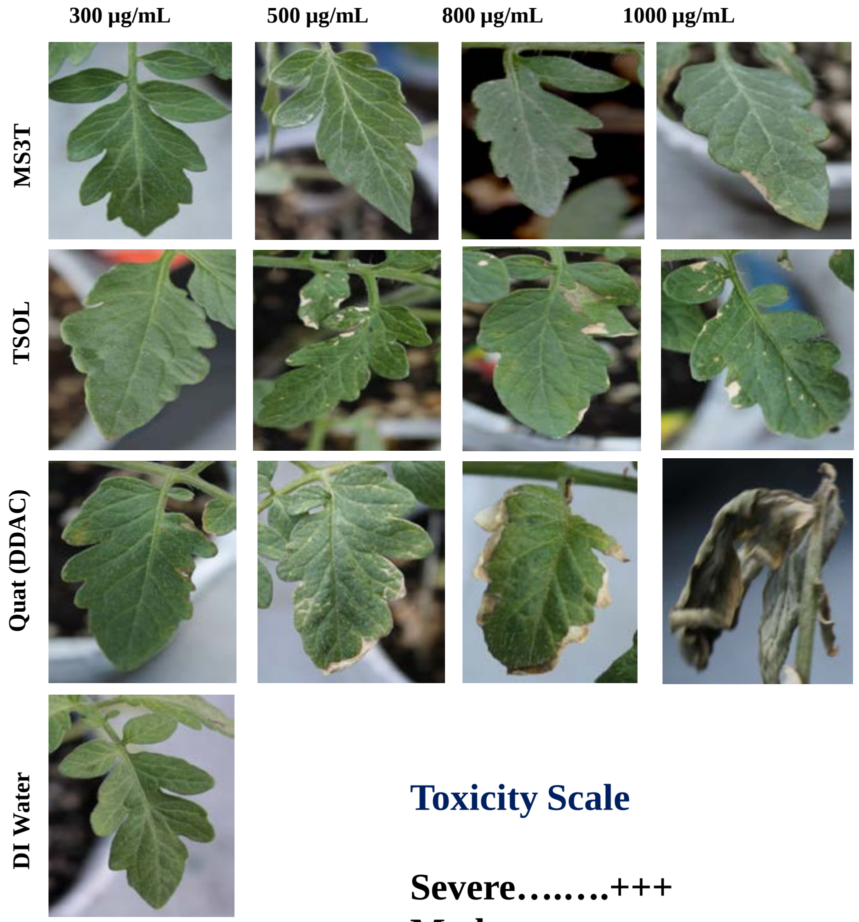
Colony Forming Unit (CFU) or Viability assay, X. alfalfae



Phytotoxicity of MS3T- After 72 hrs

Sample Conc. (ppm)	Samples		
	MS3T	TSOL	DDAC
Control (DI water)	-	-	-
300	-	-	+
500	-	+	++
800	-	+	++
1000	+	++	+++

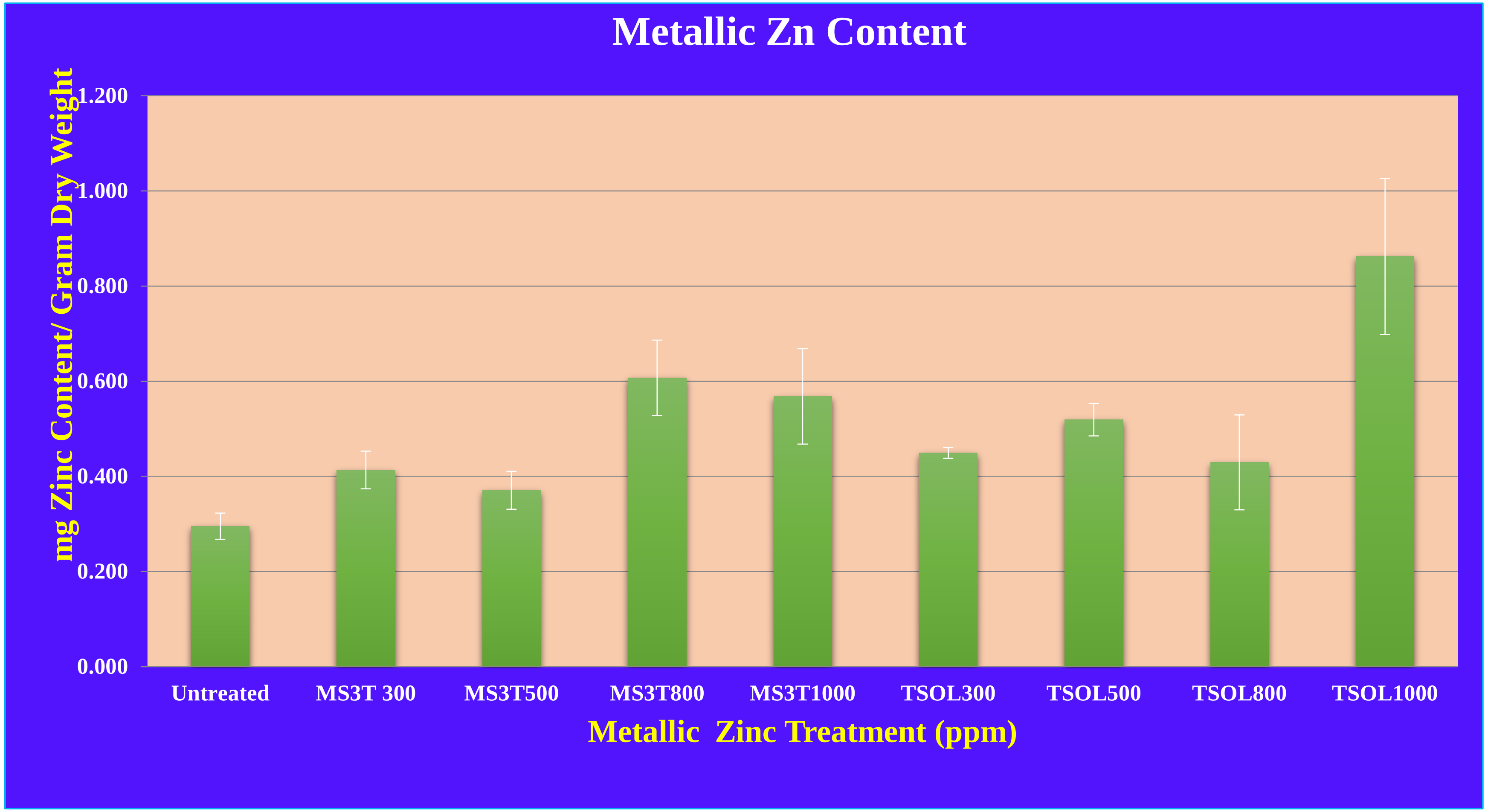
- 25 days-old Florida 91, a heat-tolerant hybrid Heirloom Tomato (Solanum lycopersicum), a model plant system.
- Treated with 300, 500, 800 and 1000 ppm of MS3T, TSOL and DDAC (metallic zinc or DDAC concentration).
- Toxicity was observed for all the concentrations of DDAC, slight toxicity was observed with leaf burn in edges at 500µg/mL for TSOL while no toxicity was observed at 300µg/mL. Slight Toxicity was observed for MS3T at 1000 ppm.
- **MS3T appear to have reduced toxicity**



Toxicity Scale

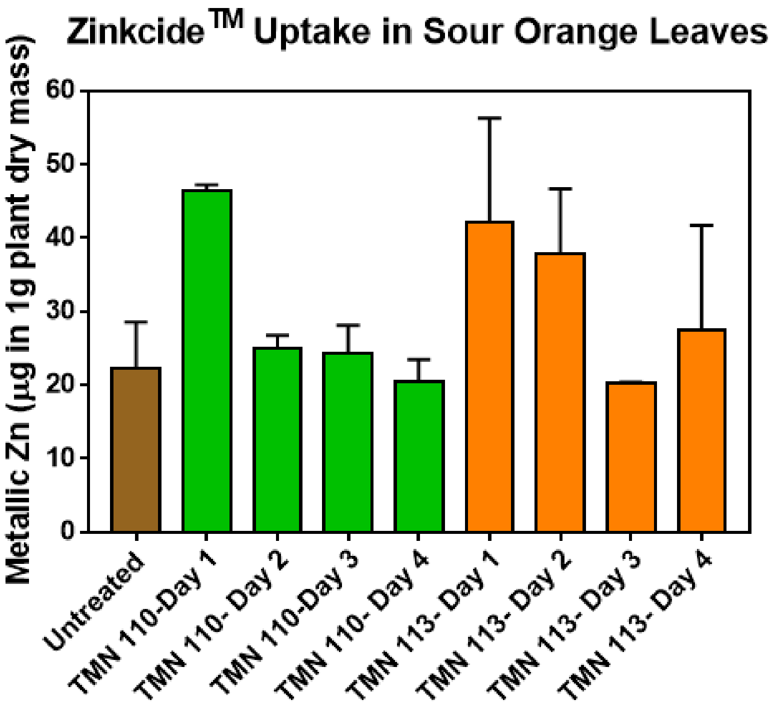
Severe……..+++
Moderate…...++
Less……….…+
No toxicity….. –

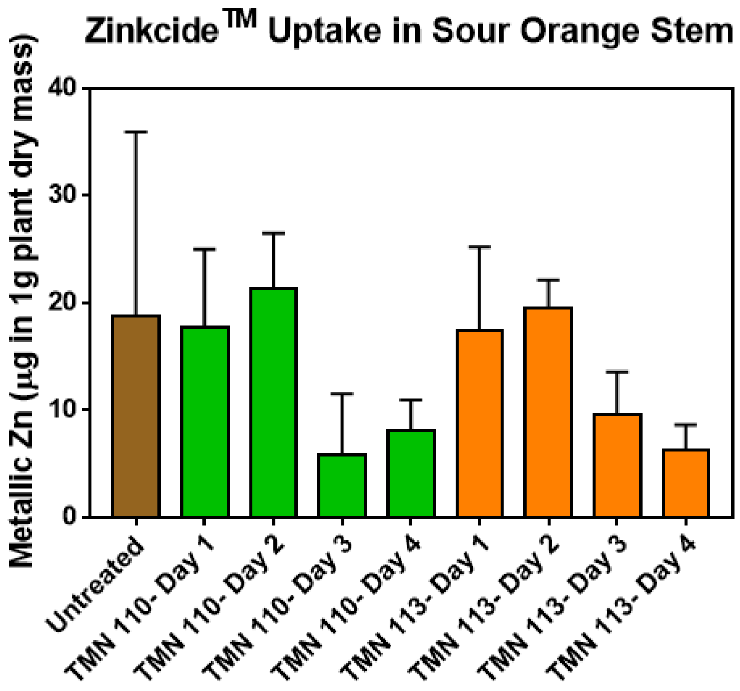
Zinc uptake in tomato plants

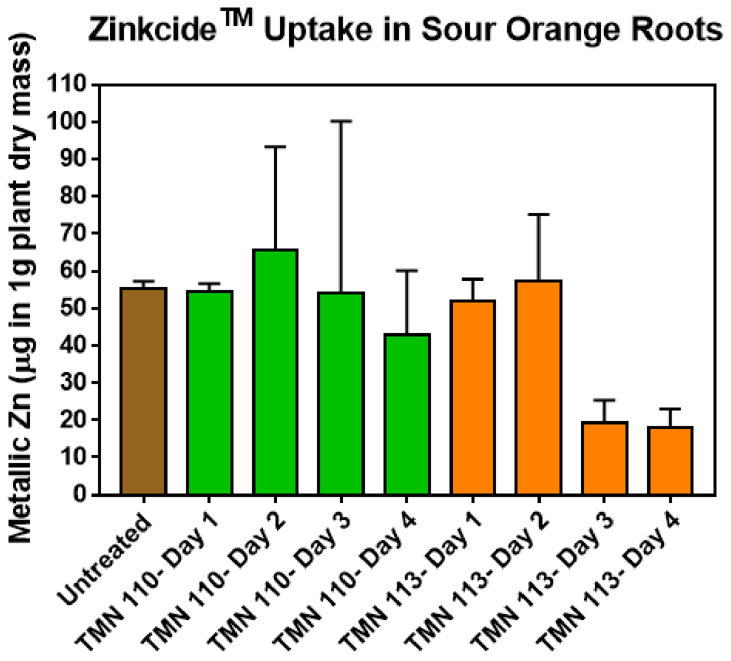


- ➢ Significant difference in uptake of Zn metal was observed at 800 and 1000 ppm of treated plants as compared to untreated sample.
- ➢ Atomic Absorption Spectroscopy (AAS) data suggest systemic movement of zinc from MS3T and T-SOL formulation to tomato plant.

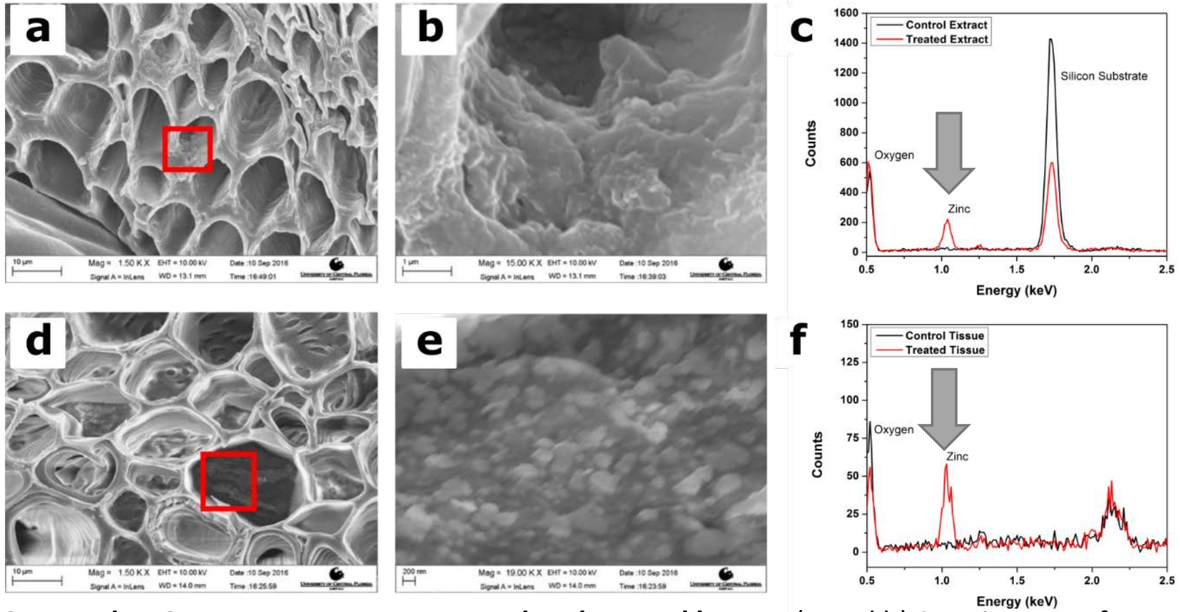
Zinkicide™ Plant Uptake Studies





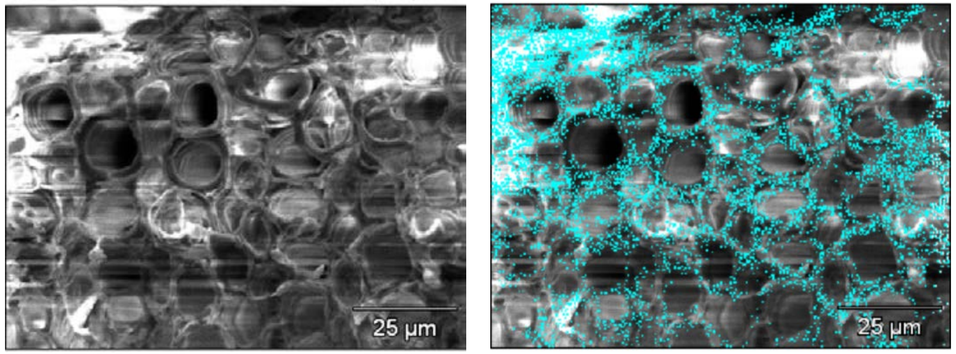


Zn uptake by citrus leaves (sour orange seedling)



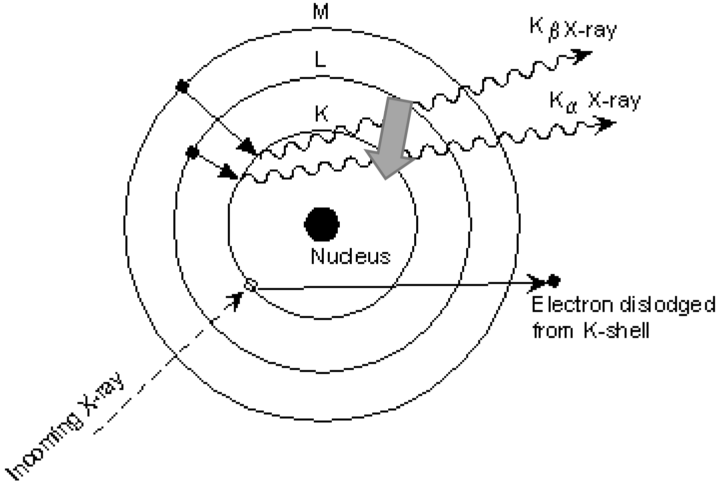
SEM and EDS measurements on treated and control leaves. (a and b) SEM images of control leaf tissue. (c) EDS measurements on control and treated leaf extract. (d and e) SEM images of the treated leaf tissue. (f) EDS measurements on the control and treated leaf tissue.

SEM (left) **with EDS overlay** (right) of Zn in the plant tissue of the leaf midrib:



Principle of EDS measurements

Detecting Xray Emitted from electronic transitions in Zn atom



2014 Citrus Canker Trial

Treatment	Metallic Cu (lb/ac)	Incidence old lesions (%)	Incidence young lesions (%)	Total incidence (%)
1) Kocide 2000	1.4	11.8 bc z	3.8 bz	15.6 cdez
2) Kocide 3000	0.9	11.8 bc	4.6 b	16.4 cde
3) Nordox 75WG	1.0	16.8 b	4.4 b	21.2 bcd
4) Nordox 75WG	2.0	10.2 bc	5.8 b	16.0 cde
5) Nordox 75WG 5 apps, MB 5 apps	1.0/0.14	16.0 b	7.0 b	23.0 bcd
6) Magna-Bon 100 ppm	0.14	12.4 bc	6.0 b	18.4 bcd
7) Nordox 30/30 WG 1.5 lb	0.50	15.8 b	8.8 b	24.6 b
8) Nordox 30/30 WG 3.0 lb	1.0	12.2 bc	5.8 b	18.0 bcd
9) ChampION++ 30 WDG	0.9	13.8 b	8.0 b	21.8 bcd
10) MV-1 CuSiNG pH 4.0	0.20	16.4 b	4.4 b	20.8 bcd
11) MV1-CuSiNG pH 4.0 plus Quat 1X	0.20	10.6 bc	7.0 b	17.6 bcd
12) MV1-CuSiNG pH 4.0 plus Quat 2X	0.40	9.4 bcd	5.6 b	15.0 de
13) Fixed-Quat- A –I DDAC (10138 ppm) 1:50	--	12.6 bc	2.6 b	15.2 de
14) Fixed-Quat-A-I DDAC(10138 ppm) 1:100	--	18.0 b	5.6 b	23.6 bc
15) CS-CuSiNPs (Si core)	0.20	11.6 bc	4.0b	15.6 cde
16) Zinkicide formulation SG4	--	3.0 d	6.2 b	9.2 def
17) Zinkicide formulation SG6	--	4.6 cd	2.4 b	7.0 ef
18) Untreated check (UTC)	--	45.0 a	17.8 a	62.8 a

2015 Citrus Canker Trial

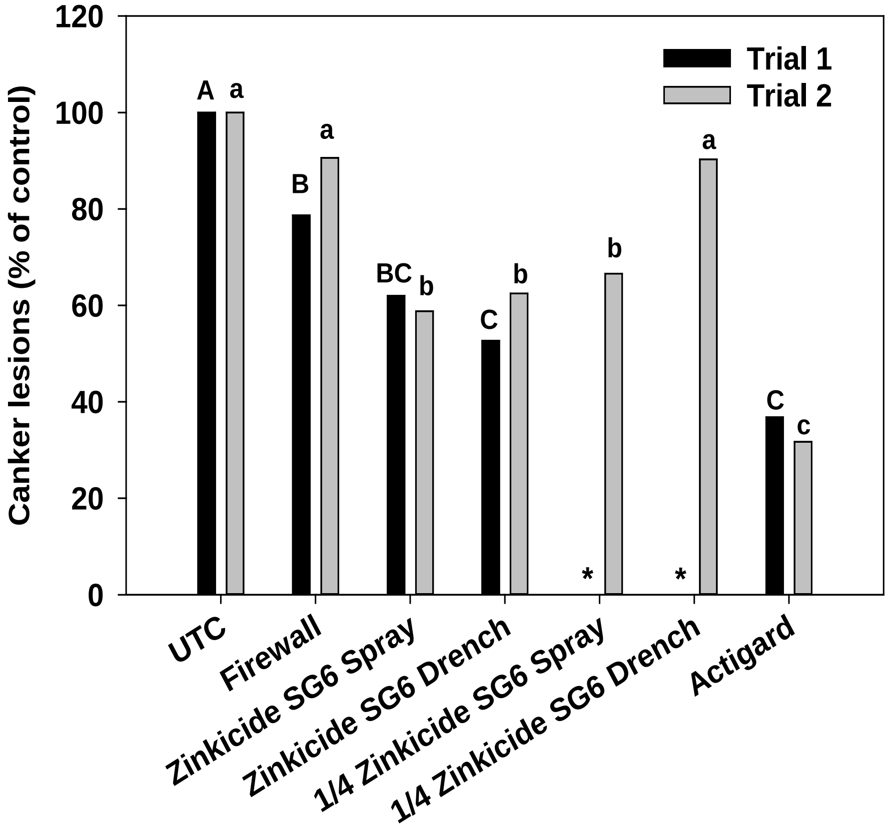
Treatment	**Metallic Cu (%)**	**Incidence old lesions (%)**	**Incidence young lesions (%)**	**Total incidence (%)**
1) Nordox 75WG	75	10 cde [z]	20 b [z]	29 c [z]
2) Nordox 30/30 WG - 1.5 lb	30	8.2 cde	13 bc	21 defg
3) Nordox 30/30 WG - 3.0 lb	30	9.0 cde	14 bc	23 def
4) Nordox 45/15 WG	45	6.2 e	14 bc	20 defg
5) Magna-Bon 100 ppm	5	16 bc	8.8 c	25 cd
6) Zinkicide SG4	--	6.2 e	11 c	17 fgh
7) Zinkicide SG6	--	5.6 e	10 c	16 gh
8) Zinkicide SG4 – half rate	--	5.2 e	8.4 c	14 h
9) Zinkicide SG6 – half rate	--	11 cde	13 bc	24 de
10) T-Sol-S	--	10 cde	14 bc	23 de
11) T-Sol-SP	--	11cde	13 bc	24 cde
12) T-Sol-G	--	8.4 cde	13 bc	21 defg
13) T-Sol-GP	--	9.0 cde	12 bc	21 defg
14) T-Sol-U	--	5.8 e	12 bc	17 fgh
15) T-Sol-UP	--	7.4 de	11 c	18 efgh
16) CuSiNG-Quat (Copper-Fixed-Quat)	--	13 bcd	12 bc	25 cd
17) Fixed-Quat A-II (200 ppm)	--	14 b	12 bc	26 cd
18) CS-CuSiNP (ZnO core)	5	11 cde	14 bc	25 cd
19) SG0025	--	23 a	12 bc	36 b
20) Untreated check 1(UTC)	--	23 a	37 a	60 a

2016 Citrus Canker Trial

Treatment	**Metallic Cu (%)**	**Incidence old lesions (%)**	**Incidence young lesions (%)**	**Total incidence (%)**
1) Nordox 75WG	75	28 ef [z]	28 cdef [z]	56 def [z]
2) Nordox 30/30 WG	30	30 def	27 def	56 def
3) Nordox 30/30 WG	30	30 def	27 def	56 def
4) Nordox 75WG 1.0 lb Cu 5 sprays, 0.5 Cu second 5 sprays	75	29 def	28 cdef	57 de
5) Zinkicide SG4 – ag grade	--	34 bc	30 bcd	64 bc
6) Zinkicide SG6 – ag grade	--	34 bc	30 bcd	64 bc
7) Zinkicide SG4 – ag grade	--	35 b	31 bcd	66 b
8) Zinkicide SG6 – ag grade	--	32 bcd	31 bc	64 bc
9) Zinkicide SG4	--	34 bc	33 b	67 b
10) Zinkicide SG6	--	26 fg	25 fg	51 g
11) T-Sol-U	--	34 bc	30 bcde	64 bc
12) T-Sol-UP	--	32 bcd	29 bcde	61 dc
13) CuSiNG-Quat (Copper Fixed-Quat)	--	30 def	29 bcde	59 cde
14) DDAC Fixed-Quat A-II (200 ppm)	--	28 ef	27 def	55 efg
15) CS-CuSiNP (ZnO core)	--	29 def	30 bcde	58 de
16) MS3T	--	26 fg	26 ef	52 fg
17) MS3T – double rate	--	23 g	23 g	46 h
18) Clay plus DDAC (200 ppm)	--	30 def	27 def	57 def
19) Clay plus T-Sol (1.0 lb/ac)	--	30 cde	26 def	57 def
20) Untreated check 1(UTC)	--	50 a	43 a	93 a

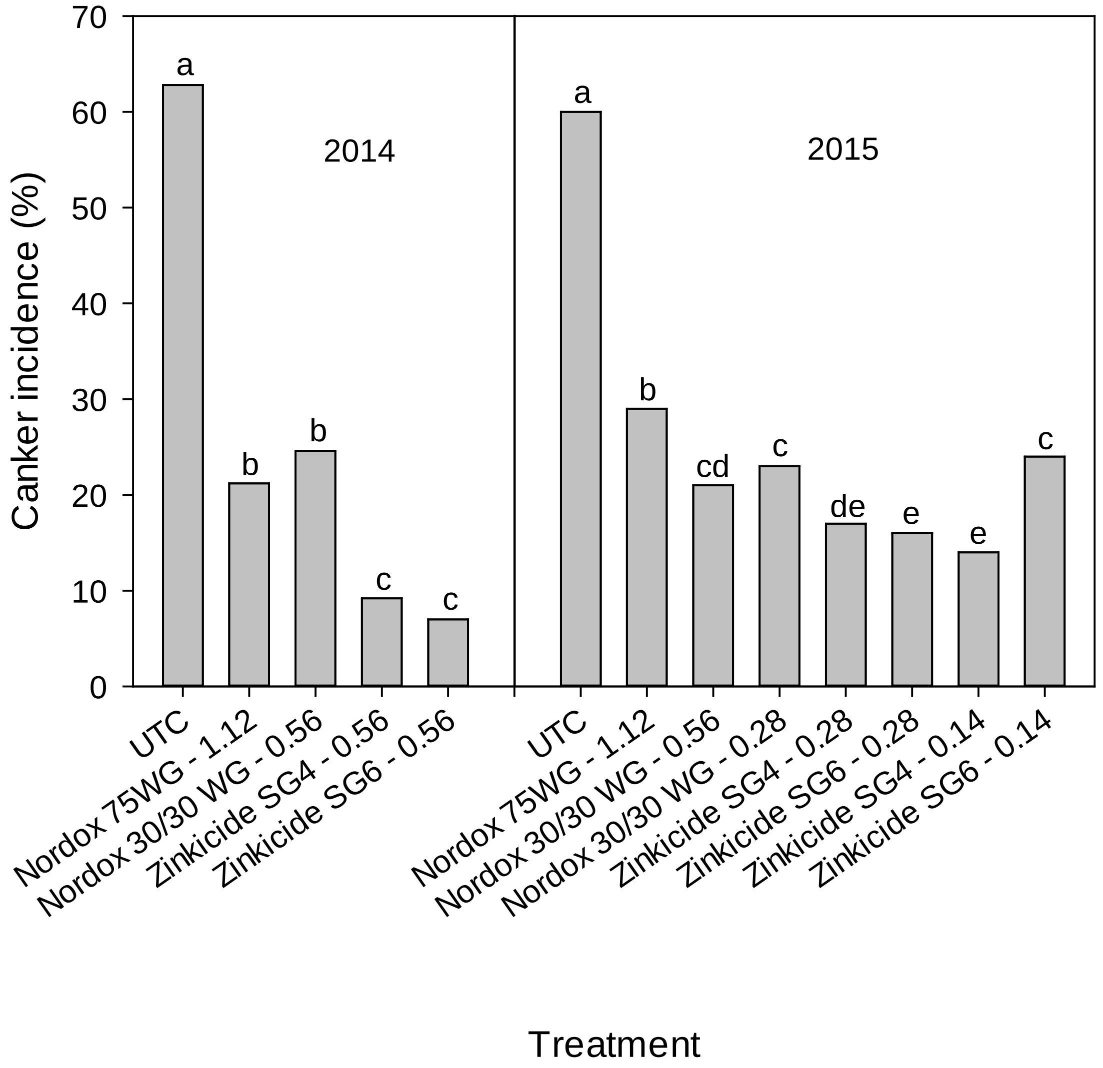
Citrus Canker – Greenhouse Trials

- Root uptake (soil drench)
  - Phloem availability?
- 4x reduction in total metal for local systemic activity



Credit: Jim Graham and Evan Johnson, CREC, UF

Citrus Canker control – Field Trials



Credit: Jim Graham and Evan Johnson, CREC, UF

What about Zinkicide efficacy against HLB?

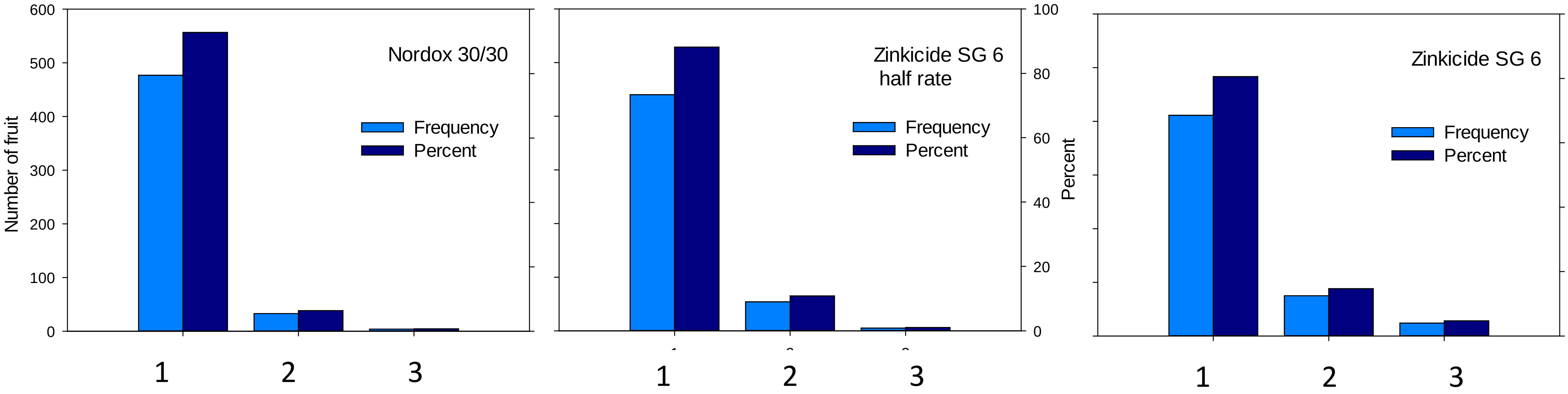
- HLB field trials underway
  - Sweet orange and Grapefruit
  - Foliar Spray, Soil Drench, and Combination
  - New planting, young trees, mature trees

- First year – too low a rate.
  - Increased all rates in rate trials

- Canker trial is a defacto HLB trial

Credit: Jim Graham and Evan Johnson, CREC, UF

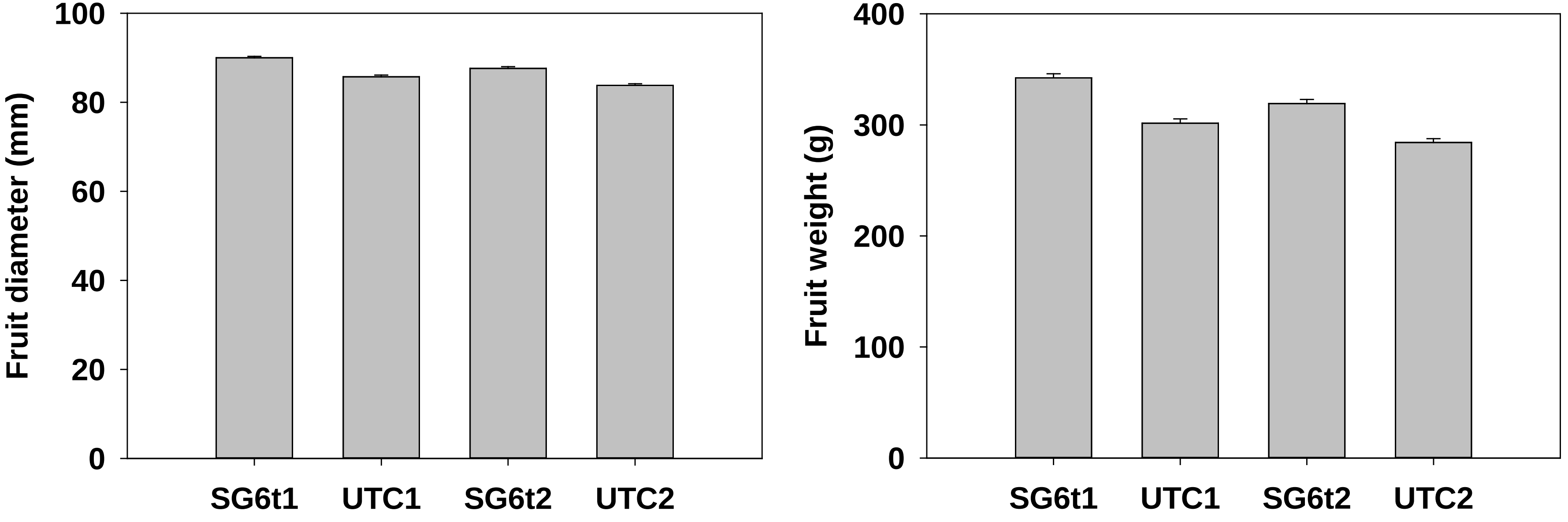
Grapefruit – Fruit size dose response



- Fruit per box – Size 1 = 56-64; Size 2 = 40–48; Size 3 = 32-36

Credit: Jim Graham and Evan Johnson, CREC, UF

HLB – Grower Demo Trial



Credit: Jim Graham and Evan Johnson, CREC, UF

List of CRDF funded projects

Project Title	Funding period	Team members	Funded amount
Development of nanoparticle/nanogel formulation for the prevention of citrus canker disease	03/05/2009 - 03/04/2010	S. Santra (PI); James H. Graham (Co-PI)	$90,000.00
Copper loaded silica nanogel technology for long term prevention of citrus canker disease	10/01/2010 - 09/30/2013	S. Santra (PI); James H. Graham (Co-PI)	$106,044.00
Soluble core-shell copper loaded silica nanoparticle formulation with improved Cu bioavailability	10/01/2012 - 09/30/2015	S. Santra (Co-PI); James H. Graham (PI)	$30,600.00
Fixed-Quat: A novel alternative to Cu fungicide/bactericide for preventing citrus canker	04/01/2013 - 07/31/2016	S. Santra (PI); James H. Graham (Co-PI)	$260,050.00
Zinkicide: A novel therapeutic zinc particulate based formulation for preventing citrus canker and HLB	04/01/2014 - 11/30/2015	S. Santra (Co-PI); Evan G. Johnson (PI); James H. Graham (Co-PI); Leonardo De La Fuente (Co-PI)	$70,937.00
T-SOL™ antimicrobial for the management of citrus canker and HLB	07/01/2015 - 06/30/2017	S. Santra (PI); Evan G. Johnson (Co-PI); James H. Graham (Co-PI)	$240,224.00
New non-phytotoxic composite polymer film barrier as ACP repellent for controlling HLB infection	04/01/2014 - 03/31/2017	S. Santra (PI); James H. Graham (Co-PI); Michael Rogers (Co-PI); Mike Irey (Co-PI)	$350,000.00

Total: $1,147,855.00

Products (publications and patents)

Publications (Peer-reviewed)

1. Graham J. H.*, Johnson E. G., Myers M. E., Young M., Rajasekaran P., Das S., and Santra S., "Potential of nano-formulated zinc oxide for control of citrus canker on grapefruit trees", Plant Disease, 2016, 100(12): 2442-2447.
2. Rajasekaran P., Kannan H., Das S., Young M. and Santra S*, "Comparative analysis of copper and zinc based agrichemical biocide products: materials characteristics, phytotoxicity and in vitro antimicrobial efficacy", AIMS Environmental Science, 2016, 3(3): 439-455.
3. Young M. and Santra S*, "Copper (Cu)-Silica Nanocomposite Containing Valence-Engineered Cu: A New Strategy for Improving the Antimicrobial Efficacy of Cu Biocides", Journal of Agricultural and Food Chemistry 2014, 62(26): 6043-6052.
4. Maniprasad P and Santra S*, "Novel copper loaded core-shell silica nanoparticles with improved copper bioavailability: synthesis, characterization and study of antibacterial properties" Journal of Biomedical Nanotechnology 2012, 8(4), 558-566.

Patents (issued)

1. Santra S, "Silica-based antibacterial and antifungal nanoformulation", EP Patent # 2,367,552 (issued on 07/20/2016). Validation in France, Italy, Greece, Netherlands, Spain, Portugal and Poland.
2. Santra S, and Berroth M., "Compositions including a vacancy-engineered(VE)-ZnO nanocomposite, methods of making a composition, method of using a composition", US Patent # 9,215,877 (issued on 12/22/2015)
3. Santra S, "Multifunctional Silica-Based Nanoformulations, Methods of Making Nanoformulations, and Methods of Using Nanoformulations", US Patent # 8,992,960 (issued on 03/31/2015)
4. Santra S, "Multifunctional Silica-Based Nanoformulations, Methods of Making Nanoformulations, and Methods of Using Nanoformulations", Australia patent # 2011238639 (issued on 01/29/2015)
5. Santra S, "Multifunctional Silica-Based Compositions and Gels, Methods of Making Them, and Methods of Using Them", Mexico Patent #329942, issued 5/7/2015
6. Santra S, "Silica-based antibacterial and antifungal nanoformulation", Mexico patent # 322,306 (issued on 07/24/2014)
7. Santra, S., "Silica-based antibacterial and antifungal nanoformulation", US Patent # 8,632,811 (issued on 01/21/2014)
8. Santra S, Silica-based antibacterial and antifungal nanoformulation", US patent # 8,221,791 (issued on 07/07/2012)

Commercial Sponsored Research and Licensing status

<u>Registrant:</u> GOWAN Company (http://www.gowanco.com/en-us/default.aspx)

- Licensed all Cu family of patents (US and International) in January, 2017
- Completed commercial evaluation of original Cu-silica nanogel product and entering into the registration process.
- Entered into an agreement with UCF to perform commercial evaluation of the following product concepts
  - ➢ UCF CS-CuSiNP (Core-Shell Cu)
  - ➢ UCF CS-CuSiNP 800 (Core-Shell Cu, ZnO core)
  - ➢ UCF MV 1- CuSiNG (Mixed-Valence Cu-silica nanogel)
  - ➢ UCF Copper Fixed-Quat
  - ➢ UCF Fixed-Quat A II

<u>Registrants:</u> Brandt (http://www.brandt.co/);
TradeMark Nitrogen (http://www.trademarknitro.com/)

- Entered into a 3-year agreement with UCF to develop Zinkicide™
- Zinkicide is now 100% agri-grade
- Entered into the pre-registration process
- Focus is to bring this product to market ASAP to help our citrus growers (highest priority)

Education/Outreach and Extension

What is MISA center ?

- USDA-NIFA recognized Center of Excellence
- Interdisciplinary Research and Education/Extension
- Aligned with UCF’s goal of becoming “America’s leading partnership University”
- Collaborative center engaging institutions from across the world

MATERIALS INNOVATION FOR SUSTAINABLE AGRICULTURE

MISA

http://nanoscience.ucf.edu/misa/index.php

Vision

To advance knowledge and promote innovations for sustainable agricultural practices

Mission

- Promote interdisciplinary research culture for stimulating innovations in materials research
- Develop new technologies for protecting sustainability of agriculture industry challenged by emerging threats of plant diseases and unpredictable weather patterns
- Establish industry-academia close collaboration for educational and technological knowledge sharing
- Establish interdisciplinary education and extension program to help growers in making informed decision
- Promote sustainable outcomes by adopting innovative approaches through engagement of stakeholders, scientists, engineers, industry partners and regulatory agencies

The 2nd Annual MISA Symposium and MS3T-SAB meeting

November 5-7, 2017

University of Central Florida

Thank you!

Extra slides

2016 Canker trial

- Location: Fort Pierce, St. Lucie County
- Citrus variety/rootstock: 8 yr-old 'Ray Ruby' grapefruit
- Windbreaks: trial was located in 11-acre block surrounded on all sides by a windbreak consisting of 3.5 yr-old *Corymbia torelliana* that was approx. 25 ft. tall
- Tree spacing: 12 ft x 25ft spacing; 145 trees per acre
- Randomized block: 5 plots of 5 trees/plot (5 trees in a row, 25 trees per treatment). Each plot is approx. 0.17 acres
- Spray volume and application: One gallon of spray per tree applied with a handgun (to incipient run-off from foliage) equivalent to 145 gal/acre
- Spray dates: 19-Apr, 10-May, 31-May, 20-Jun, 11-Jul, 1-Aug, 22-Aug, 12-Sep, 10-Oct, 24 Oct

Evan Johnson and James H. Graham
University of Florida
CREC, Lake Alfred

Rainfall at Indian River FAWN station

Monthly rainfall (inches)	Jan	Feb	Mar	April	May	June	July	Aug	Sept.	Oct
St. Lucie 2016	8.7	2.4	3.5	2.6	9.1	4.7	4.3	9.3	5.7	4.0
Indian River 2015	2.1	2.1	1.6	4.9	0.7	9.4	7.3	7.6	10.0	2.0
Ft. Pierce[z] average			2.3	3.3	6.6	6.8	6.5	5.9	3.8	1.9

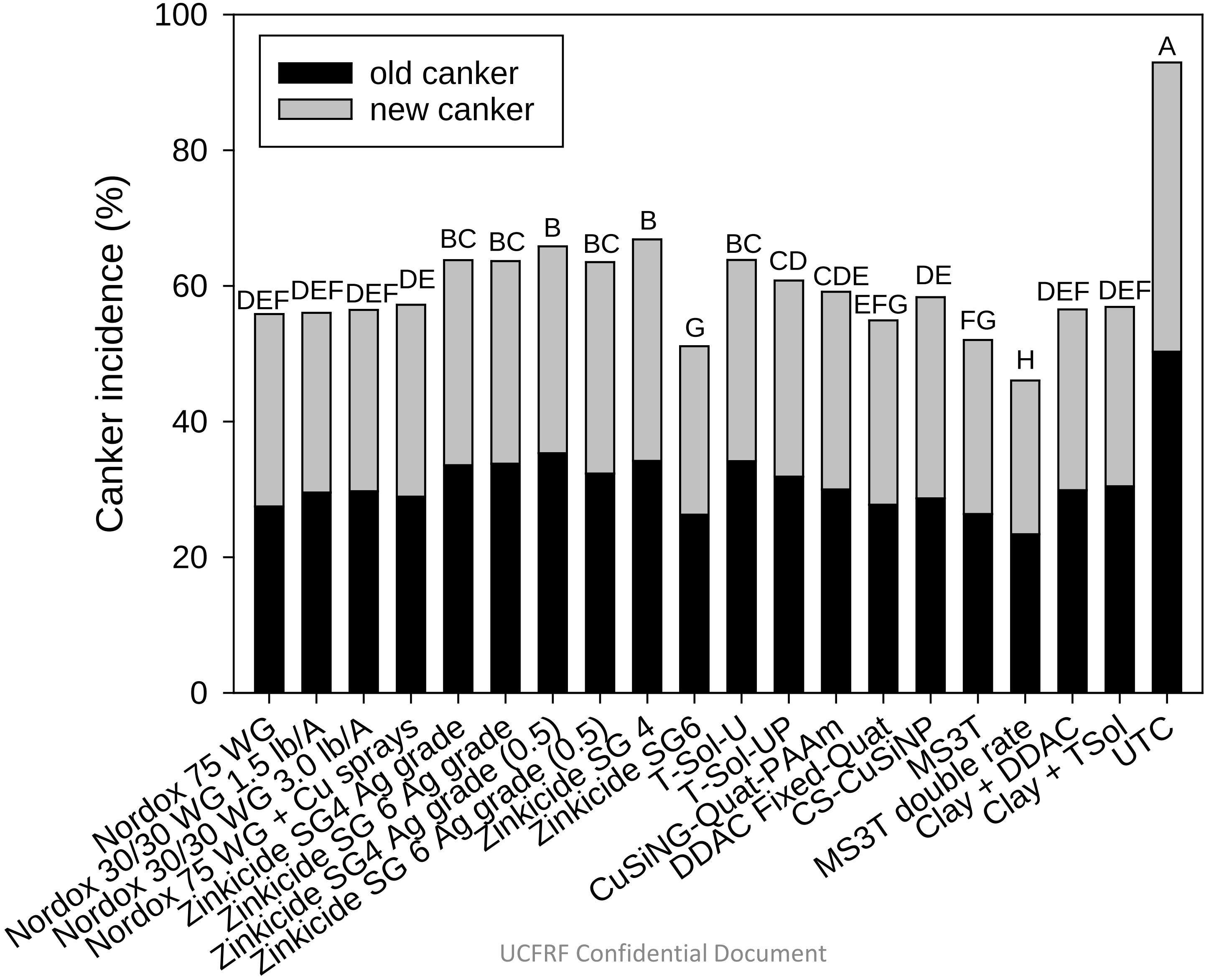
[z]Average monthly rainfall from 2000-2010 obtained from FAWN at IRREC

It was an extremely warm and wet January-March 2016 with active flush throughout the winter

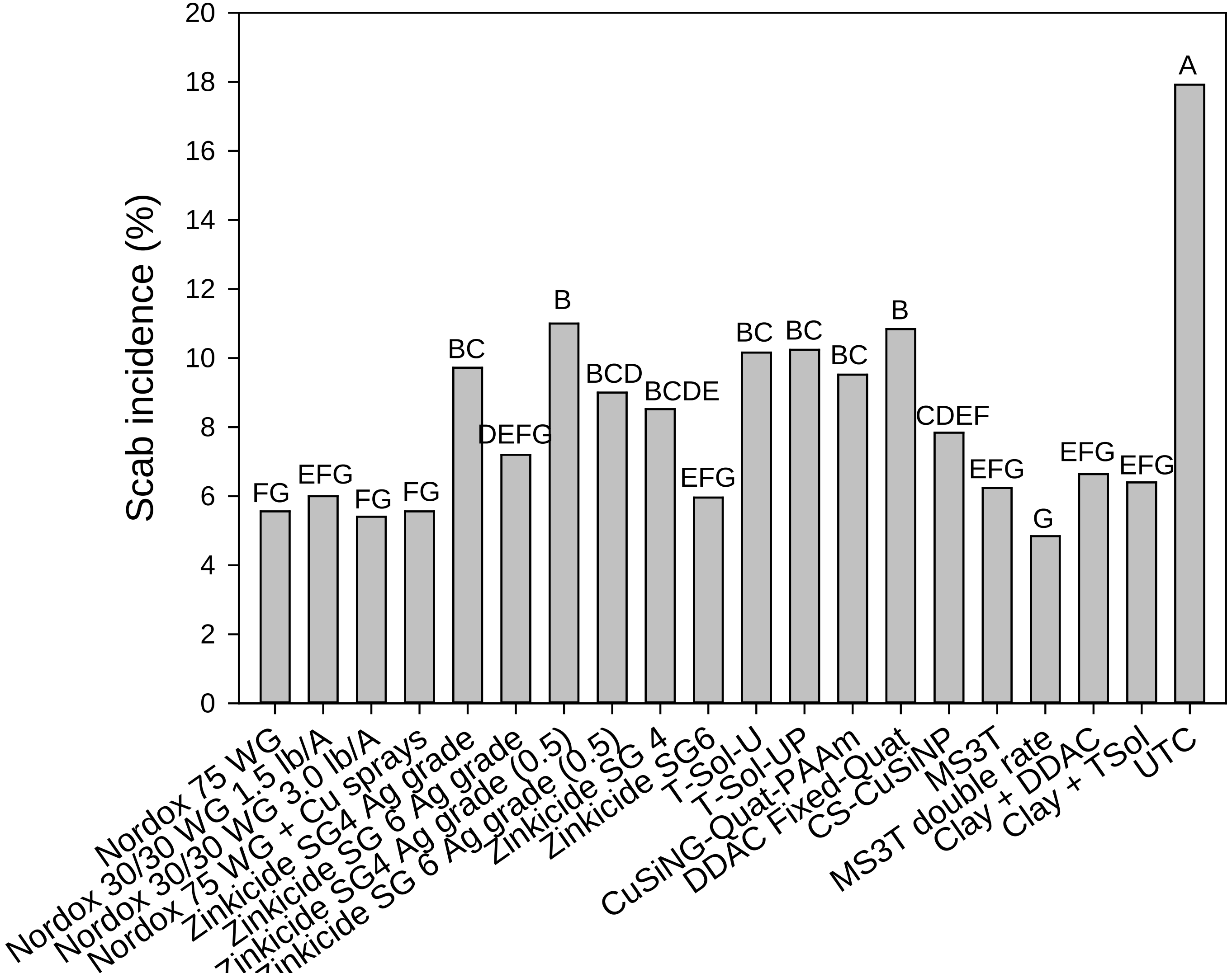
Formulations and rates of products in the Estes grapefruit trial 2016

Treatment	Supplier	Metallic Cu (%)	Rate (lb or oz/acre)	Metallic Cu or Zn (lb/ac)
1) Nordox 75WG	Brandt	75	1.33	1.0
2) Nordox 30/30 WG	Brandt	30	1.5	0.45
3) Nordox 30/30 WG	Brandt	30	3.0	0.9
4) Nordox 75WG 1.0 lb Cu 5 sprays, 0.5 Cu second 5 sprays	Brandt	75	1.33/0.66	1.0/0.5
5) Zinkicide SG4 – ag grade	UCF-Zn			1.0
6) Zinkicide SG6 – ag grade	UCF-Zn			1.0
7) Zinkicide SG4 – ag grade	UCF-Zn			0.5
8) Zinkicide SG6 – ag grade	UCF-Zn			0.5
9) Zinkicide SG4	UCF-Zn			1.0
10) Zinkicide SG6	UCF-Zn			1.0
11) T-Sol-U	UCF-Zn			0.5
12) T-Sol-UP	UCF-Zn			0.5
13) CuSiNG-Quat-P	UCF-Cu+Quat			0.5
14) DDAC Fixed-Quat (200 ppm)	UCF-Quat			--
15) CS-CuSiNP	UCF-Cu	5	0.5	0.20
16) MS3T	UCF-Zn+Quat			0.5
17) MS3T – double rate	UCF-Zn+Quat			1.0
18) Clay plus DDAC (200 ppm)	UCF-Clay+Quat	--	--	--
19) Clay plus T-Sol (1.0 lb/ac)	UCF-Clay+Zn			1.0
20) Untreated check 1(UTC)	---	--	--	--

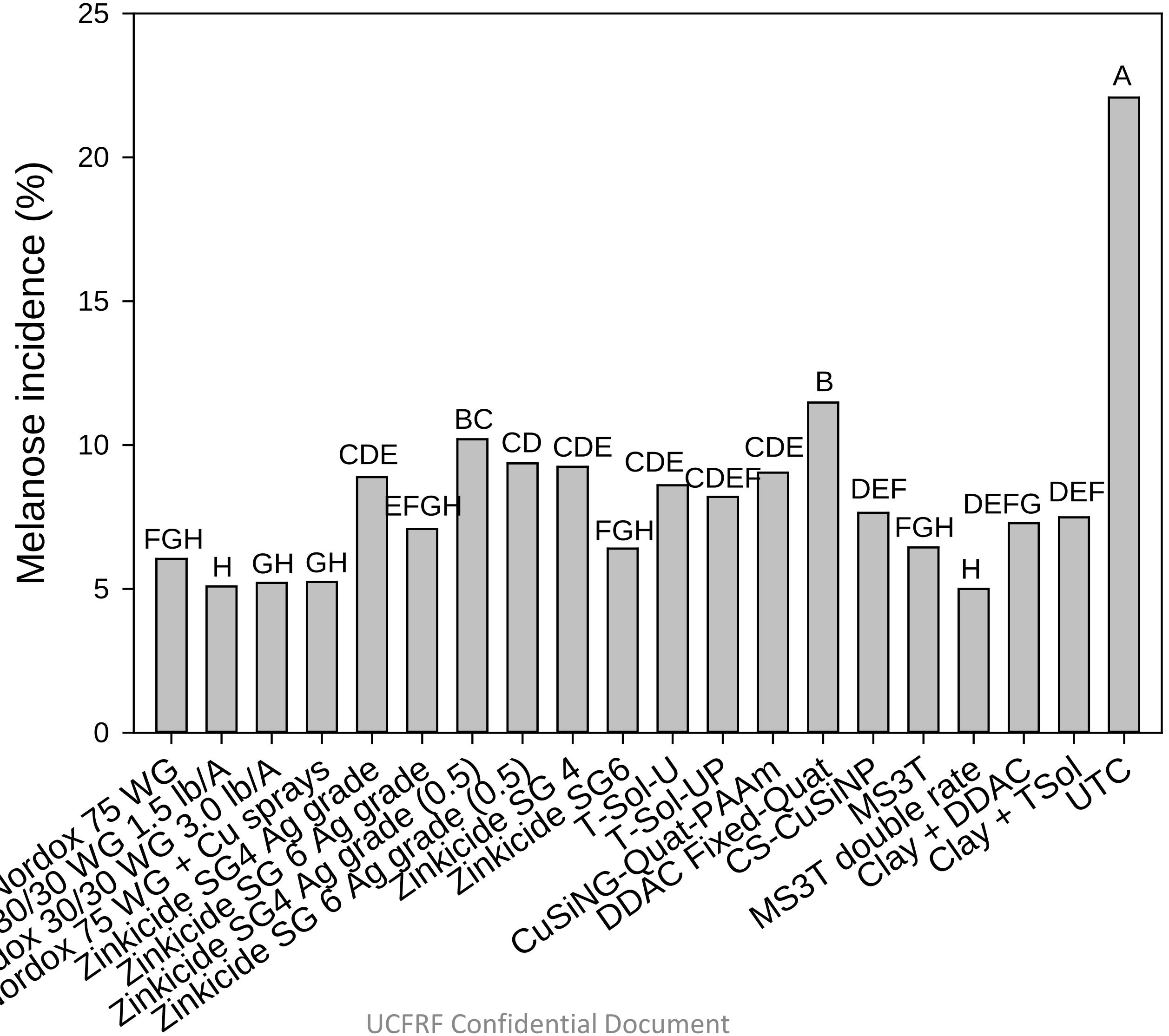
Grapefruit Canker



Grapefruit scab



Grapefruit melanose



MS3T uptake studies in citrus plants

Location: Fort Pierce, FL
Citrus variety/rootstock: 8 yr-old 'Ray Ruby' grapefruit

Objective is to develop rapid detection methodology for measuring MS3T concentration in citrus leaf tissue (Residual)

<u>Analytical techniques</u>

- X-ray fluorescence (XRF) spectroscopy
  - Direct determination of Zn content
- Near infrared (NIR) and Fourier transform infrared (FTIR) spectroscopy
  - Identify specific absorbance bands in spectra
  - Analyze via principal component analysis (PCA) for sample groupings
  - Build calibration model to measure Zn content and/or other compounds in leaf tissue

Warren Edmunds and Nicole Labbé
Center for Renewable Carbon
The University of Tennessee

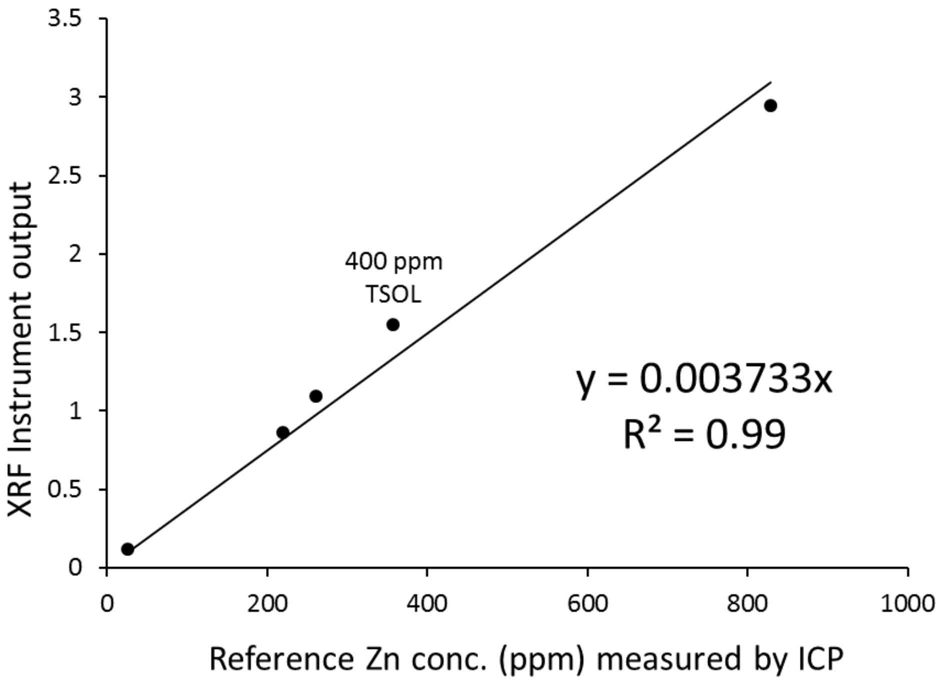
Mikhael Soliman and Laurene Tetard
NanoScience Technology Center
University of Central Florida

XRF Calibration Curve to Measure Zn Content

- Citrus leaf calibration standards were prepared by soaking leaves in different concentrations of Zn (TSOL) solution.
- Samples were milled and measured by XRF to obtain XRF output signal.
- Zn content (Reference) in the leaves was measured with ICP-OES after acid digestion.

Zn (TSOL) uptake samples preparation

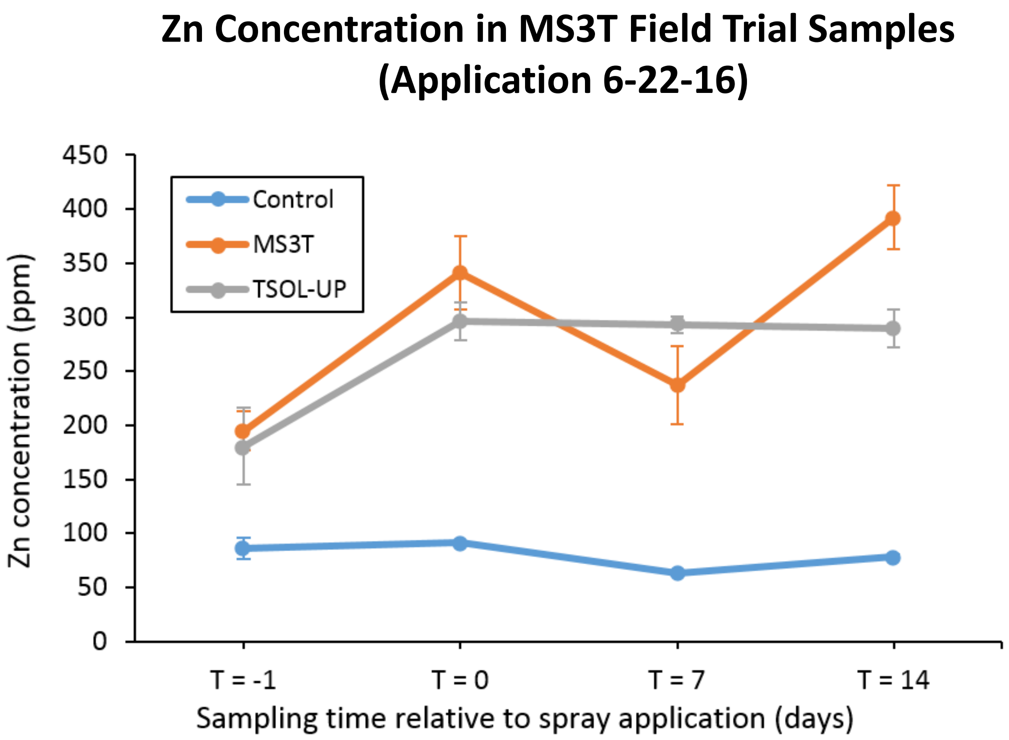
Zn calibration curve for XRF



Robust linear calibration was obtained for Zn

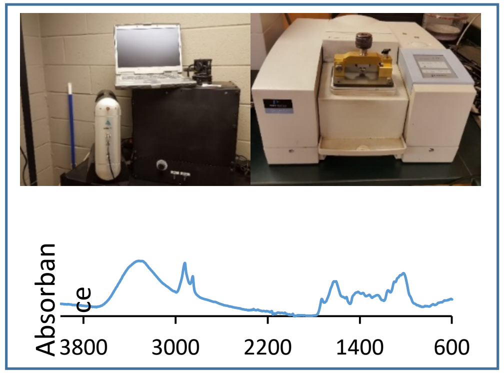
MS3T Field Samples – Zn Conc. Determined by XRF

- Zn concentration in MS3T field trial samples (spray application: 6-22-2016) measured by XRF.
- Zn increased after spray application (T= -1 vs. T= 0) by 75% and 64% in MS3T and TSOL-UP samples, respectively.
- Zn accumulations were observed in MS3T and TSOL-UP samples from previous applications.



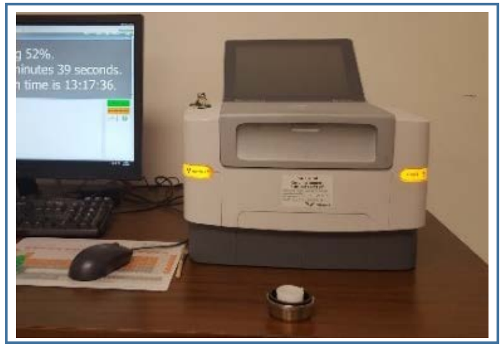
Spectroscopic Model Development

Near/Infrared spectra of citrus leaves

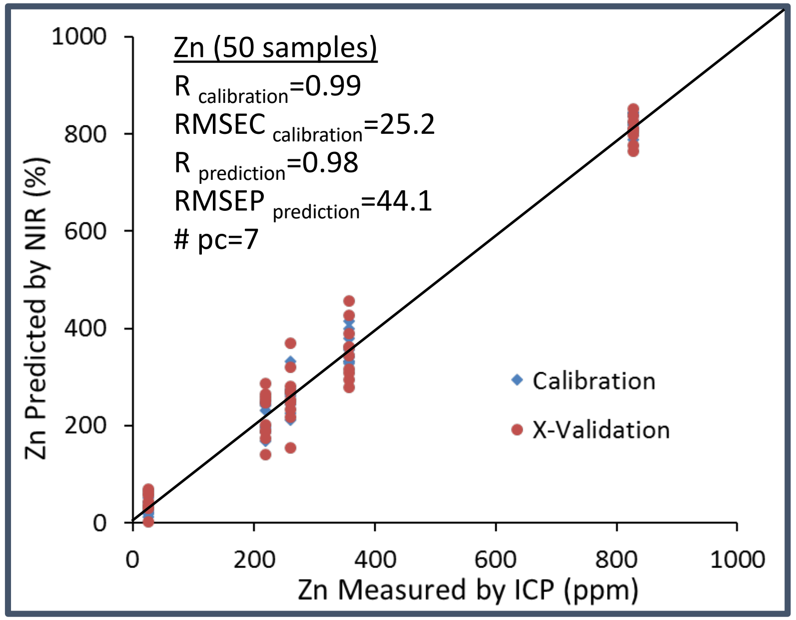


+

Zn content of citrus leaves by XRF



Predictive Models



Models Performance

- Robust to measure [Zn] in citrus leaves
- Rapid (less than 1 min)
- Minimum sample preparation
- Inexpensive
- Non destructive
- Chemical-free

Future Studies

- Validate Zn concentration in MS3T field samples using ICP-OES
- Build regression models using NIR and FTIR spectroscopic data to predict zinc or MS3T concentration in citrus leaf tissue
- Compare analytical performance of the different spectroscopic methods tested
- Continue work towards monitoring MS3T concentration in leaf tissue from field trials

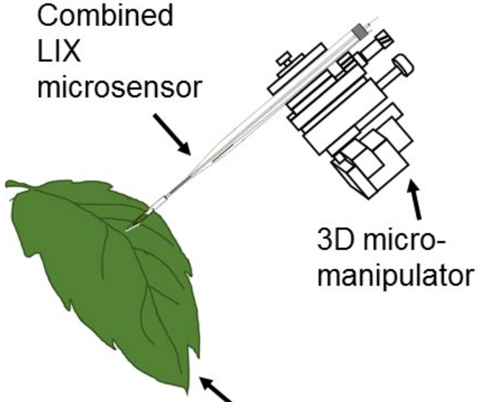
Tracking Zn movement in planta

Development of needle-type electrochemical microsensors for Zn tracking in plant system

Goal

To develop a needle-type ion-selective microsensor for tracking the systemic movement of Zn^{2+} in citrus plants

- Fabrication, characterization, and application
- Zn^{2+} Monitoring → Systemic movement, Phloem concentration, and Half-life



Objectives

1. Fabrication of Zn^{2+} liquid ion-exchange (LIX) membrane microsensors
2. Characterization of the microsensor → LOD, working ranges, ion interferences, and lifetime
3. Optimization for Zn^{2+} Monitoring → Lab setting vs. field application

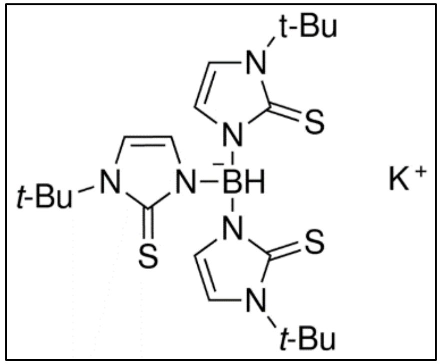
Woo Hyoung Lee

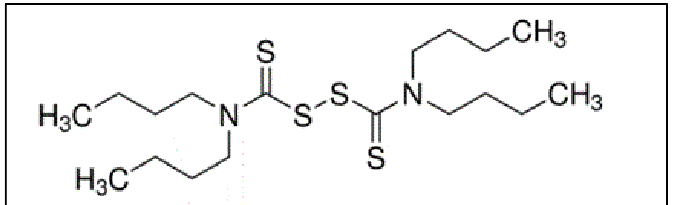
Department of Civil, Environmental, and Construction Engineering, University of Central Florida

Karin Y. Chumbimuni-Torres

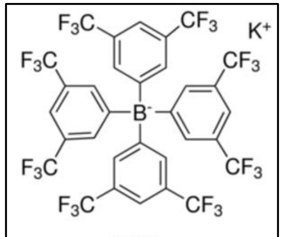
Department of Chemistry, University of Central Florida

Ion selective electrodes (ISEs)

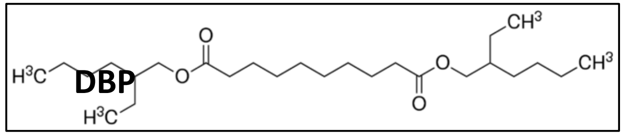




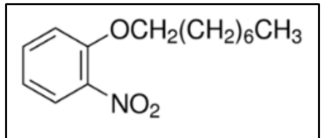
Zinc Ionophore (I)



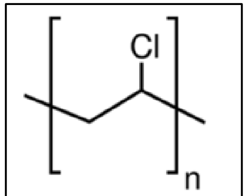
KTFPB



DBP



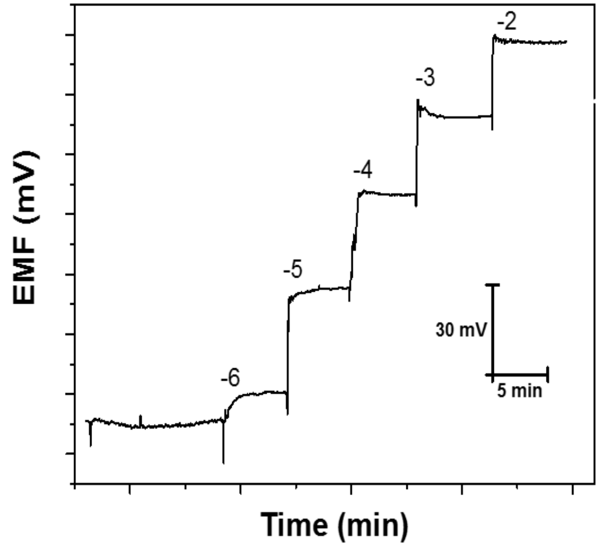
NPOE



PVC

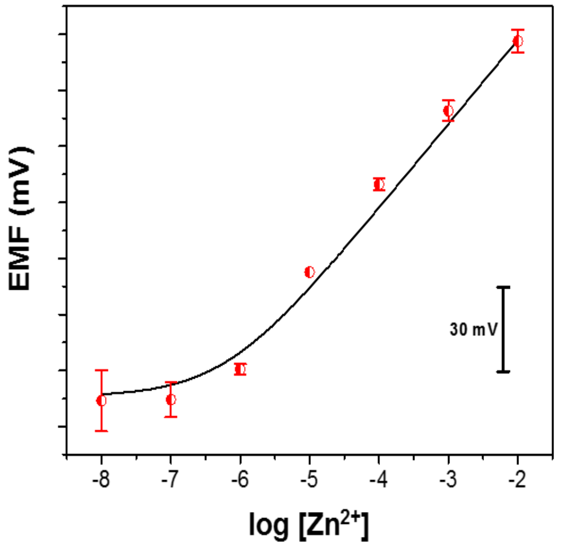
- Ionophore: Provides selectivity
- Ion-Exchanger: Provides electroneutrality
- Plasticized polymer: Provides mechanical stability

Zn2+ - Selective Microelectrodes



Nernst Equation

$$E = E_I^0 + \frac{2.303RT}{z_I F} log a_I$$



Parameters

Conditioned for 2 hour in 0.01 M $ZnSO_4$

Zinc Ionophore I : KTFPB = 2.6 : 1

Results

LOD = 3.74×10^{-7} M

Slope = 28.70 mV ($R^2 = 0.998$)

LDR = 10^{-6} to 10^{-2} M

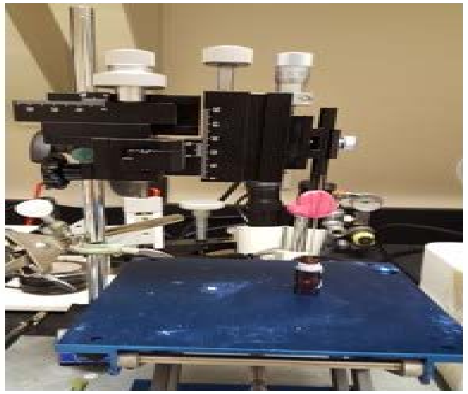
Zn^{2+} Liquid Ion Exchange (LIX) Microsensor Fabrication

Techniques for the fabrication of a novel Zn^{2+} LIX microsensor were established for measuring Zn^{2+} concentrations in the phloem of citrus leaves.

Pull and bevel

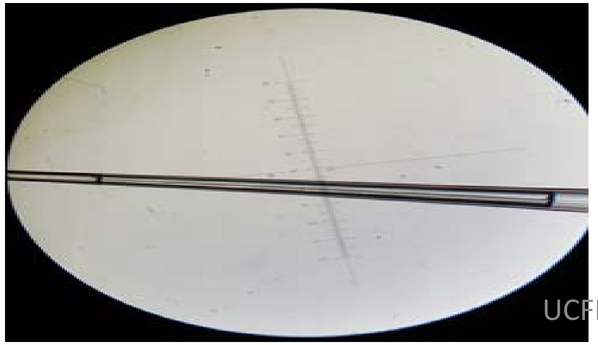
Silanization

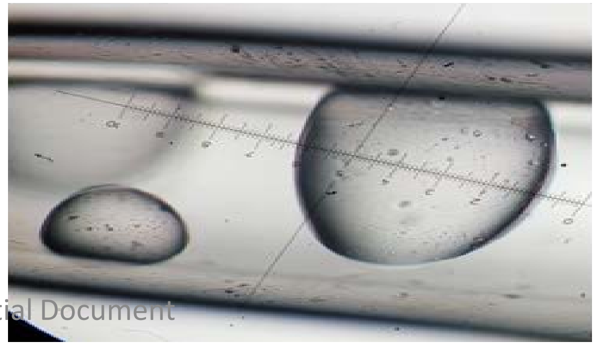
Apply LIX membrane



Challenges

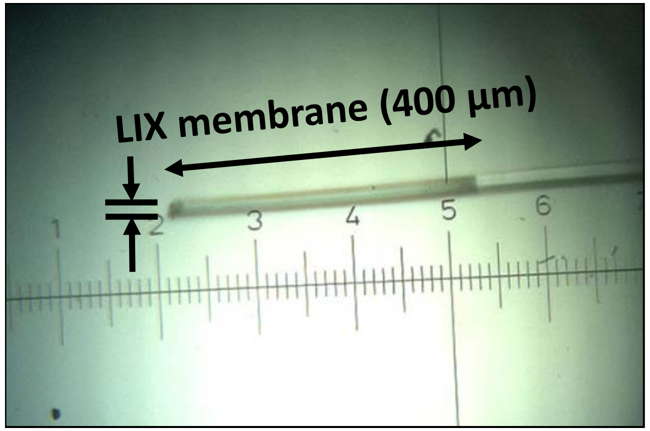
- Improper silanization
- Gap between backfilling solution and LIX
- Air bubbles



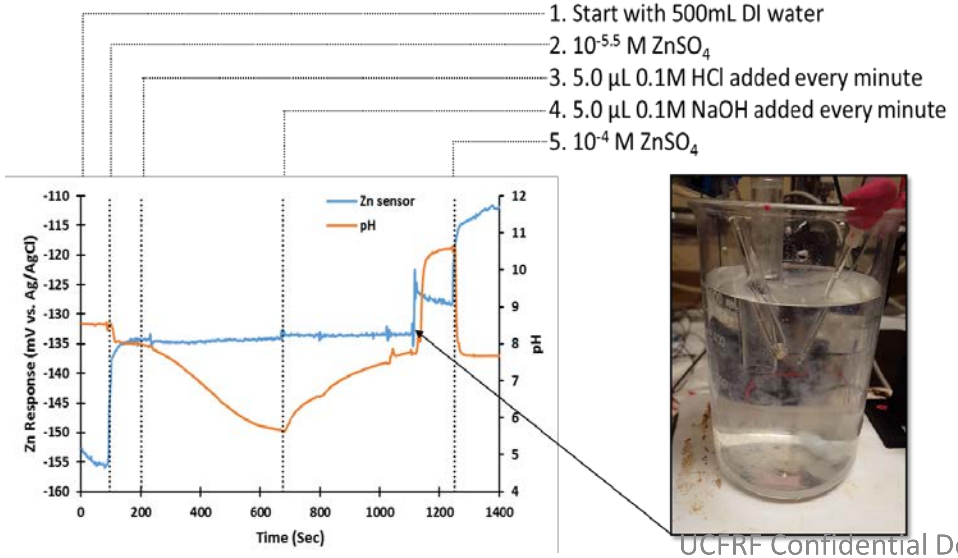


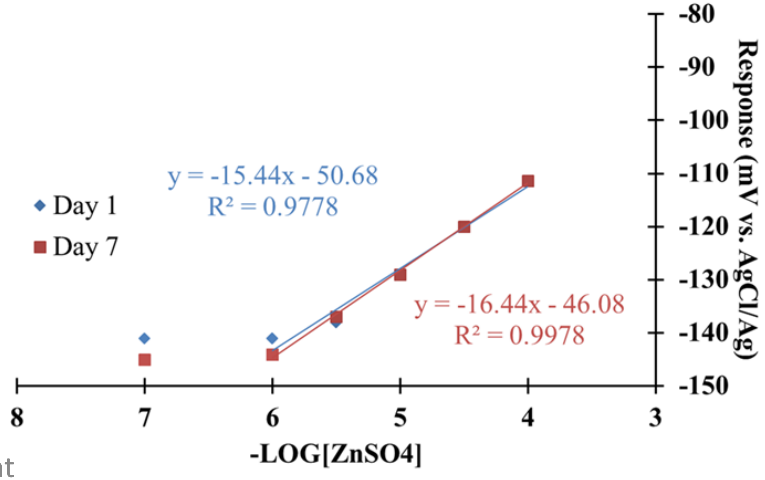
Zn^{2+} LIX Microsensor Characterization

- Good response toward Zn^{2+} ($ZnSO_4$ solution) and highly reproducible: 16.44 mV per decade
- Response time: < 30 secs
- Lifetime of the sensor: > 7 days
- No pH effect was found near neutral pHs (6 – 8).
  - ✓ Zinc precipitate formed at high pH (pH 10) and no sensor response at low pH (pH 4)
- Ionophore Zn (IV) is unstable and produces an anionic response in the presence of NO_3^{2+} →



Need modification of LIX for eliminating ion interferences





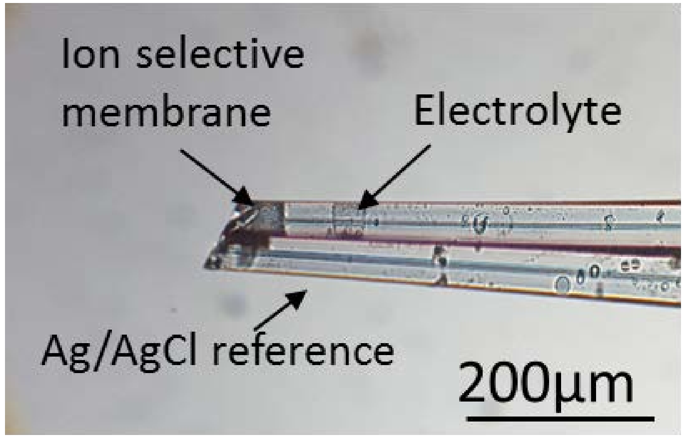
Optimization of the Microsensor for Zn^{2+} Monitoring of Plants

Durability of the sensor was tested for the application on citrus plants

- ✓ 50µm tip size is desirable for monitoring zinc movement within the vascular bundle
- ✓ Tip diameters: < 20µm → broken on leaf
  < 50µm → broken on upper stem

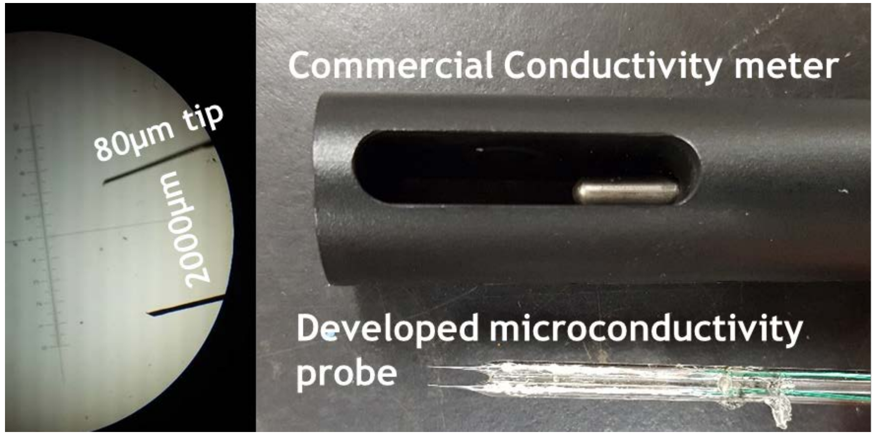
Double barrel design for field-deployable application

- ✓ A new sensor design concept was developed which fuses the reference (Ag/AgCl) electrode to the LIX membrane microsensor
- ✓ Allows reference electrode to be in close proximity to the working electrode
- ✓ Improves durability of the sensor

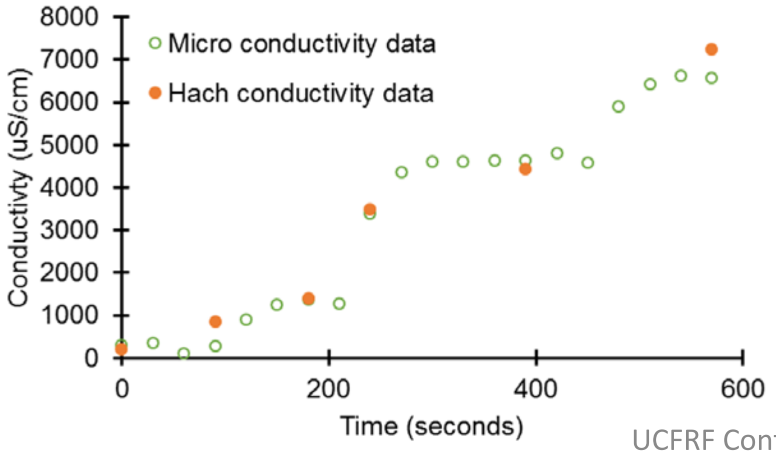


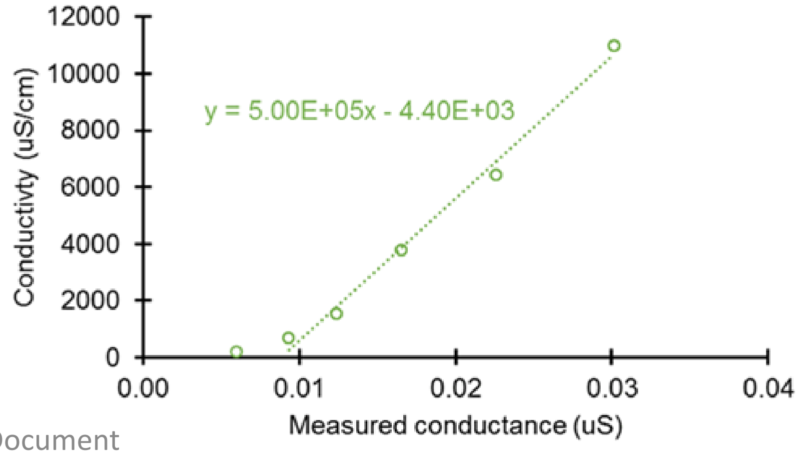
Micro-conductivity Sensor

A miniaturized conductivity meter was developed as a surrogate means for in situ monitoring of zinc concentrations in citrus plants.



- **Conductivity micro-probe**
  - Tip diameters: 80 μm
  - Approx. distance: 0.2 cm
  - Approx. Surface area: 2.0×10^{-4} cm^2
  - Cell constant: 5.00×10^5 cm^{-1}
- **Validation**
  - Micro-conductivity probe was compared to a commercial 4 point conductivity meter (Hach)

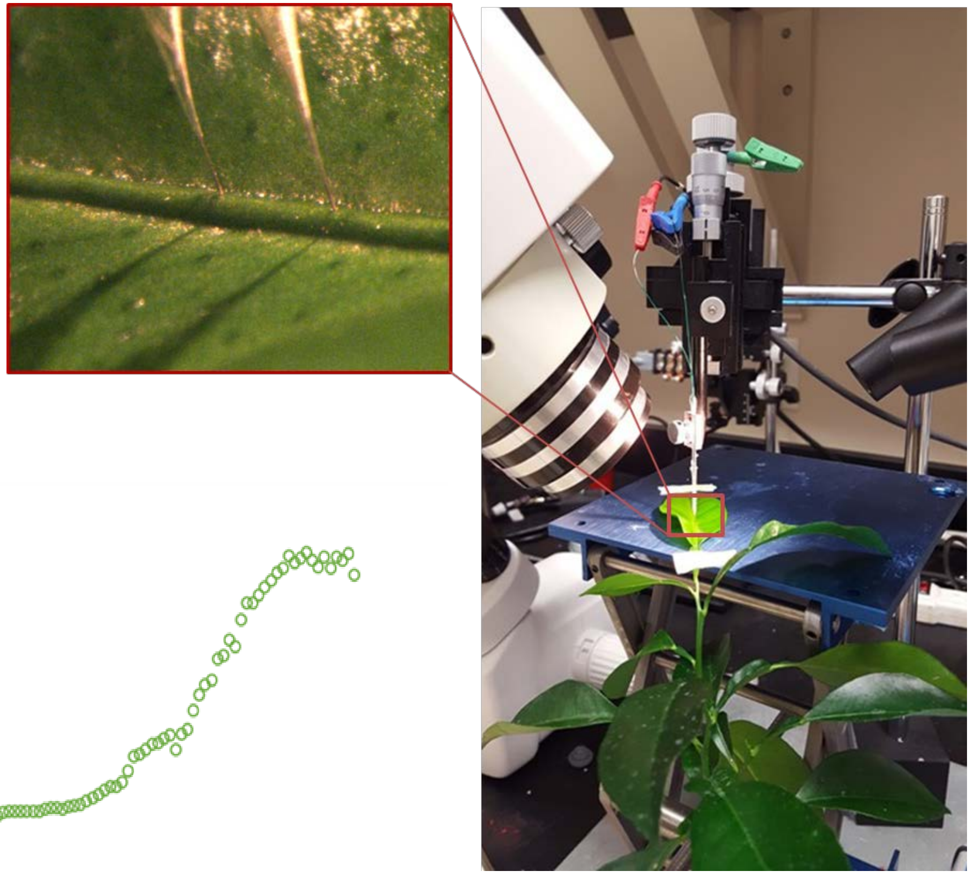


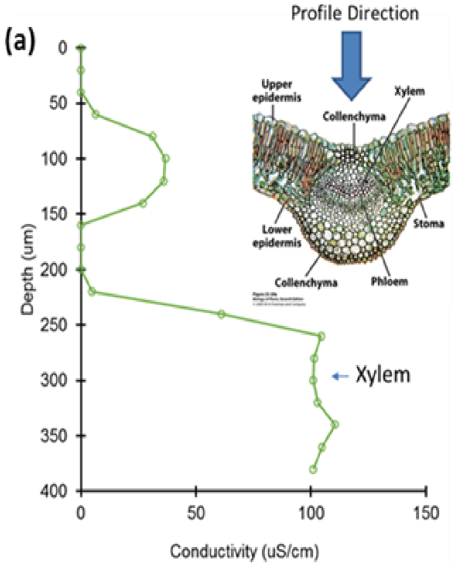


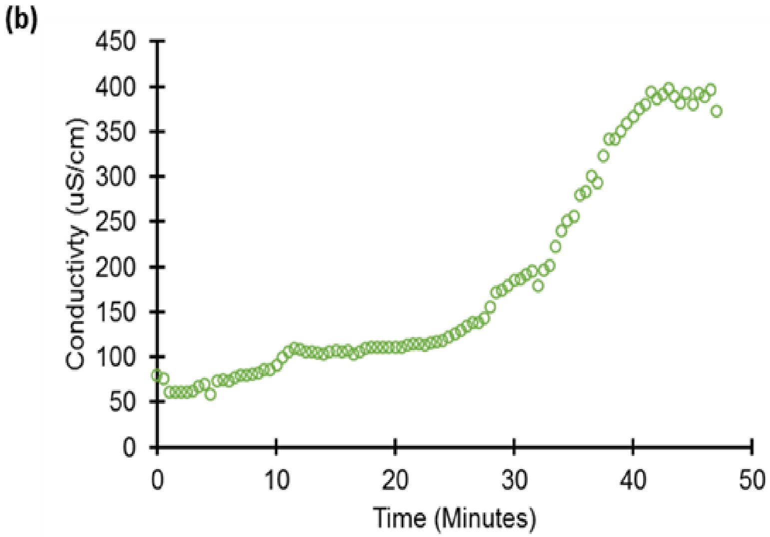
Microconductivity Sensor Application

The developed micro-conductivity sensor was used to measure conductivity changes through the vertical profile of the mid-rib of a citrus seedling leaf

- Two peaks in conductivity were found in the vertical profile of the midrib
  - ✓ The conductivity of the xylem was determined to be 100 µS/cm
- The leaf was exposed to 0.1 M NaCl
  - ✓ Conductivity increased by 300 µS/cm
  - ✓ Rate of ion movement: 0.1 cm/min.







Conclusions and Future Works

- Techniques for the fabrication and application of a novel Zn^{2+} microsensor were established for measuring Zn^{2+} concentrations in the phloem of citrus leaves.
  - Improved double barrel sensor design improved durability and simplified application to citrus plants
  - Further work on the LIX membrane selectivity needs to be addressed (Nitrate and copper interferences)

- An innovative micro-conductivity sensor was developed and applied to the midrib of citrus leaf as a surrogate method for detecting the movement of Zn^{2+} ions.

- Moving forward, the developed microsensors will be tested for their efficacy in laboratory and field settings using citrus seedlings (model system)

  - Tracking systemic movement of Zn
  - Estimating phloem concentration of Zn between spray applications
  - Estimating the half life of Zn chelate