

# Fusarium & Verticillium Wilts in Spinach: Research Update

Wilbur-Ellis/Alf Christianson Seed Co. Seed Growers' Mtg  
6 February 2013, Burlington, WA

Lindsey du Toit, Mike Derie, Emily Gatch, & Barbara Holmes  
WSU Mount Vernon NWREC



# Fusarium wilt of spinach

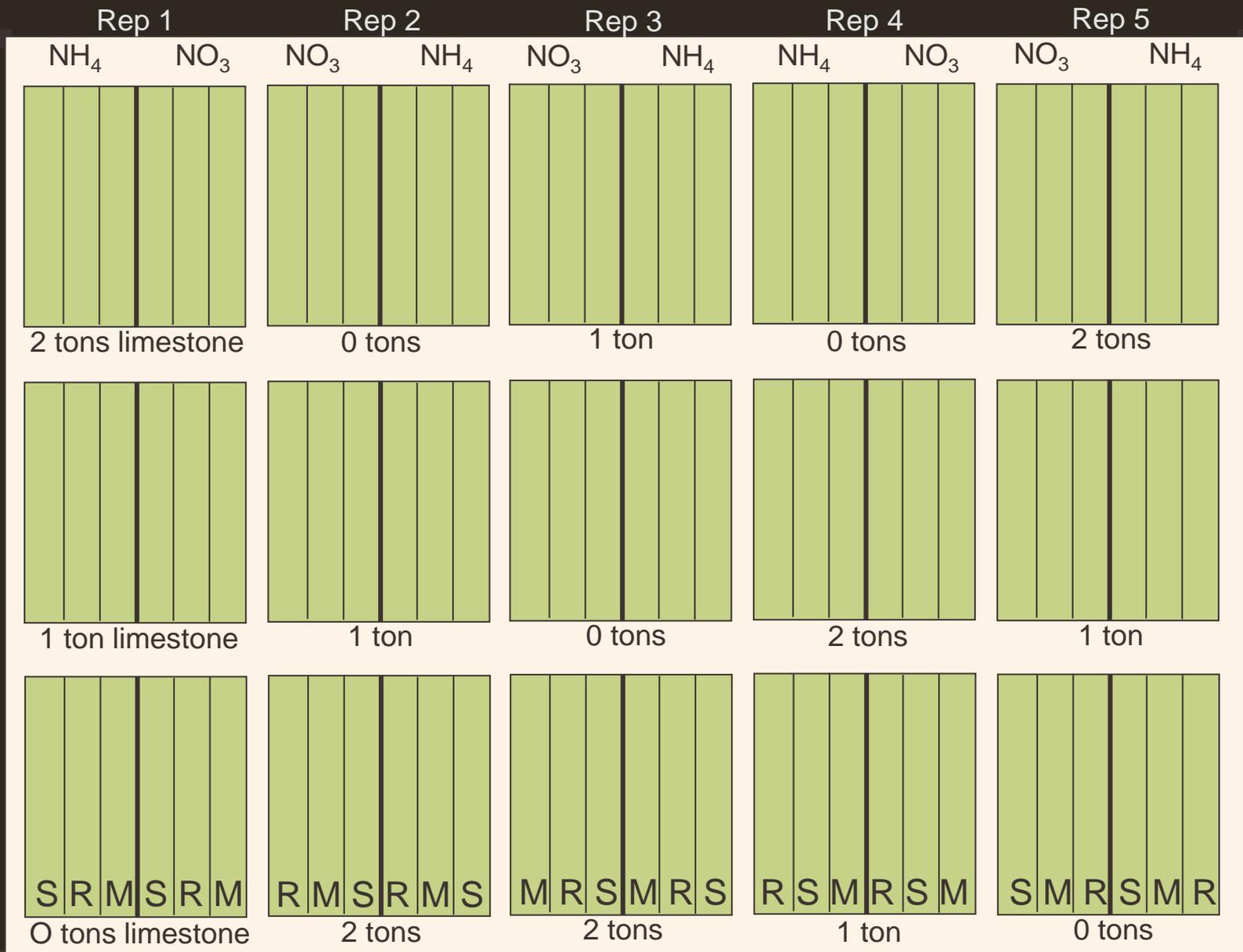
*Fusarium oxysporum* f. sp. *spinaciae*



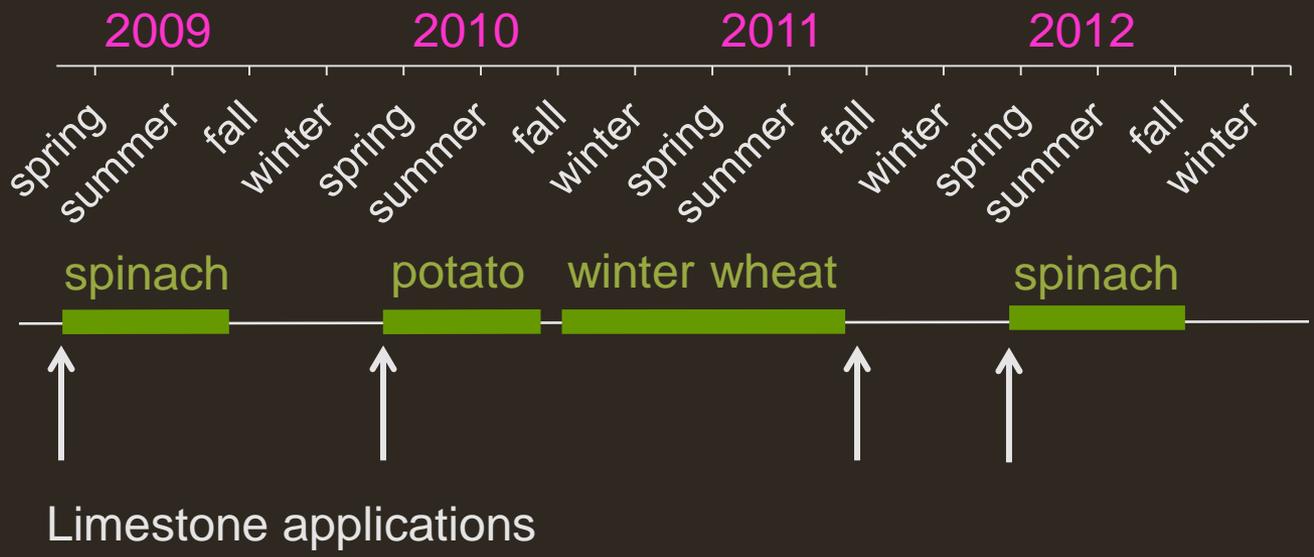
# Emily Gatch's PhD Research Objectives

1. Assess potential for enhancing suppression of Fusarium wilt using annual applications of limestone and nitrate-N fertilizers, and the effects of limestone on *Verticillium dahliae*.
2. Develop a soil bioassay for spinach seed growers to determine the relative risk of a field for Fusarium wilt, using spinach parent lines with different levels of resistance.
3. Determine the effects of micronutrients and pH on *F. oxysporum* f. sp. *spinaciae* and host-pathogen interactions.

# 2009 Field Trial







# 2012 Field Trial

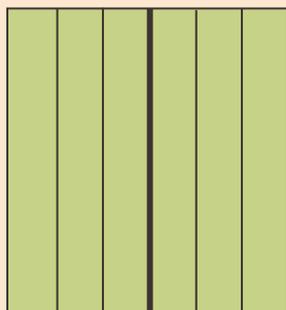
Rep 1

Rep 2

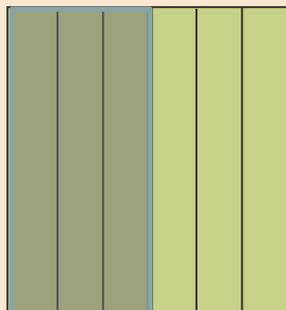
Rep 3

Rep 4

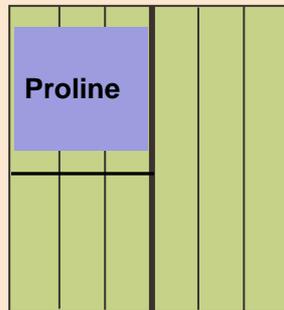
Rep 5



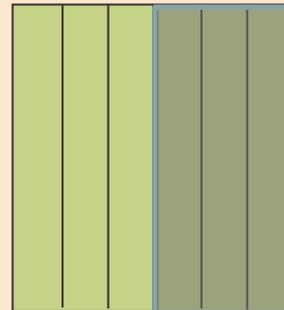
2 tons limestone



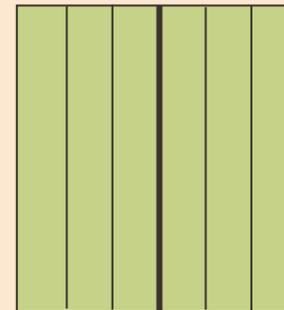
0 tons



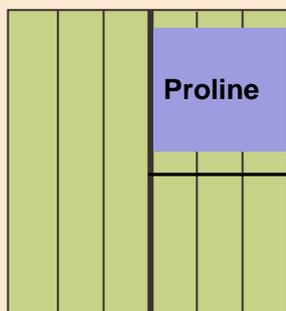
1 ton



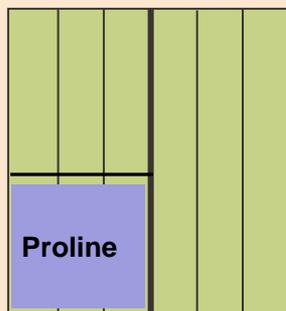
0 tons



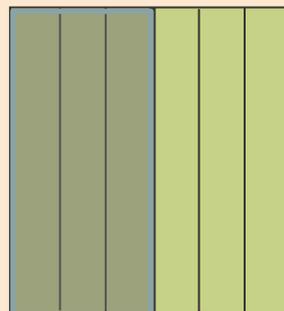
2 tons



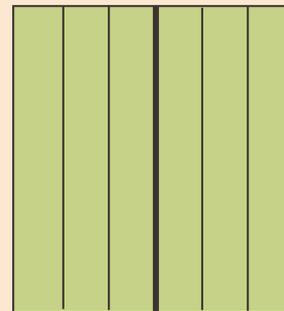
1 ton limestone



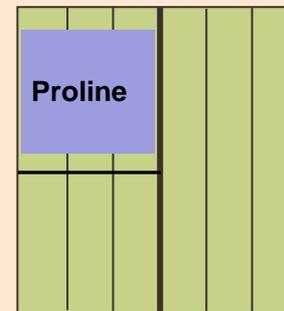
1 ton



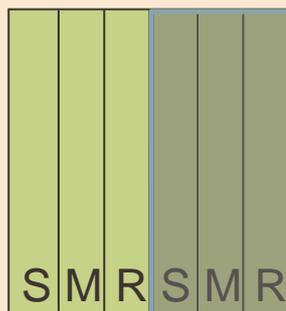
0 tons



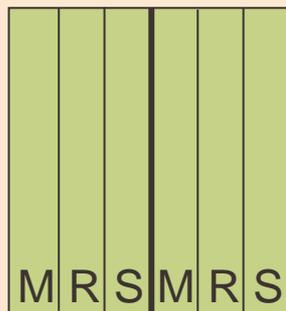
2 tons



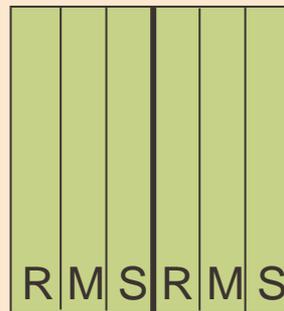
1 ton



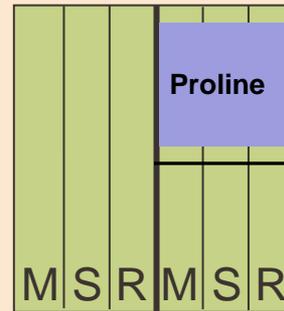
0 tons limestone



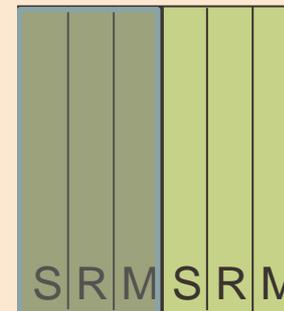
2 tons



2 tons



1 ton



0 tons



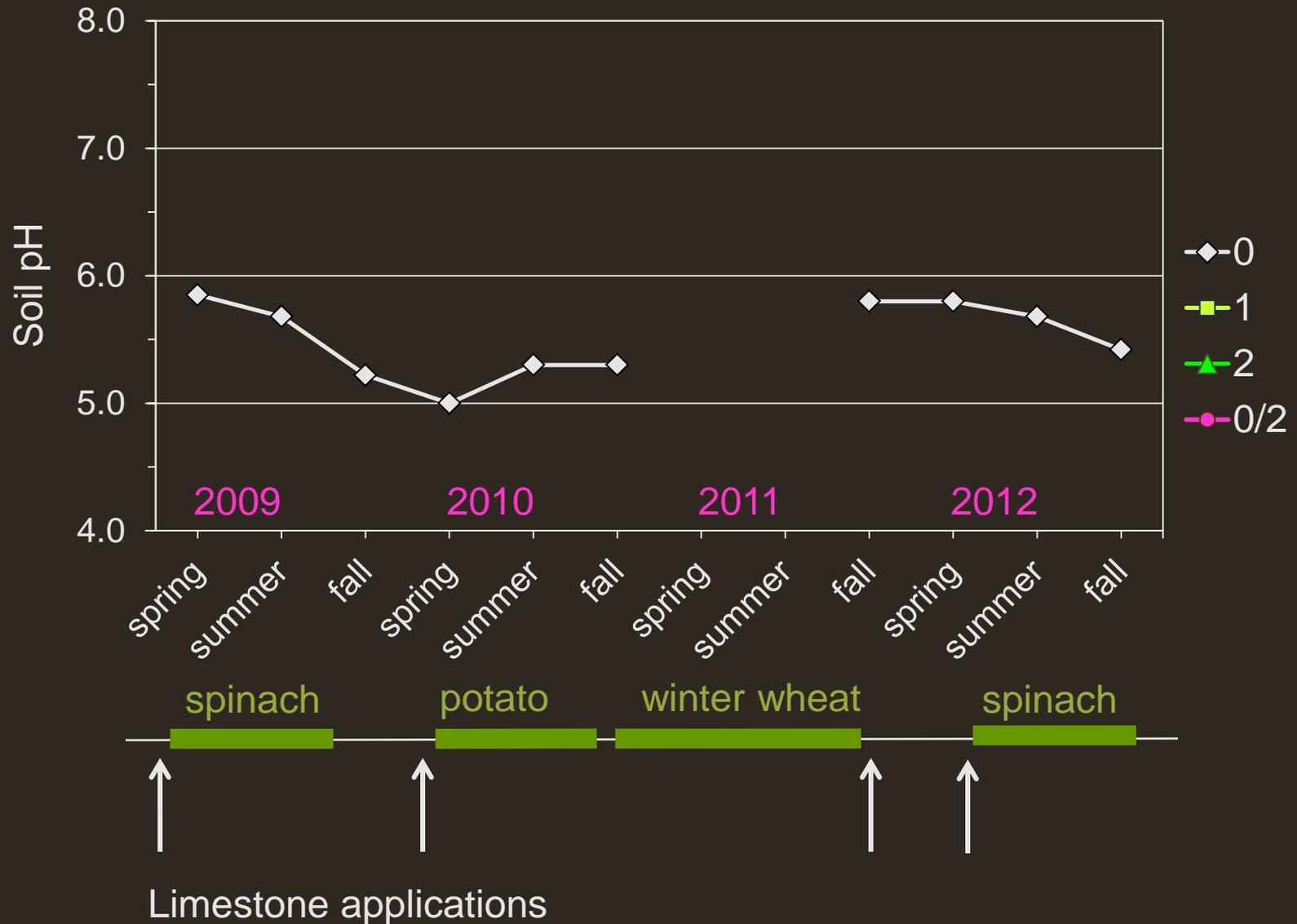
2 tons limestone/acre each year

1 ton limestone/acre each year

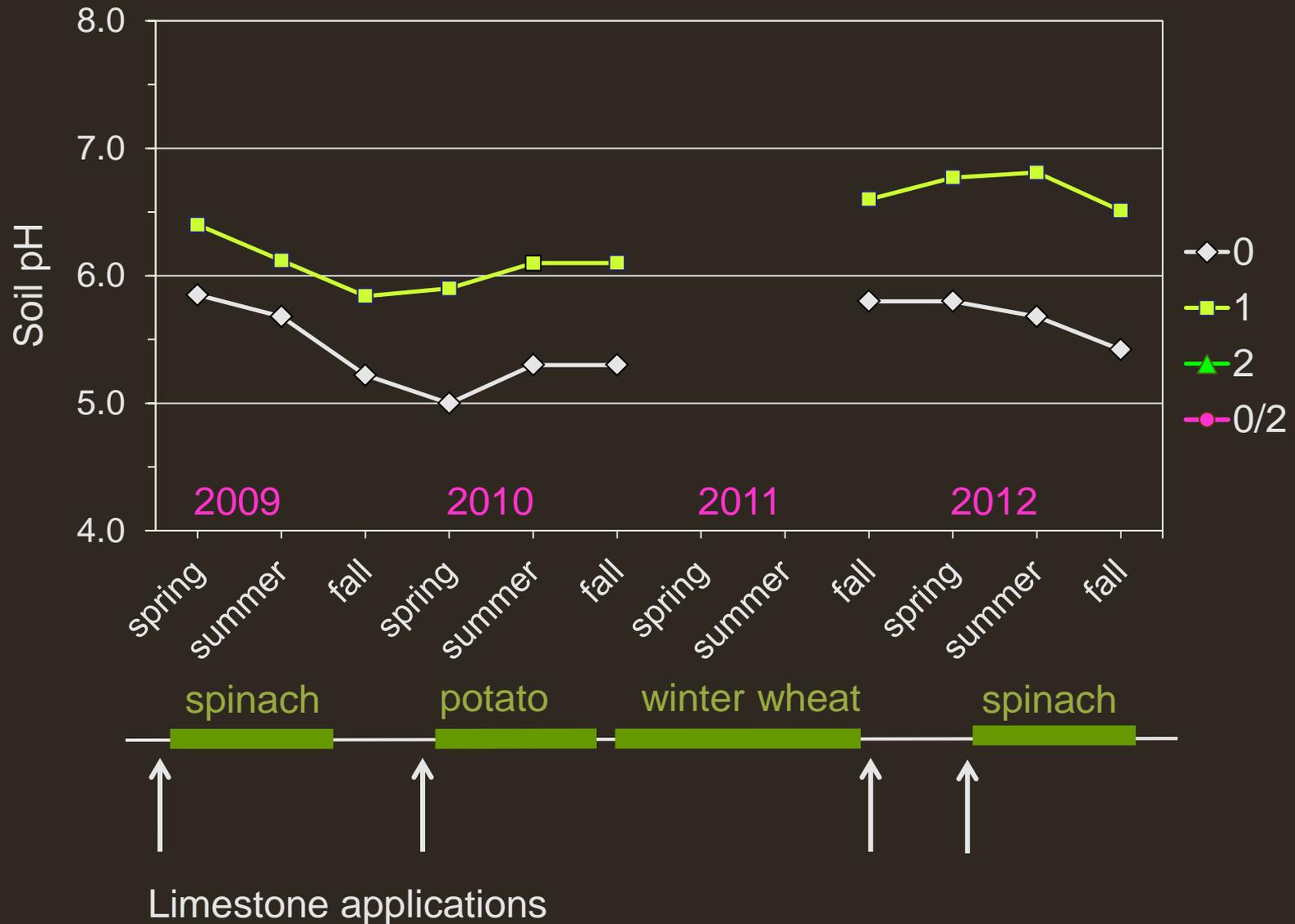
0 tons limestone/acre each year

0 tons limestone/acre each year  
+ 2 tons/acre in spring 2012

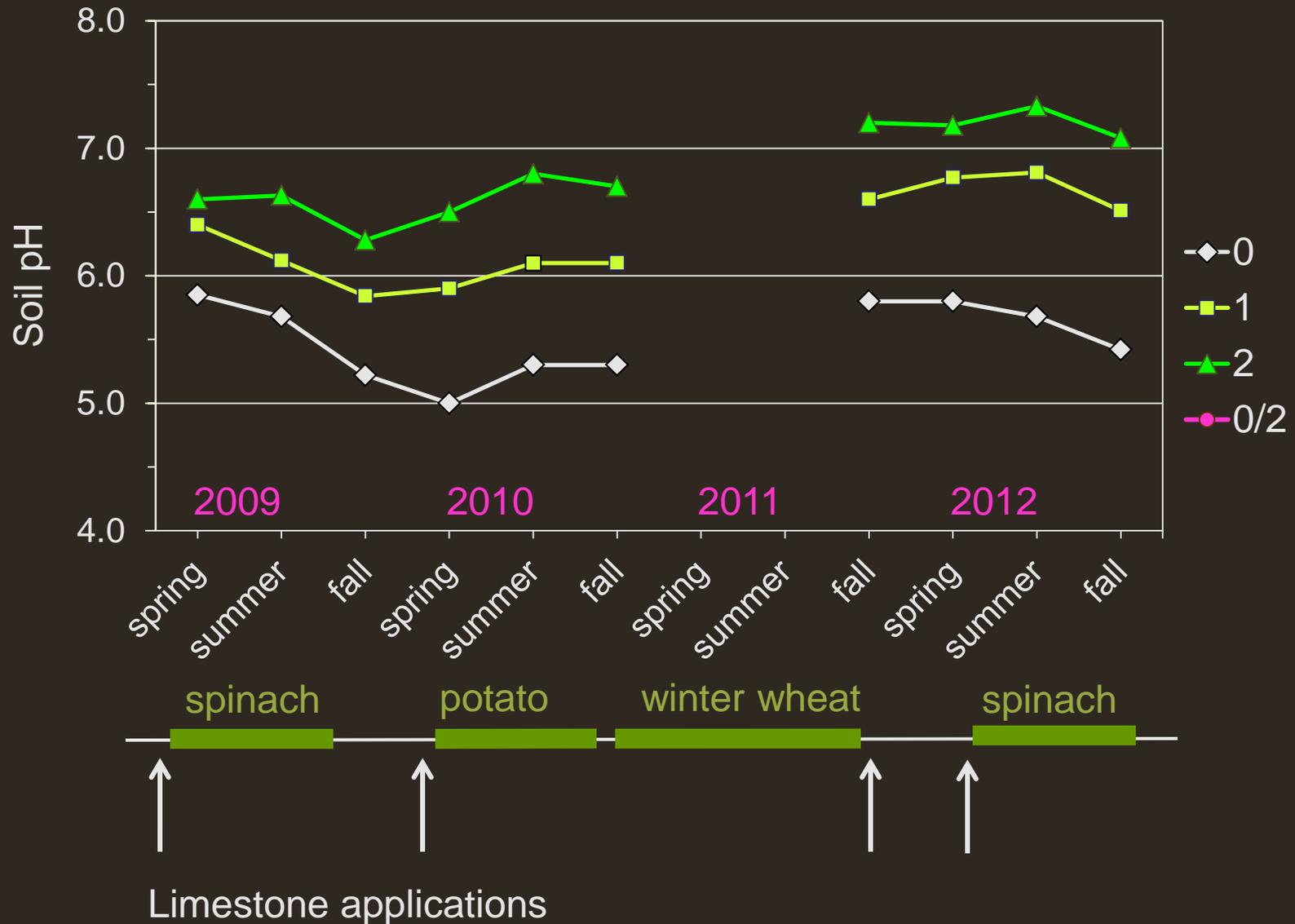
# Effect of limestone rate on soil pH: 2009-2012



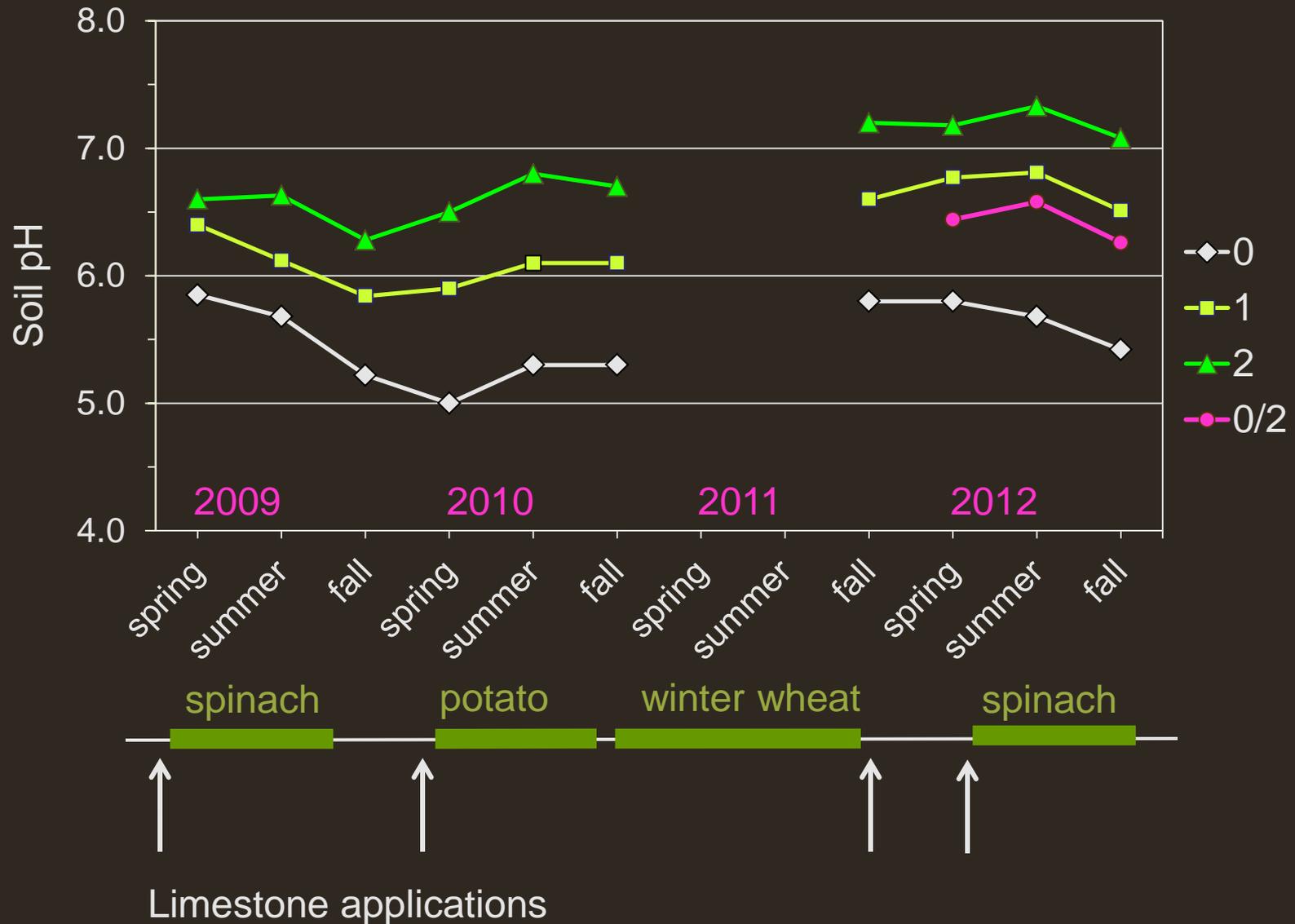
# Effect of limestone rate on soil pH: 2009-2012



# Effect of limestone rate on soil pH: 2009-2012



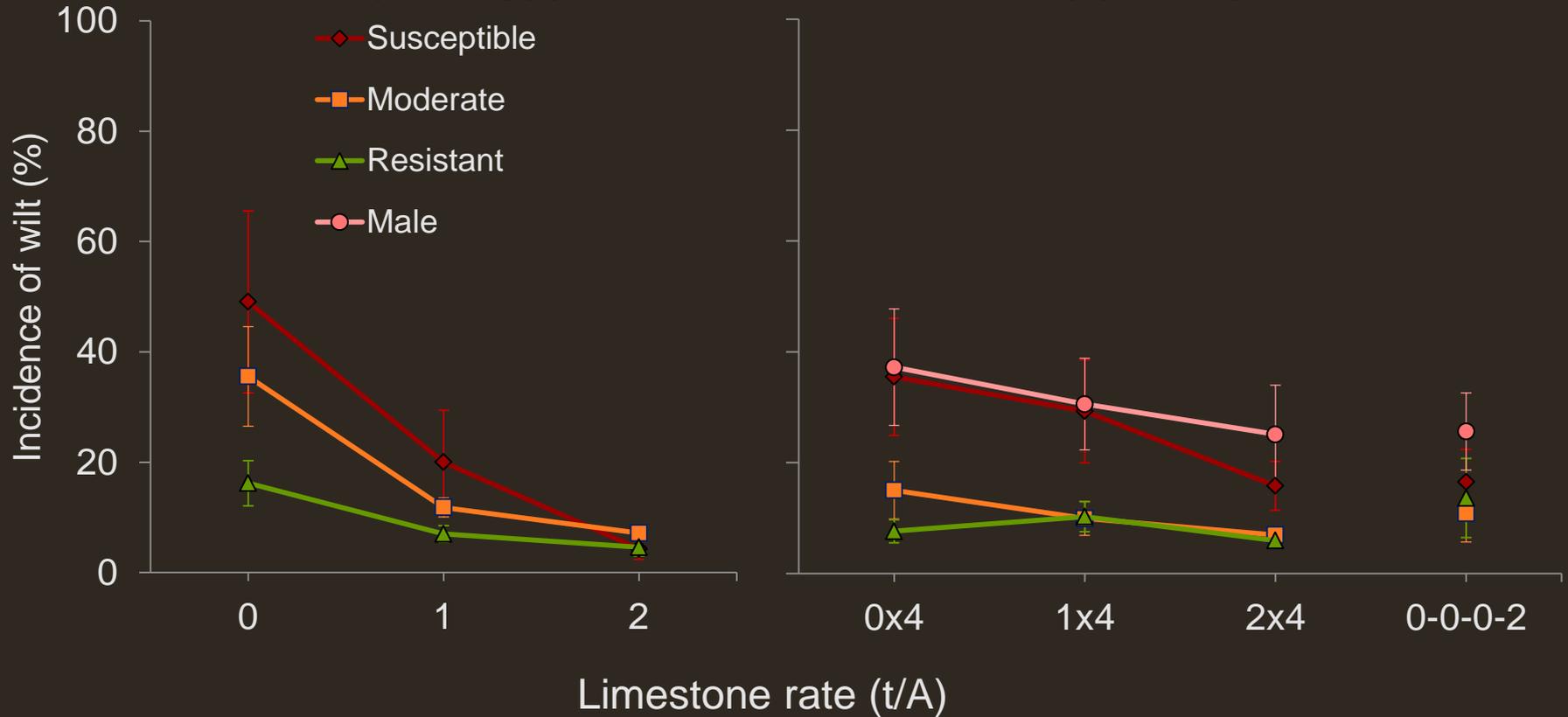
# Effect of limestone rate on soil pH: 2009-2012



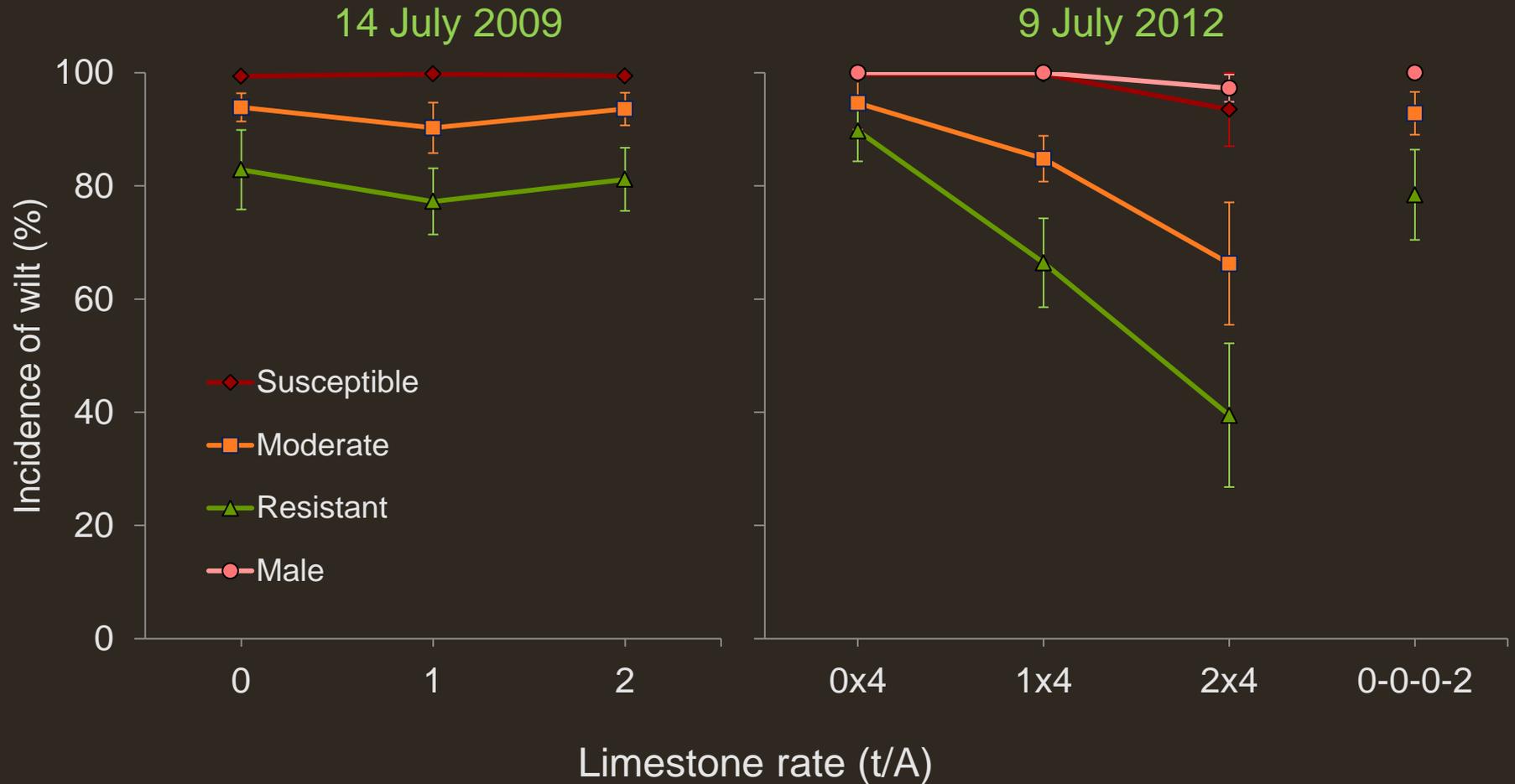
# Wilt incidence

24 June 2009

19 June 2012

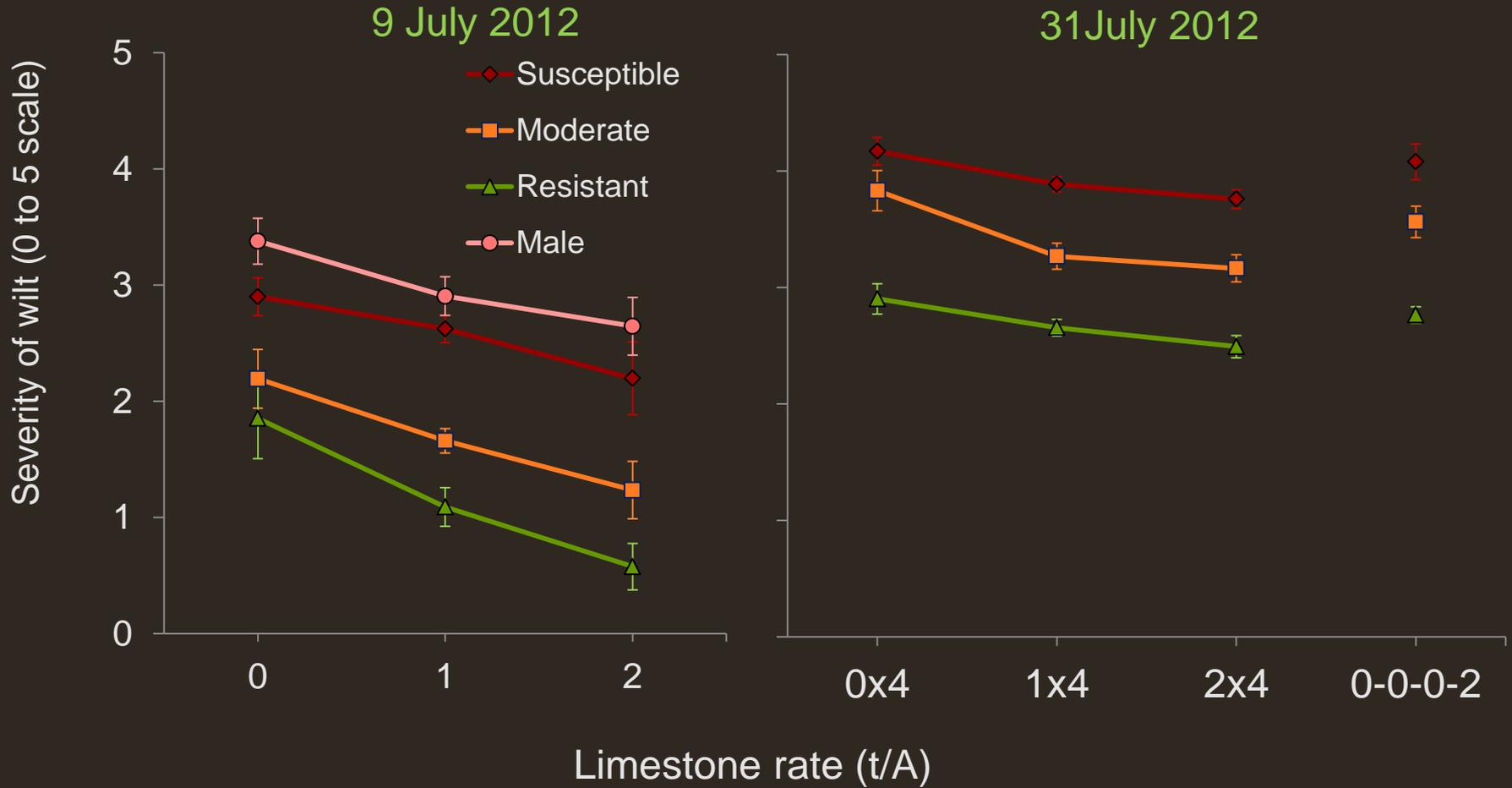


# Wilt incidence





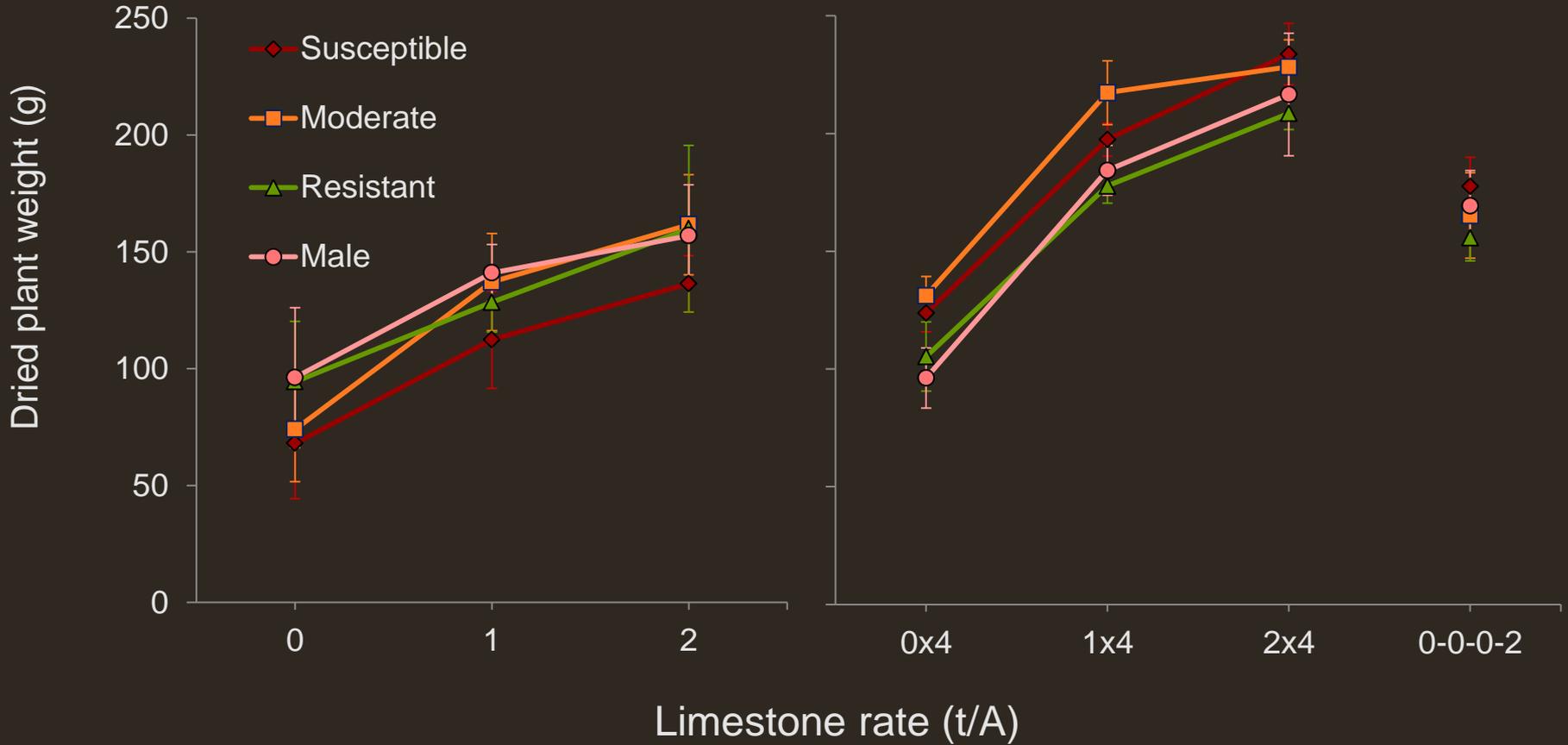
# Wilt severity



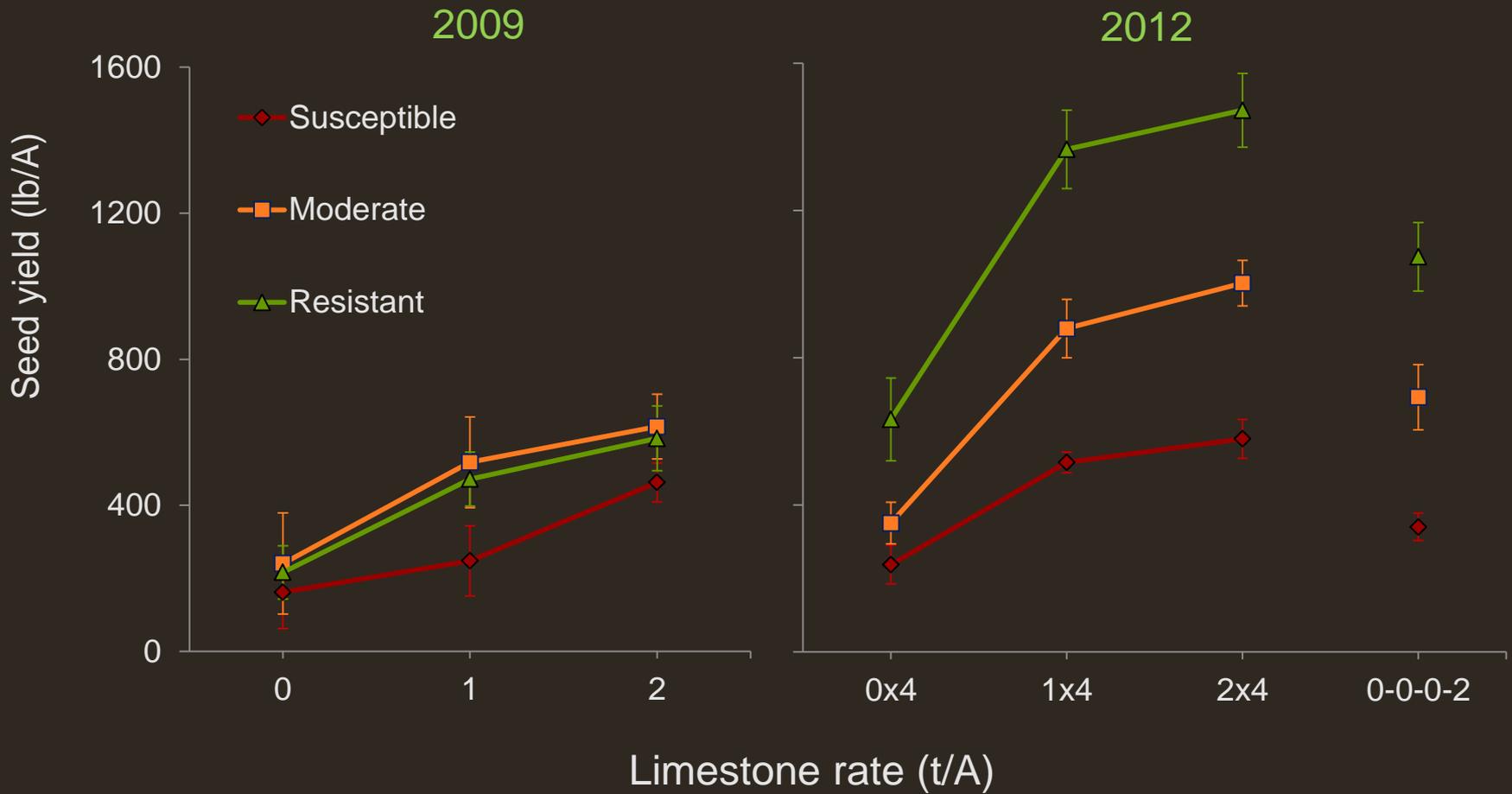
# Spinach biomass (dry plant weight)

22 July 2009

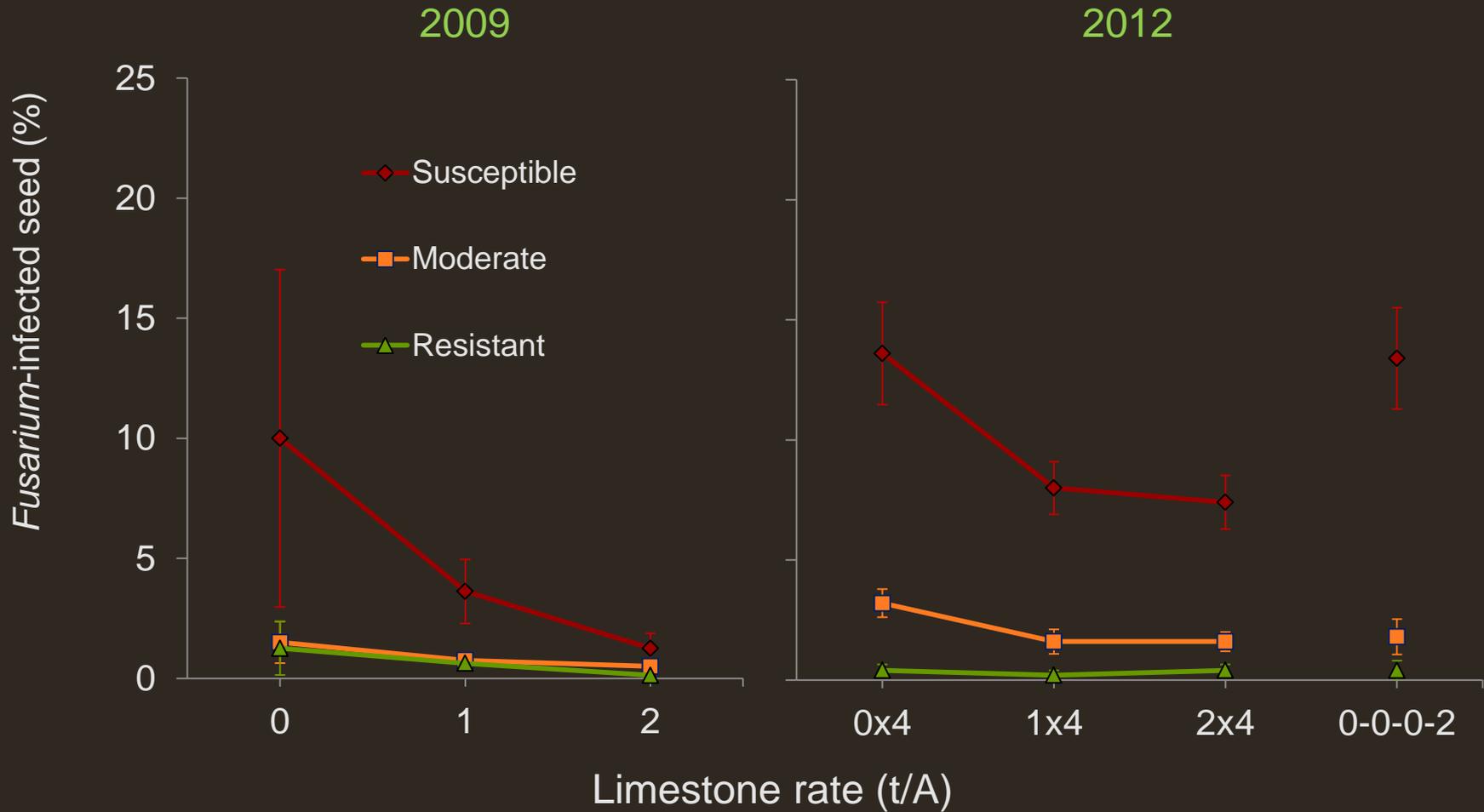
11 July 2012



# Seed yield



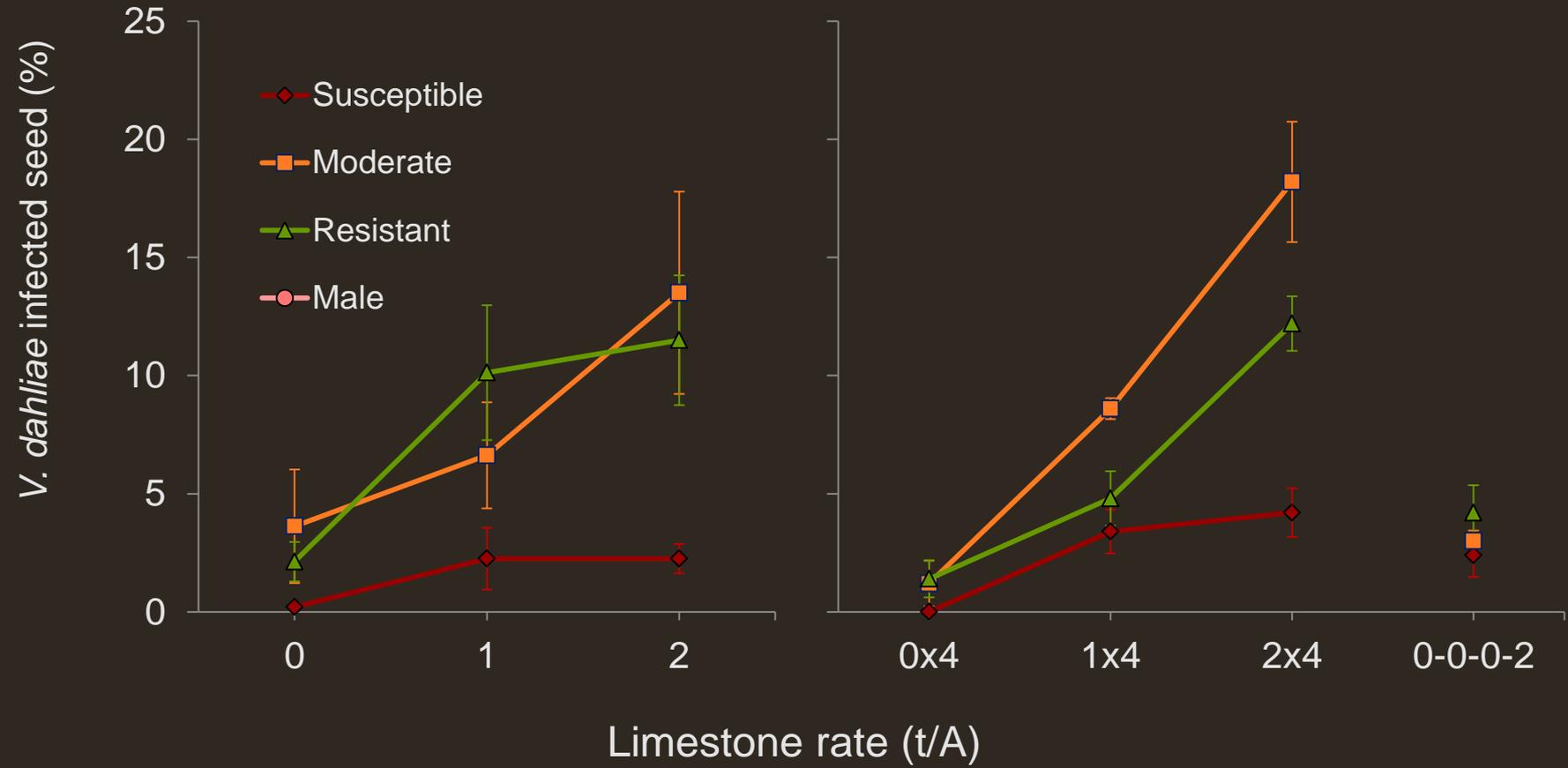
# Harvested seed (%) infected with *Fusarium*



# Harvested seed (%) infected with *Verticillium dahliae*

2009

2012



# Research Objectives

2. Develop a soil bioassay for spinach seed growers to determine the relative risk of a field site for Fusarium wilt, using spinach lines with different levels of resistance.
  - Wilt ratings
  - Spinach biomass
  - Quantify *F. oxysporum* and *V. dahliae* on selective media
  - Soil nutrient analyses: macro & micronutrients, pH, OM, CEC
  - Texture analysis
  - # of years out of spinach seed crops
  - Quantify *F. oxysporum* f. sp. *spinaciae* with a real-time PCR assay

2009-10: 26 soils submitted  
2010-11: 42 soils submitted  
2011-12: 39 soils submitted  
2012-13: 40 soils submitted

Control soils included each year:

Low (no), medium, and high Fusarium wilt risk



Soil A



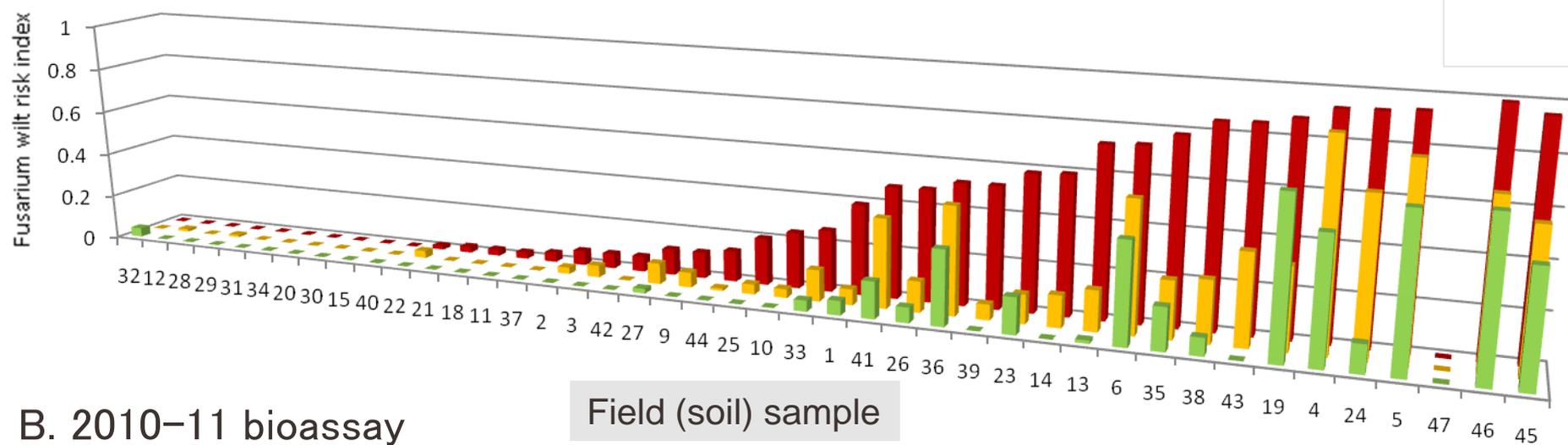
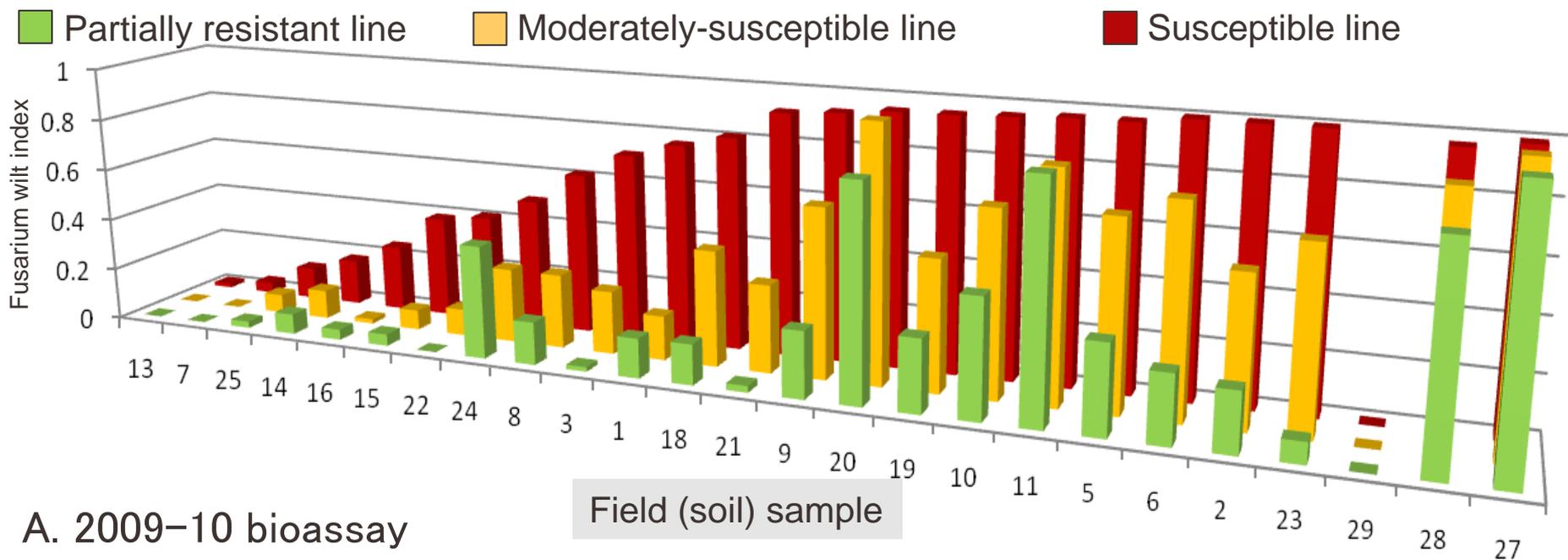
Soil C



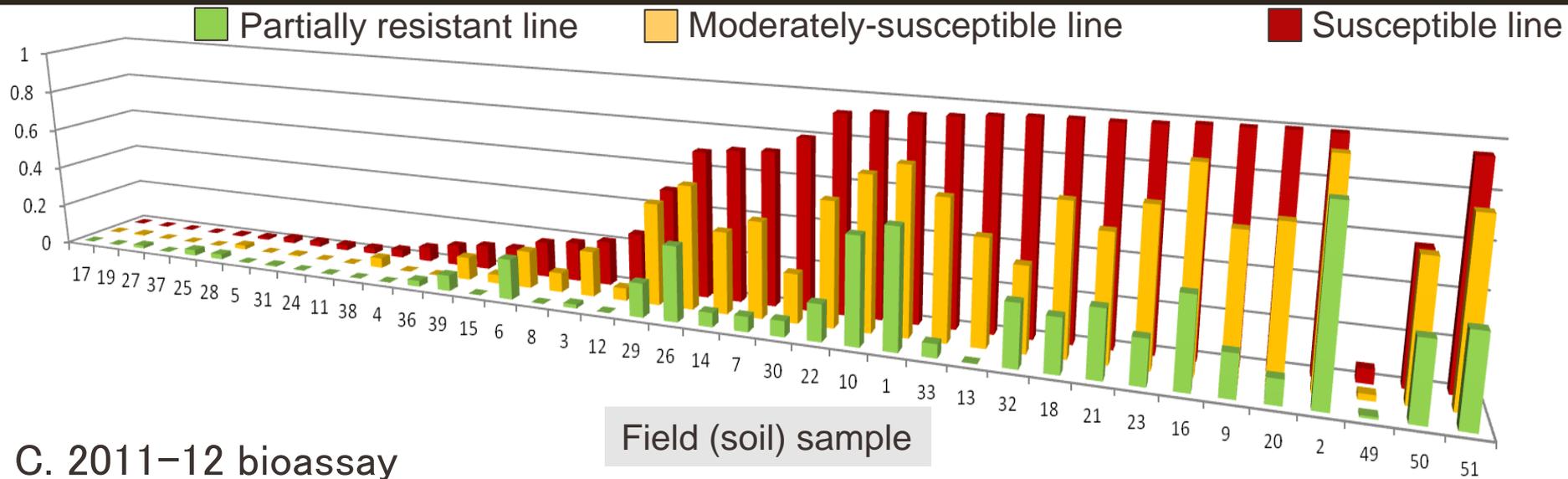
Soil B



# Risk assessment: Spinach Fusarium wilt soil bioassay



# Risk assessment: Spinach Fusarium wilt soil bioassay



C. 2011-12 bioassay

# Bioassay validation



# Acknowledgements

**Puget Sound Seed Growers' Association**

Washington State Commission for Pesticide Registration

Alfred Christianson Family Endowment, Robert MacDonald Fund

**USDA Western Region IPM, Western SARE**

Participating growers and seed companies



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**Dan Nelson, Russ Duckworth**

**Mike Derie, Barbara Holmes,**

**Avi Alcala, Sarah Meagher,**

**Ron Dralle, Jonathan Roozen,**

**Martha Sudermann,**

**John Kuhn, Anita da Costa,**

**Shawn Watkinson, Tammy Ennen,**

**Nathan McCartney, Tyler VanderPol**

# Evaluation of Proline for Management of Fusarium Wilt in Spinach Seed Crops



# Proline and Fusarium Wilts

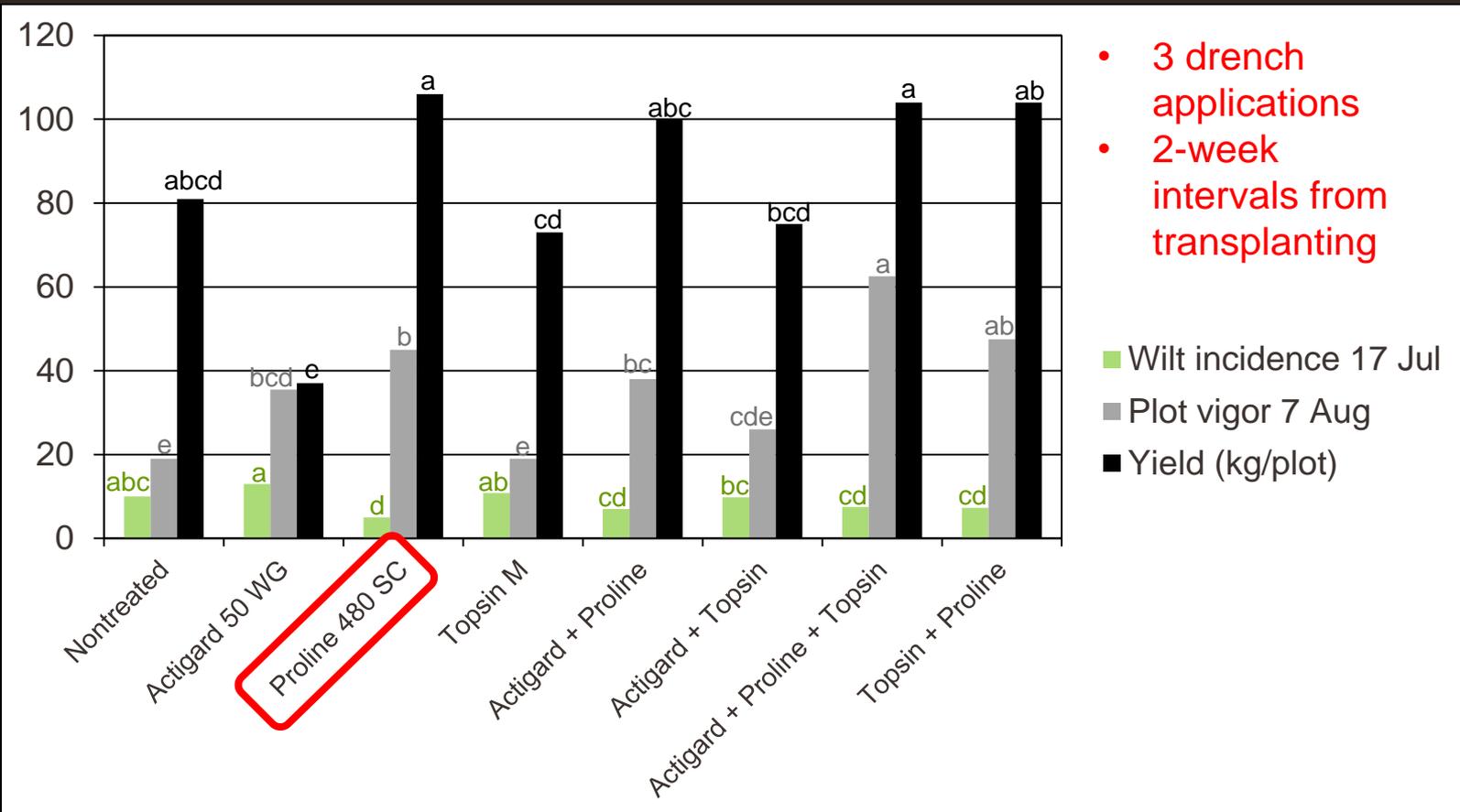
Proline 480 SC = prothioconazole (triazole, Group 3)

Spinach seed crops in DK: empirical observations?

Transplanted watermelon Fusarium wilt: drench, drip trials

Zhou & Everts. 2009. PDMR 3:V092; Zhou et al. 2010. PDMR 4:V065

Everts et al. 2011. Phytopathology 101:S50-S51 (Abstract)



# 2012 Spinach Seed Crop Fusarium Wilt Field Trial

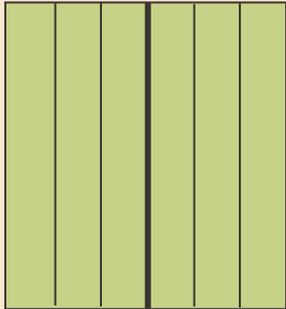
Rep 1

Rep 2

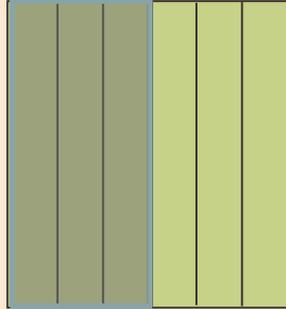
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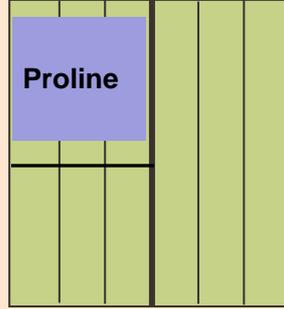
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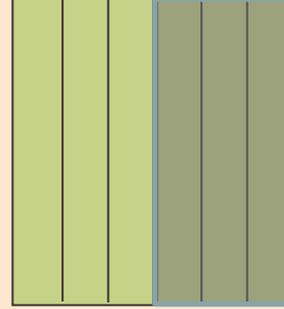
2 tons limestone



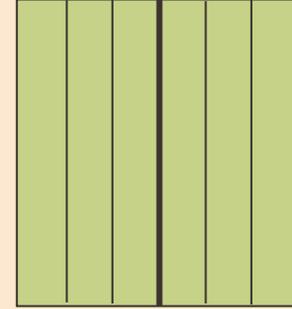
0 tons



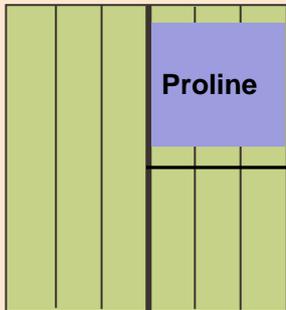
1 ton



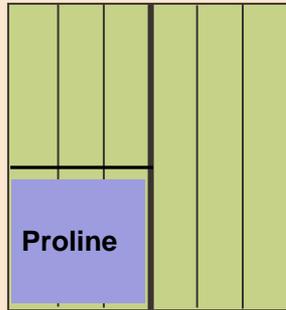
0 tons



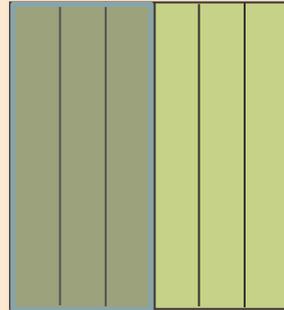
2 tons



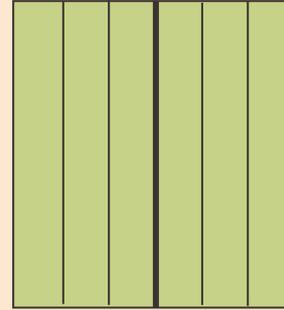
1 ton limestone



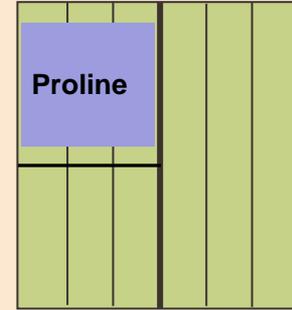
1 ton



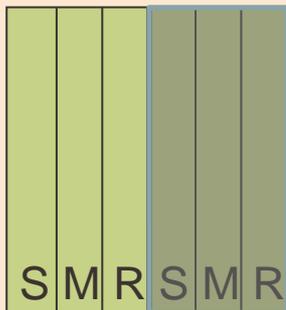
0 tons



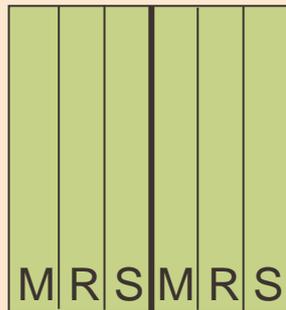
2 tons



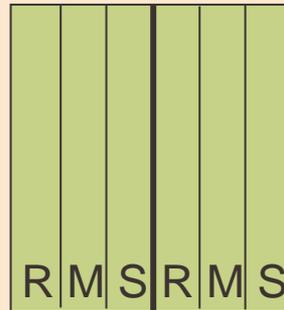
1 ton



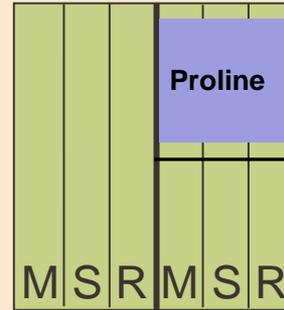
0 tons limestone



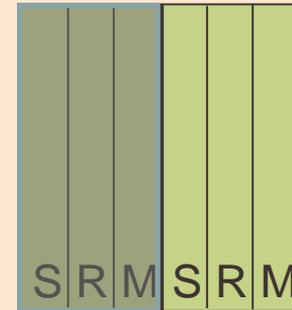
2 tons



2 tons



1 ton



0 tons

S M R S M R

M R S M R S

R M S R M S

M S R M S R

S R M S R M

# Banded Proline applications ( $\text{CO}_2$ -backpack sprayer)

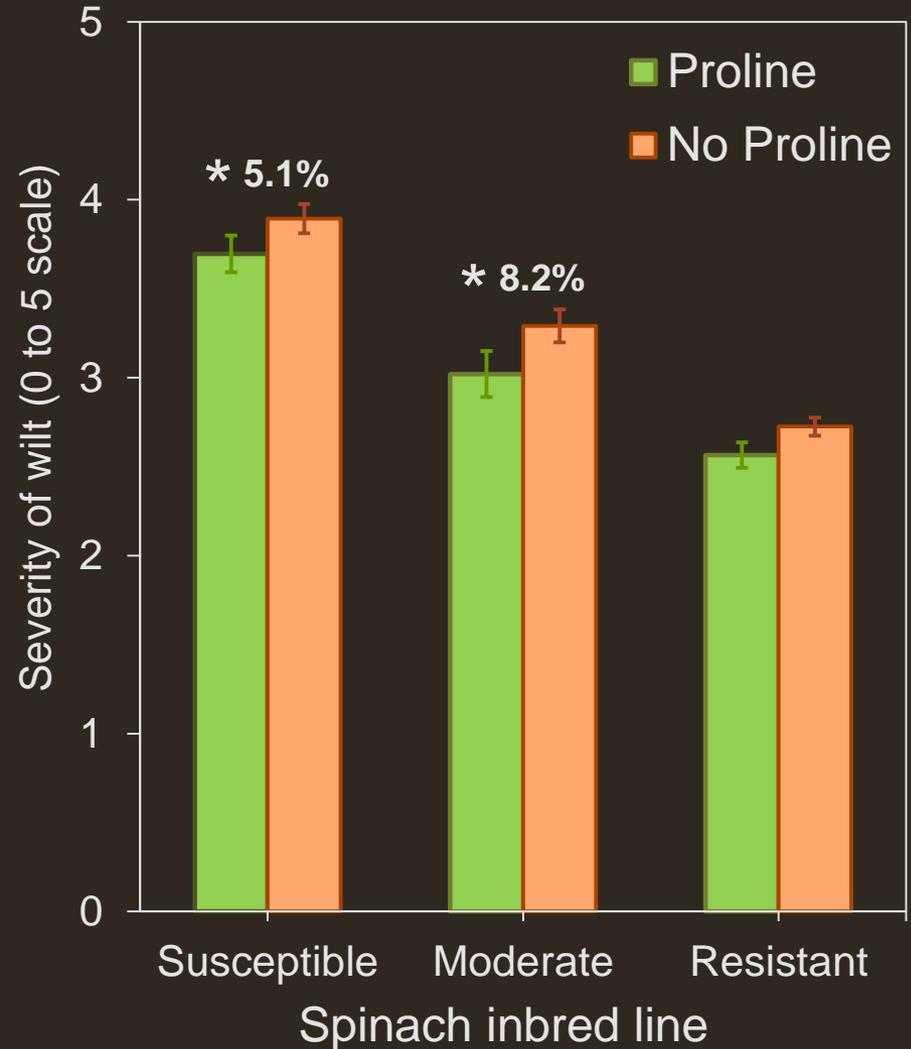
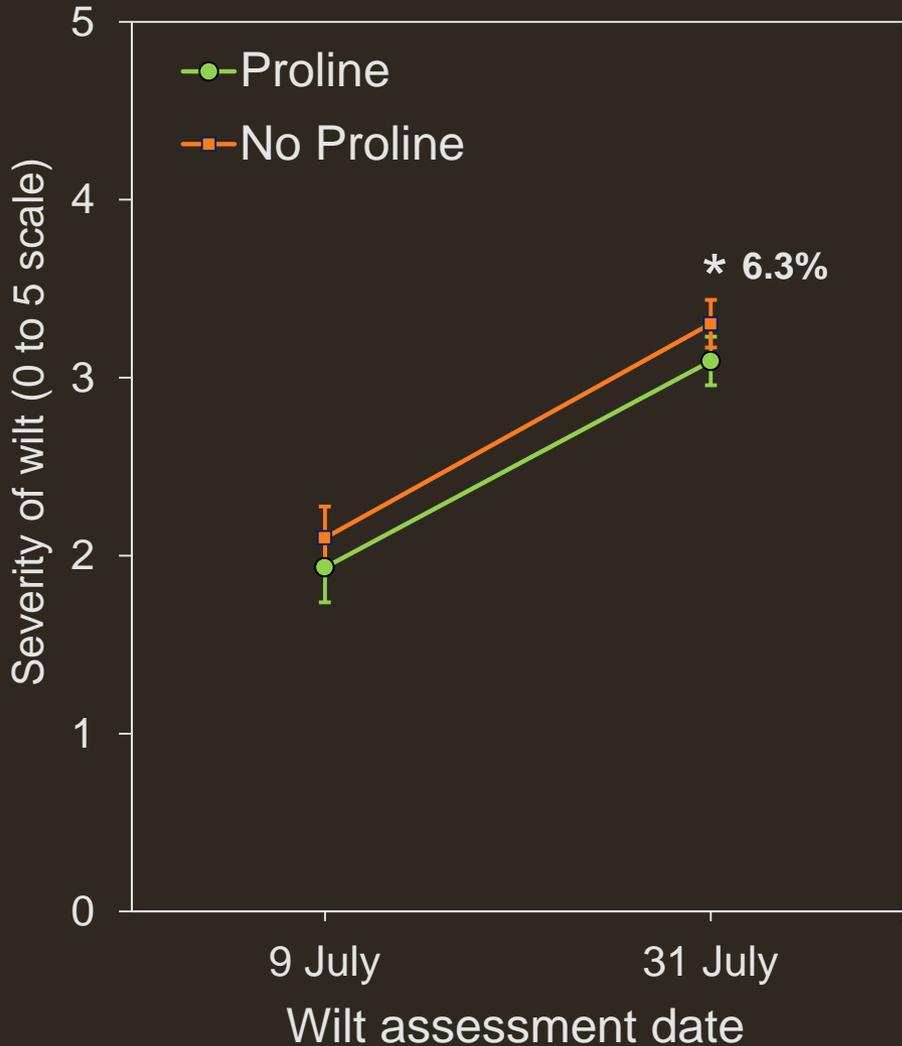
10 May = 2 days after planting spinach

31 May = 3 weeks after planting

21 June = 6 weeks after planting

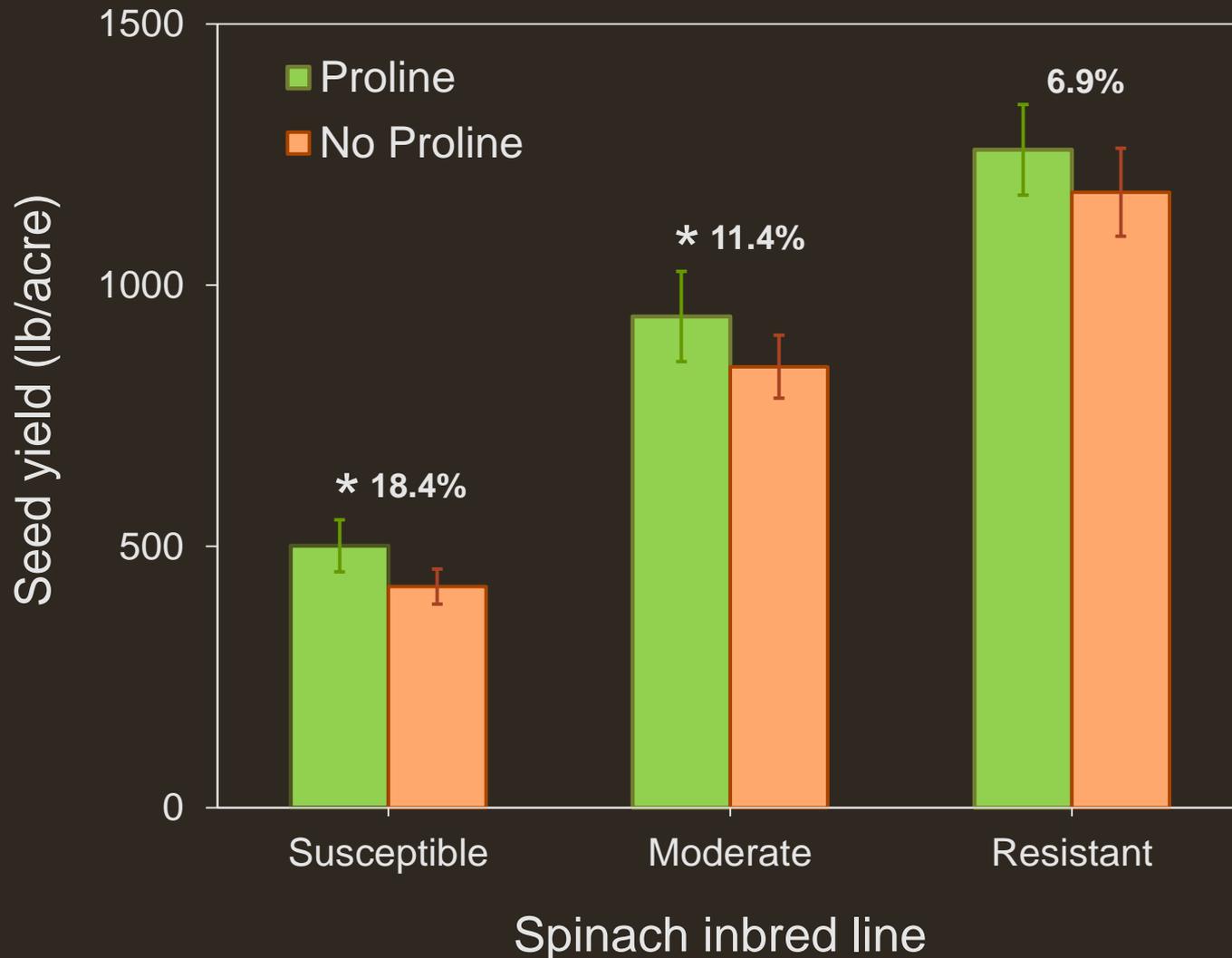


# Effect of Proline on severity of wilt



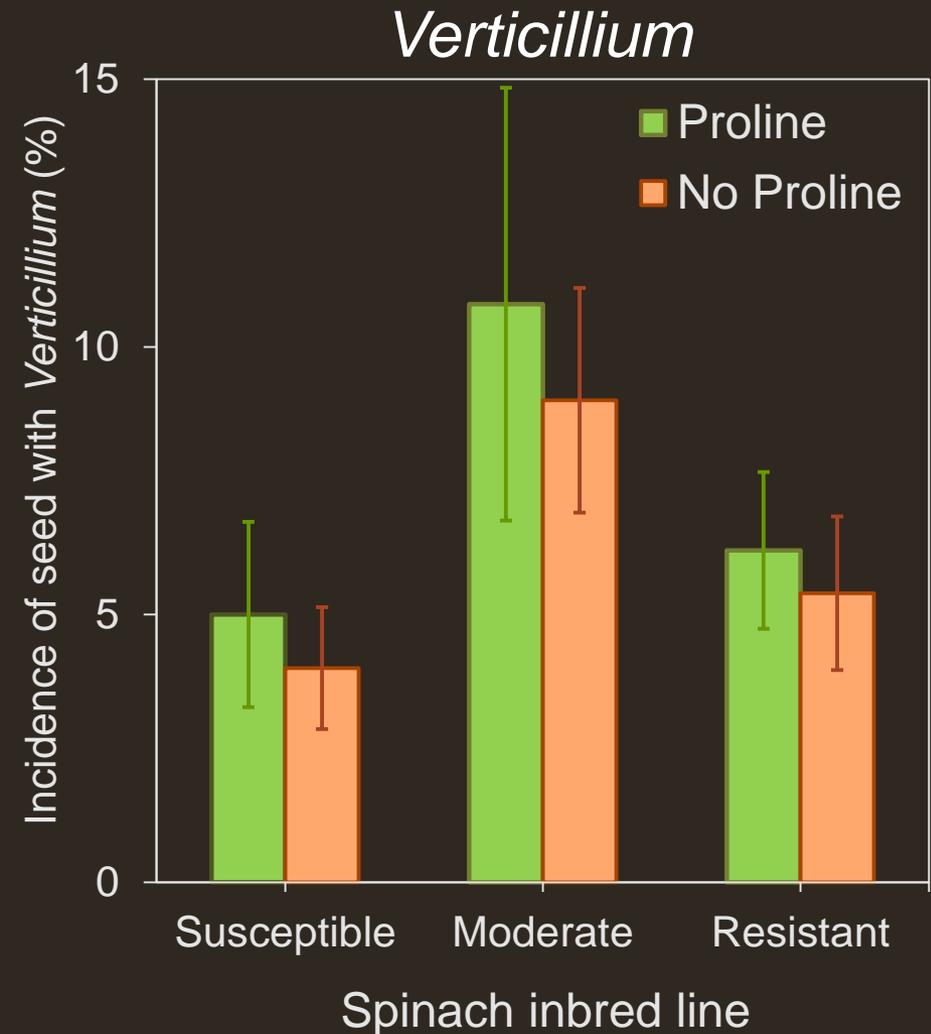
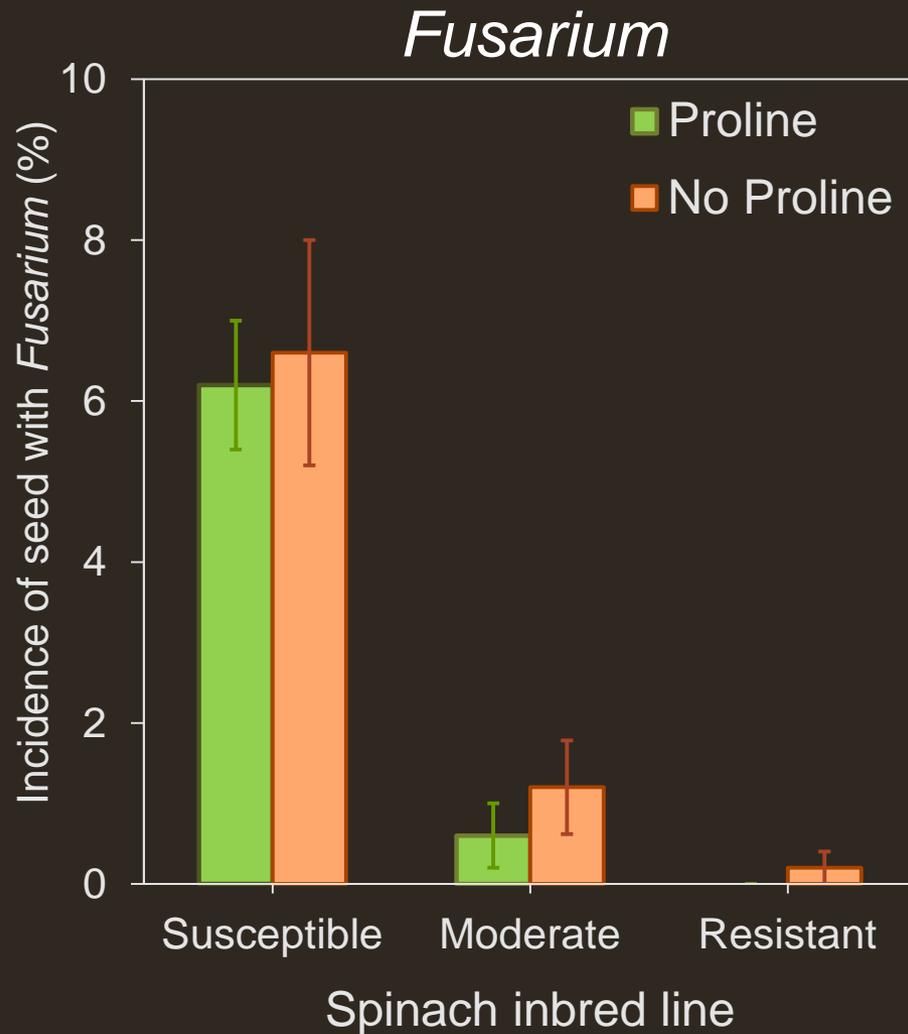
\* Denotes significant difference between Proline vs. no Proline plots at  $P \leq 0.05$

# Effect of Proline on seed yield



\* Denotes significant difference between Proline vs. no Proline at  $P \leq 0.05$

# Effect of Proline on incidence of harvested seed infected with *Fusarium* and *Verticillium*



# 2013 Spinach Seed Crop Fusarium Wilt Proline Field Trial

- Field: acid soil, spinach seed crop last 4-5 yrs, proximity to NWREC
- Treatments:
  - Proline vs. no Proline
  - 3 female spinach inbred lines (S, M, R)
- 2 tons limestone/acre (all plots)
- Apron + Thiram + Topsin seed treatment
- Proline applications:
  - Method: In-furrow drench + banded soil applications
  - Number: 3 = maximum (sugar beet)
  - Interval: 14-21 days

# 2013 Spinach Fusarium Wilt Proline Field Trial

Suggestions? Questions?  
[dutoit@wsu.edu](mailto:dutoit@wsu.edu) or 360-848-6140



# Management of *Verticillium* in Spinach



**Lindsey du Toit & Mike Derie, Washington State University**  
**Jim Correll, University of Arkansas**



AUG 1 2



SEP 3 2008

# *Verticillium* associated with spinach seed

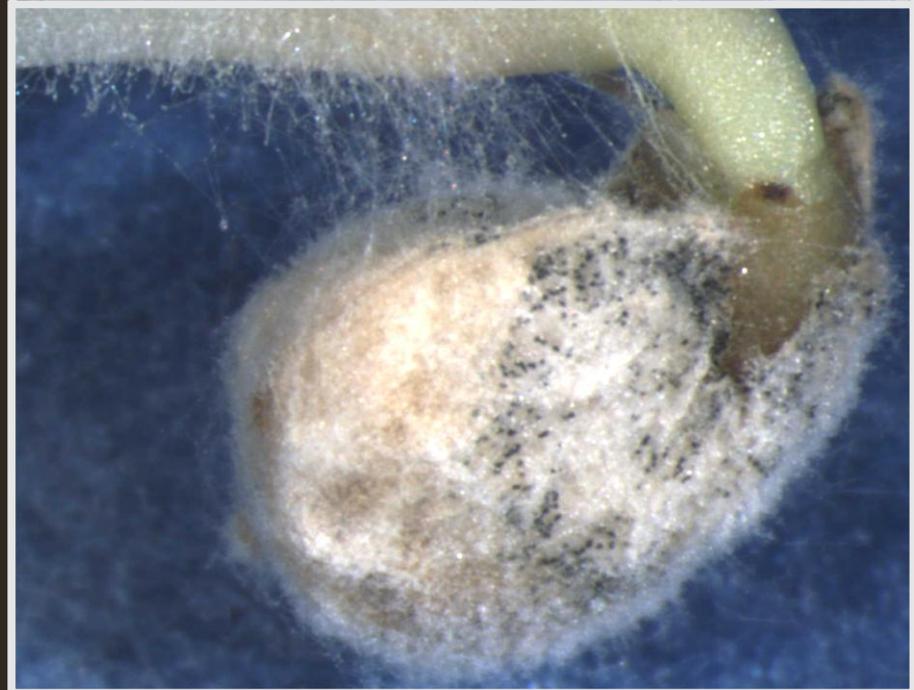
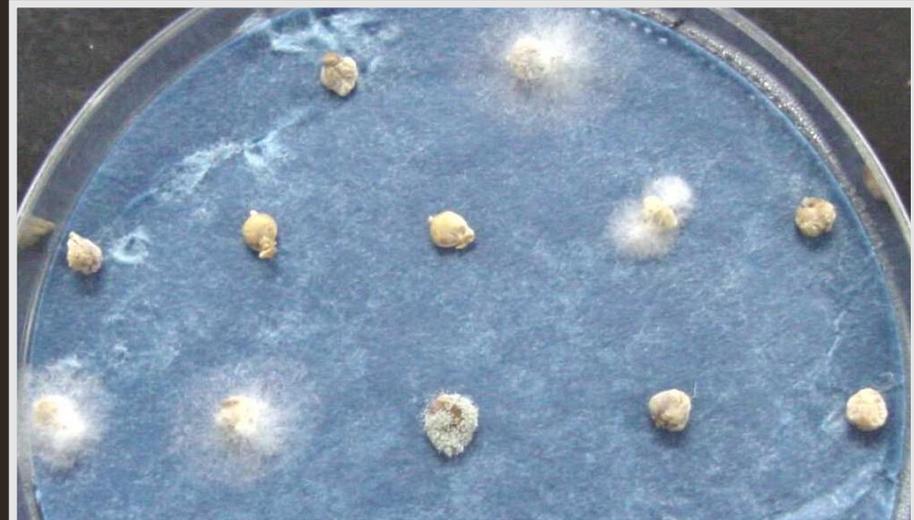
1. Prevalence of *Verticillium* in commercial spinach seed lots (90% of lots tested: US, EU, NZ, Chile)
2. Pathogenicity of seed isolates to spinach
3. Seed-to-seed transmission

Plant Disease 89:4-11

Plant Disease 89:1305-1312

PDMR 1:ST003; PDMR 3:ST020;

PDMR 4:ST038



# Spinach/lettuce connection?



Atallah et al. 2010. *Fungal Genetics & Biology*.

Gene flow among *V. dahliae* isolates from CA lettuce, pepper, strawberry, artichoke & weeds, spinach seed, & WI ornamentals (not tomato)

Relative significance of infested spinach seed?

# Evaluation of spinach seed treatments

A. Hot water & chlorine (du Toit & Hernandez-Perez, 2005. Plant Disease 89:1305-1312)

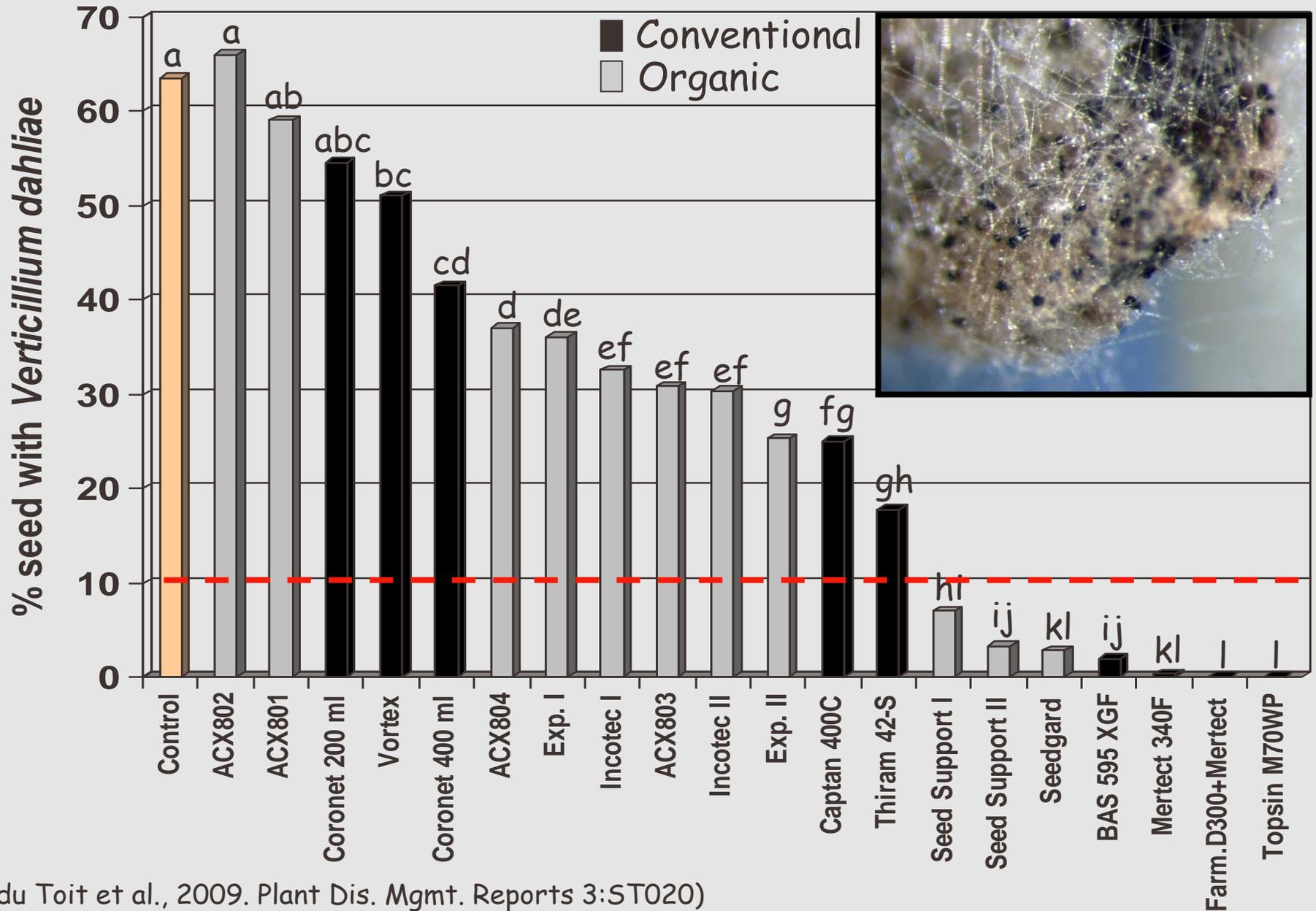
B. Efficacy of spinach seed treatments for managing seedborne *Verticillium* (du Toit et al., 2009. PDMR 3:ST020, du Toit et al., 2010. PDMR 4:ST038)

1. Reduce incidence of seed infected/infested
2. Reduce rate of seed transmission
3. Reduce rate of soil infestation from planting infested seed



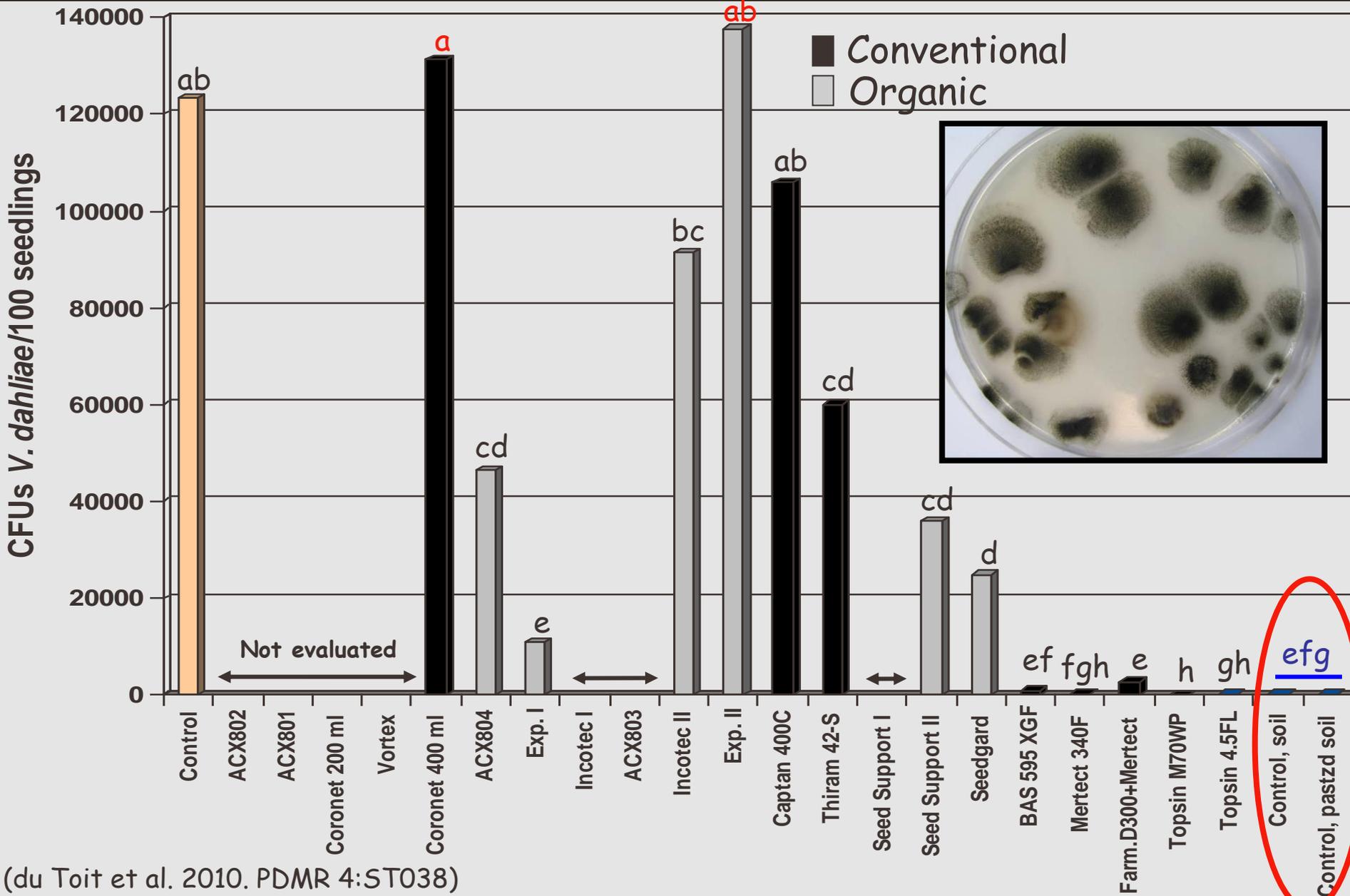
C. Influence of soil fumigation

# GH Trials: % Seeds with *Verticillium* - Trial 1



(du Toit et al., 2009. Plant Dis. Mgmt. Reports 3:ST020)

# GH Trials: Seed transmission assay - Trial 2



(du Toit et al. 2010. PDMR 4:ST038)

# Influence of soil fumigation/pH on *Verticillium* seed transmission & soil infestation

2010 WA field trial of baby leaf spinach & head lettuce

1. Main plots:

MeBr:Chloropicrin (57:43% at 350 gpa)

No soil fumigation

2. Split plots:

Acid soil pH (~5.7-5.9)

5 tons limestone/ha (pH 6.6-6.8)

3. Spinach split-split plots:

No spinach seed

Baby leaf spinach: No seed treatment

Baby leaf spinach: Apron + Thiram

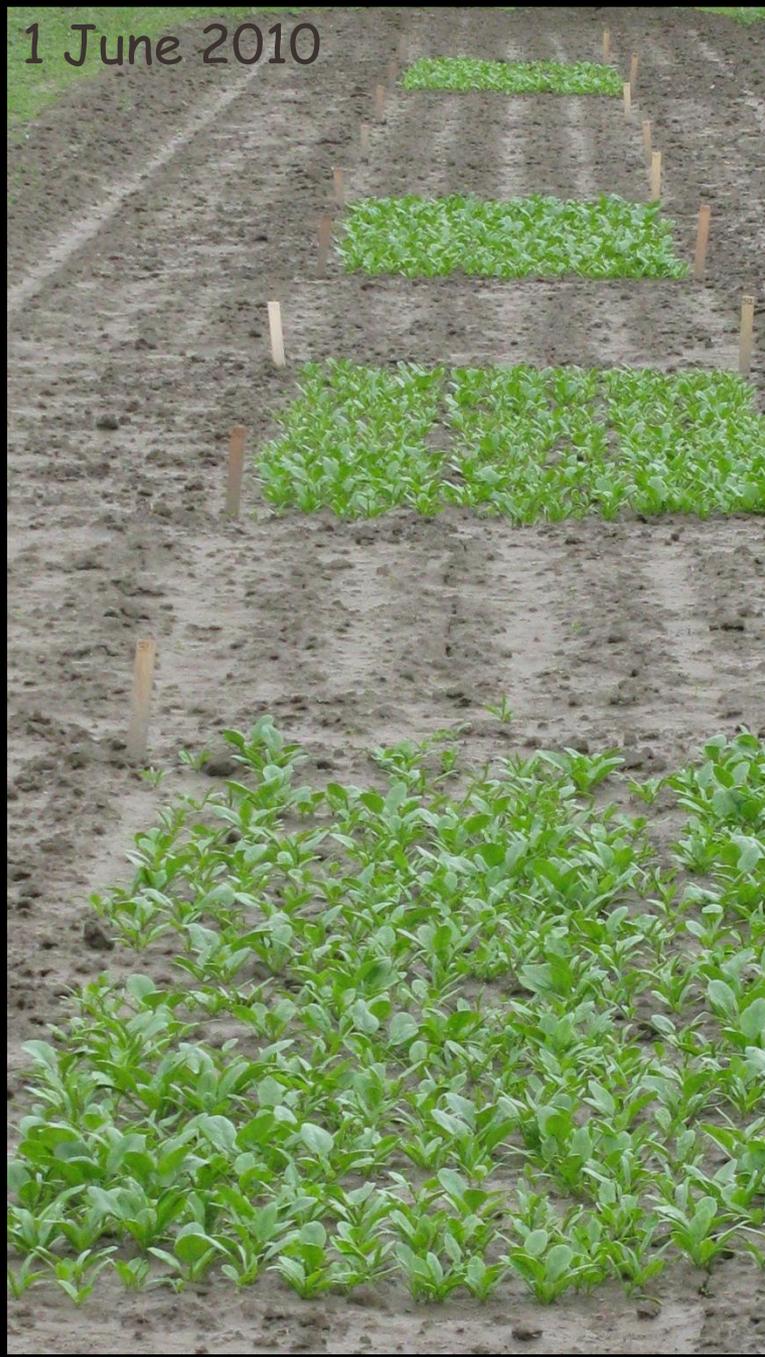
Baby leaf spinach: Apron + Thiram + Topsin 4.5 FL

Baby leaf spinach: Farmore D300 + Mertect 340F

Followed by head lettuce in each plot ('Salinas' type)



1 June 2010



14 June 2010



# 2011 & 2012 Field trials

Mount Vernon, WA (2011) & Salinas, CA (2011, 2012)

Baby leaf spinach & head lettuce, RCBD, 5 reps

## 1. Main plots:

Fumigated vs. non-fumigated

## 2. Spinach split plots:

No spinach

Non-treated seed

Apron + Thiram

Farmore D300 + Mertect 340F

Apron + Thiram + Topsin 4.5 FL (WA 2011)

Seed Support (WA 2011)

ProGro (WA & CA, 2011), ProGro + Apron (CA, 2012)

Followed by head lettuce in each plot

# Salinas, CA: 2011 & 2012

Ocean Mist Farms (2011)  
Christensen & Giannini (2012)  
New Fresh



Schafer Ag Services  
Holaday Seeds



Germain's Technology Group



Rachel

Yim

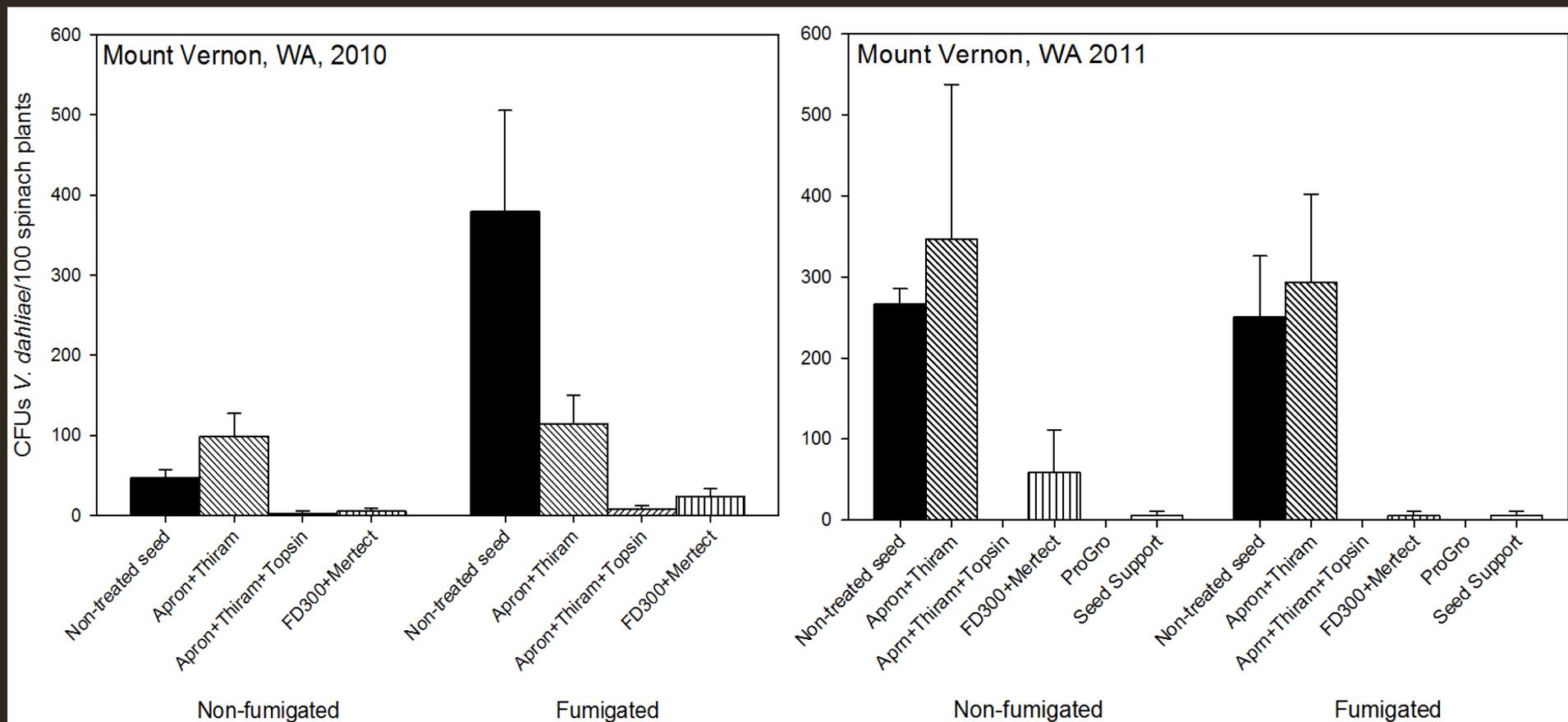
Bobby

Eugenia

# Mount Vernon, WA: 2010, 2011

## Effect of seed treatment & soil fumigation on seed transmission of *V. dahliae*

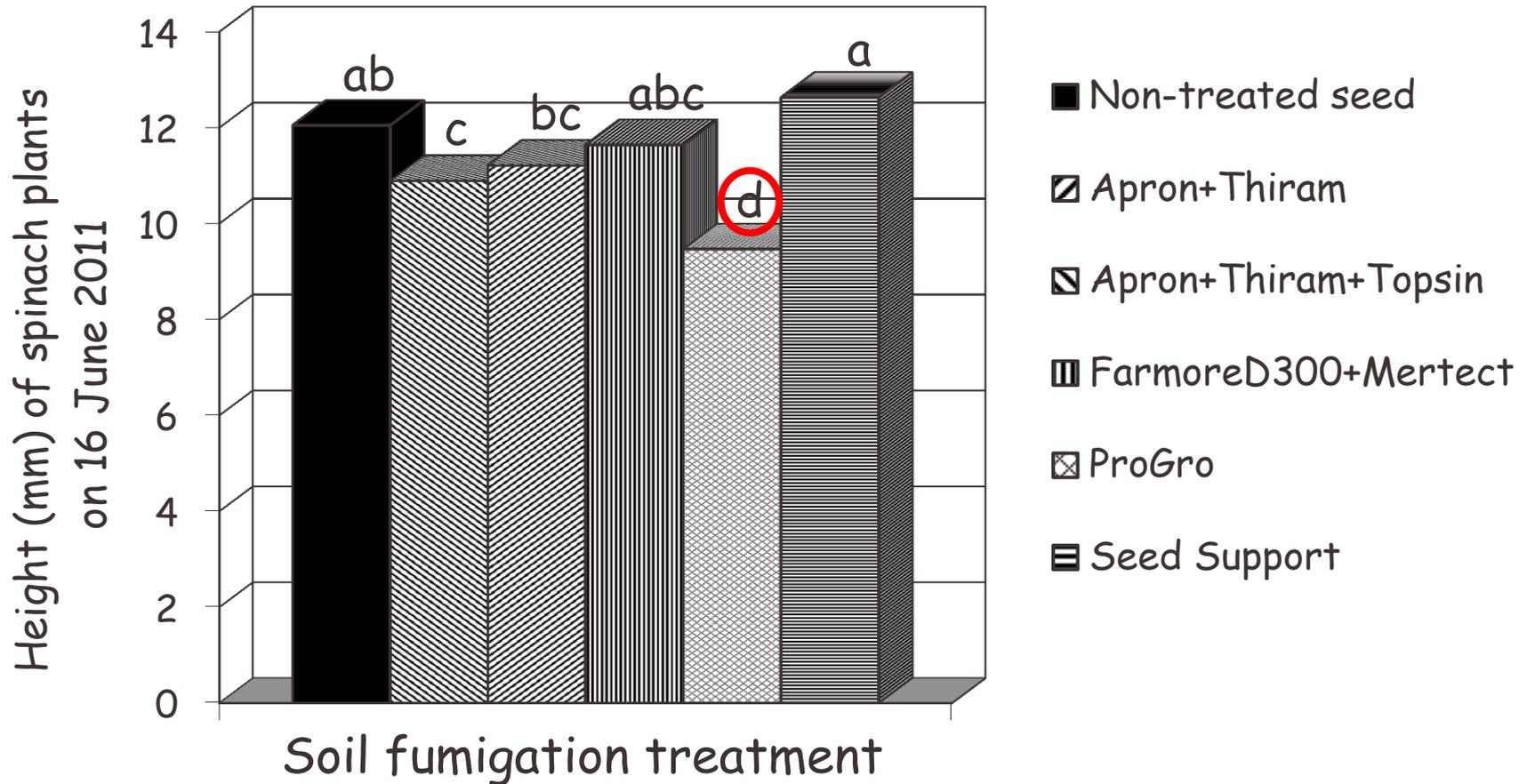
CFUs *V. dahliae*/100 spinach plants after harvest of leaves



Significant fumigation & seed treatment effects

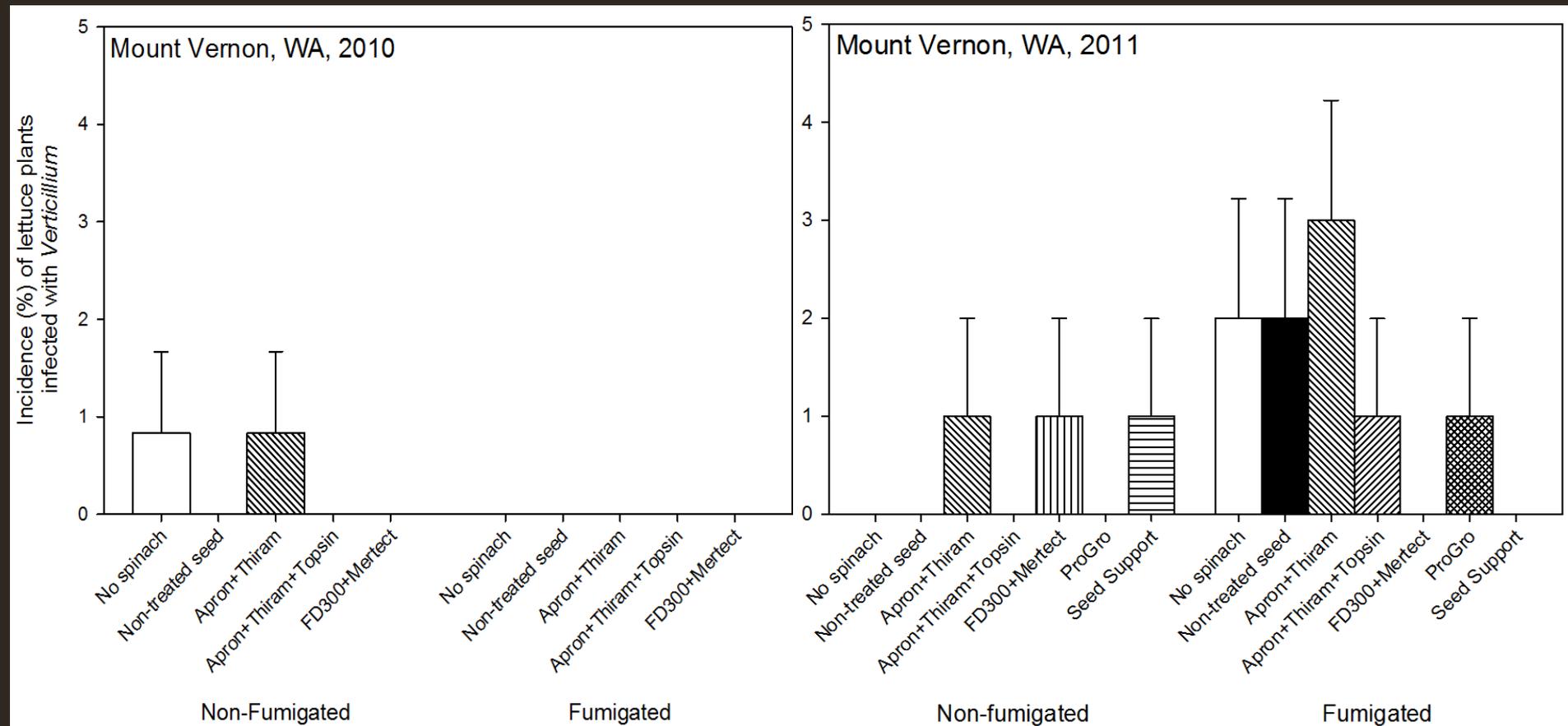
# Mount Vernon, WA: 2011

## Effect of seed treatment on spinach plant height



# Mount Vernon, WA: 2010, 2011

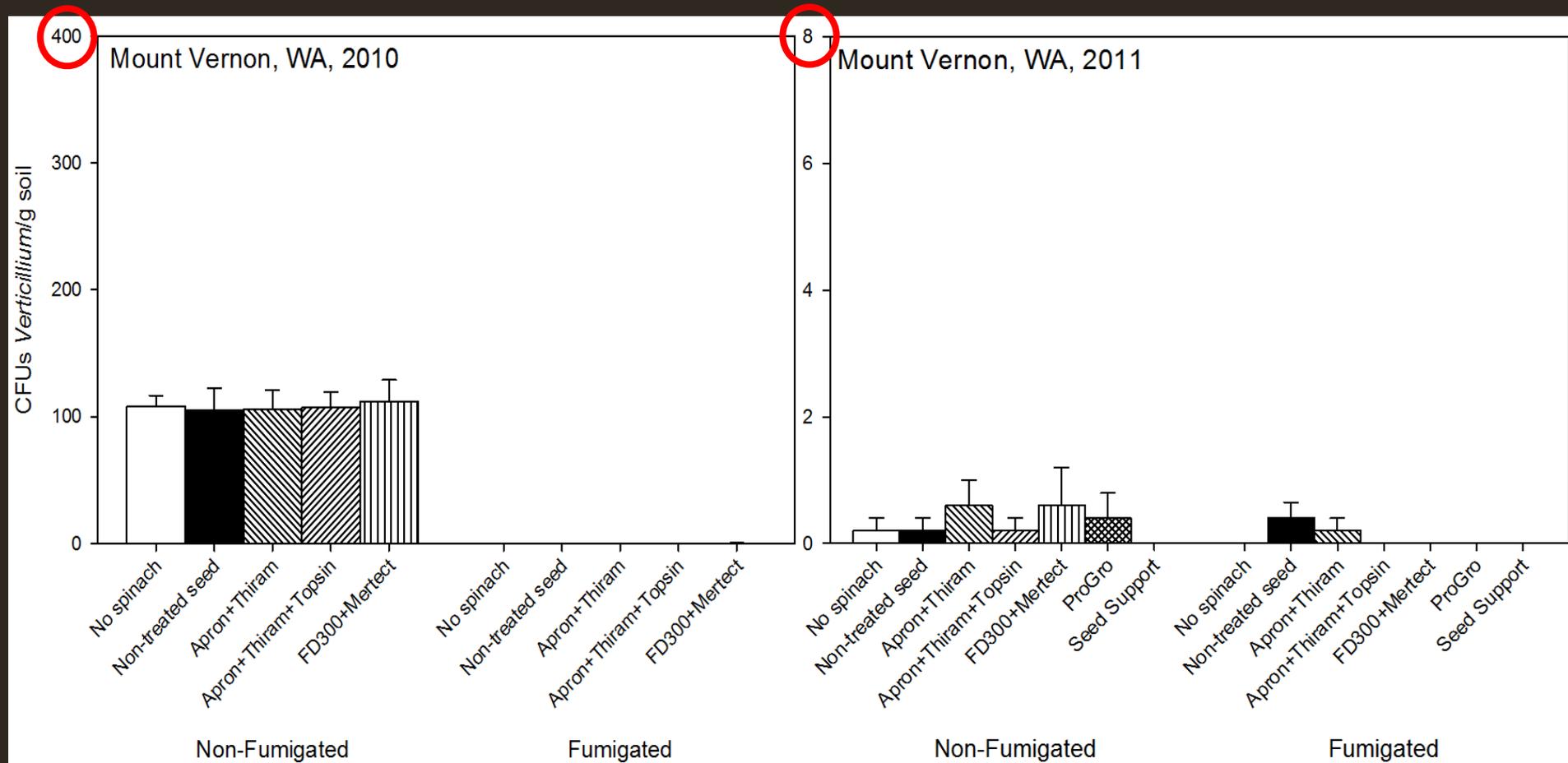
% of lettuce stems infected with *V. dahliae* at harvest



No significant seed treatment or fumigation effects,  
& no symptoms of *Verticillium* wilt on lettuce.

# Mount Vernon, WA: 2010, 2011

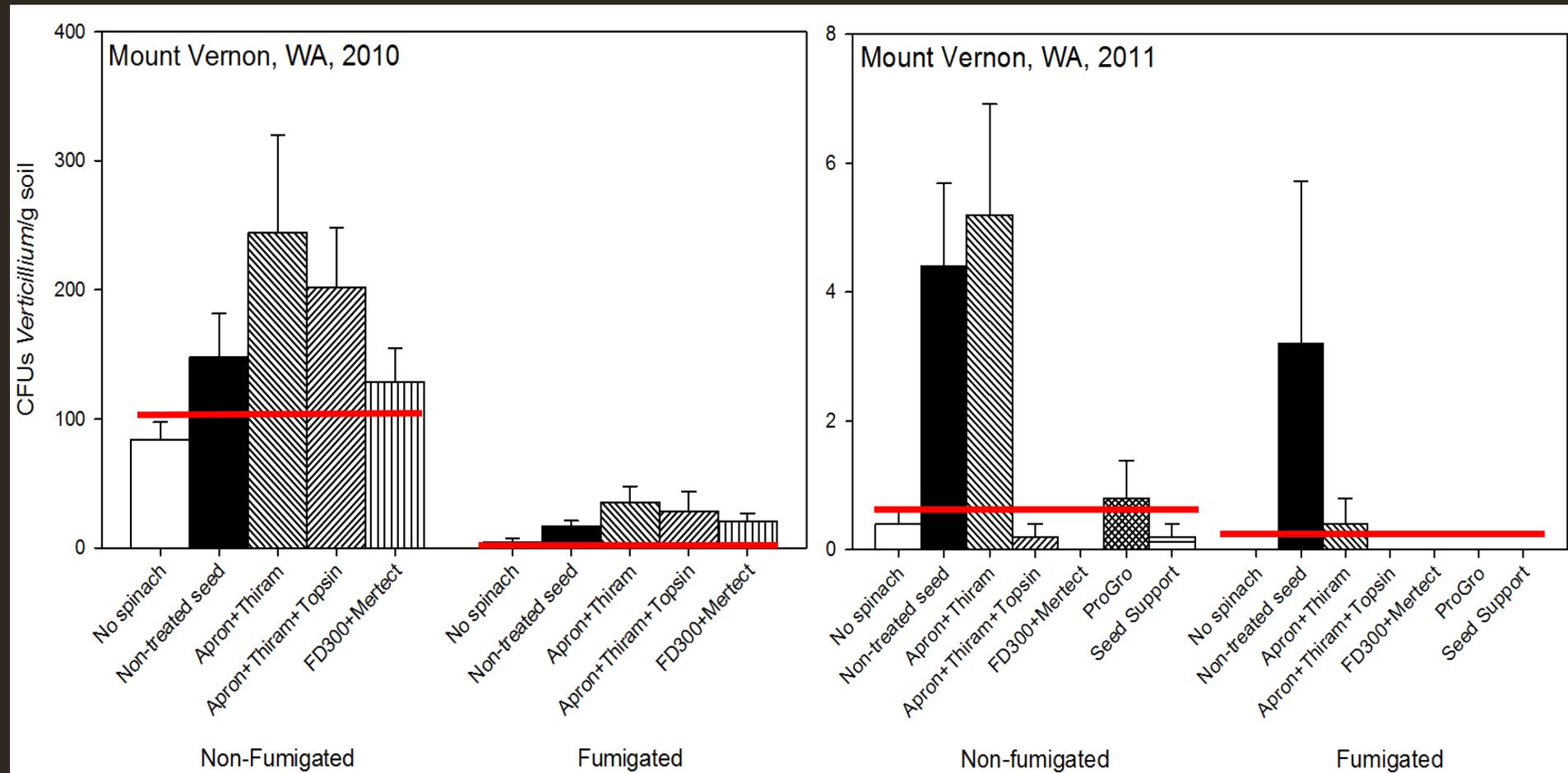
## CFU of *V. dahliae* in soil at spinach planting



Significant fumigation effect, but no seed treatment effect

# Mount Vernon, WA: 2010, 2011

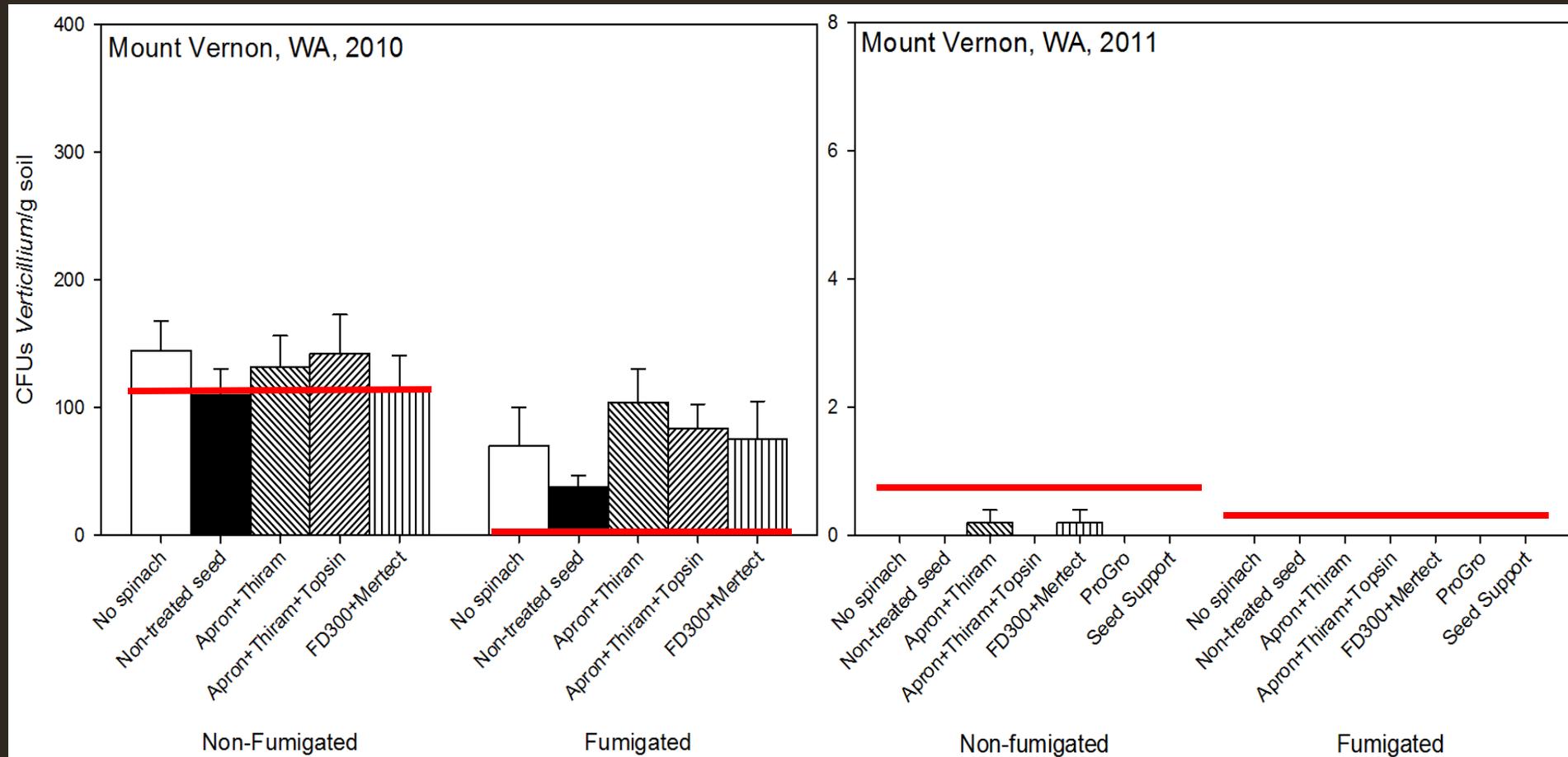
## CFU of *V. dahliae* in soil at lettuce planting



Significant fumigation & seed treatment effects in 2010,  
significant seed treatment effects in 2011.

# Mount Vernon, WA: 2010, 2011

## CFUs of *V. dahliae* in soil 2-5 weeks after harvest of lettuce & incorporation of lettuce residues

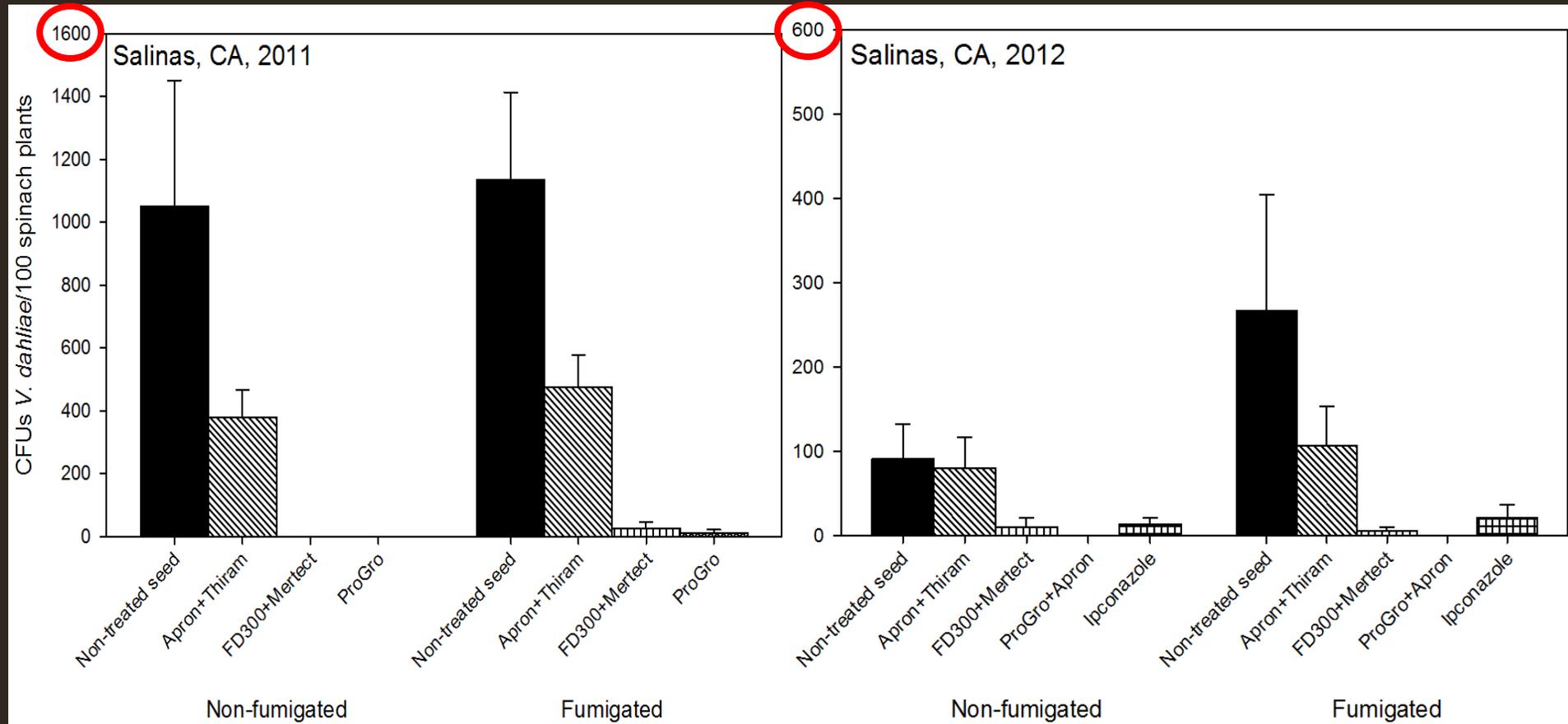


No significant fumigation or seed treatment effects in 2010.

# Salinas, CA: 2011, 2012

## Effect of seed treatment & soil fumigation on seed transmission of *V. dahliae*

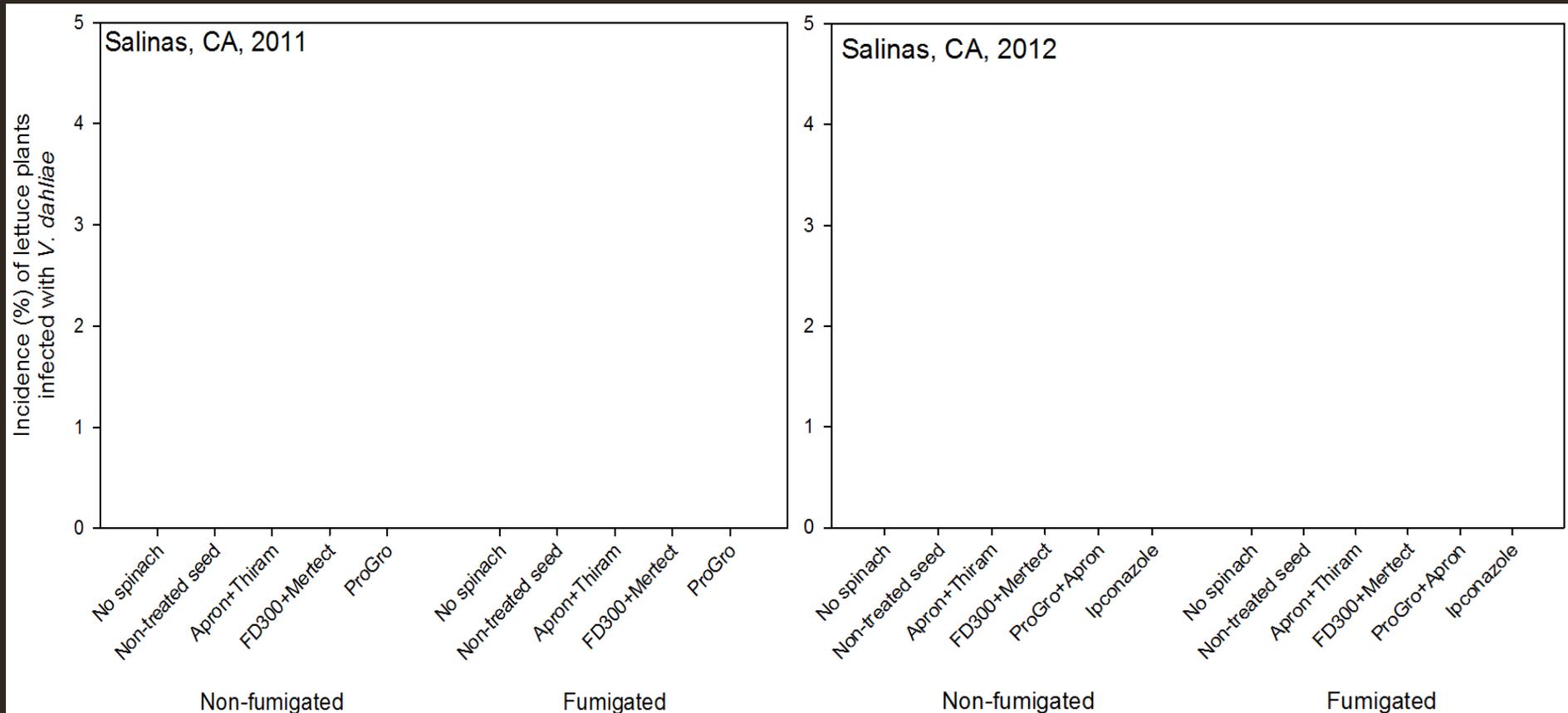
CFUs *V. dahliae*/100 spinach plants after harvest of leaves



Significant seed treatment effects,  
no significant fumigation effects

# Salinas, CA: 2011, 2012

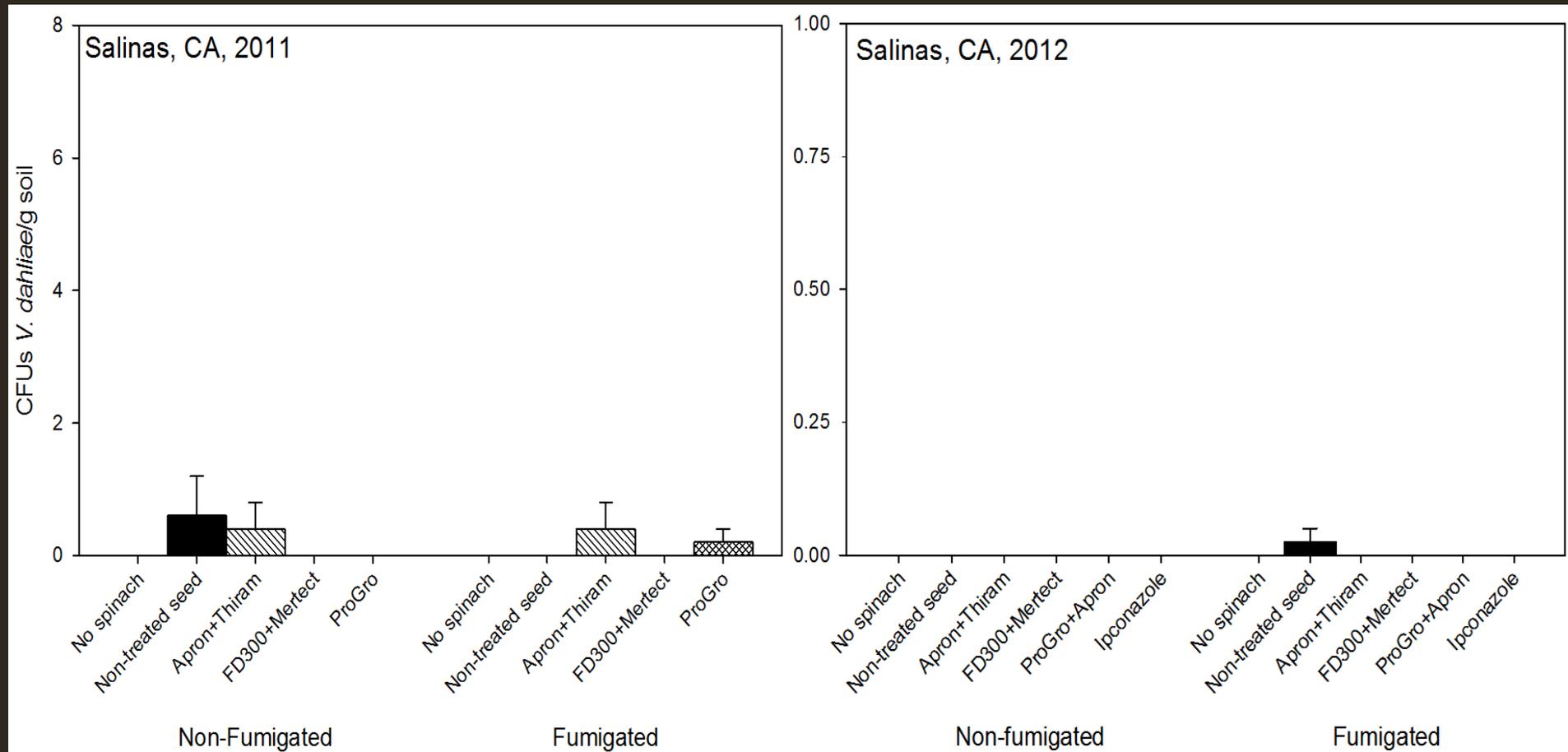
% of lettuce stems infected with *V. dahliae* at harvest



No significant seed treatment or fumigation effects,  
& no symptoms of Verticillium wilt on lettuce plants.

# Salinas, CA: 2011, 2012

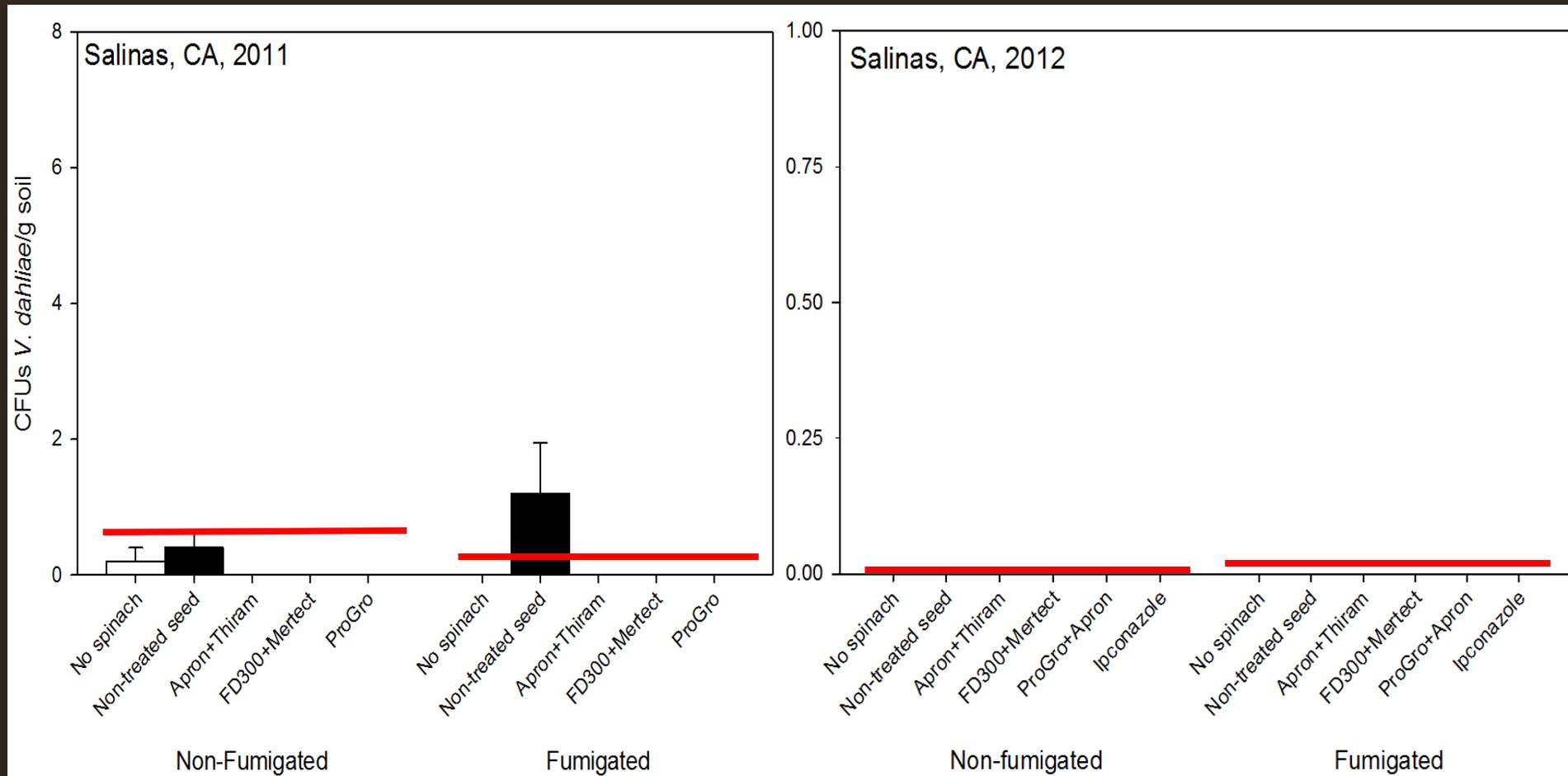
## CFU of *V. dahliae* in soil at spinach planting



No significant fumigation or seed treatment effects

# Salinas, CA: 2011, 2012

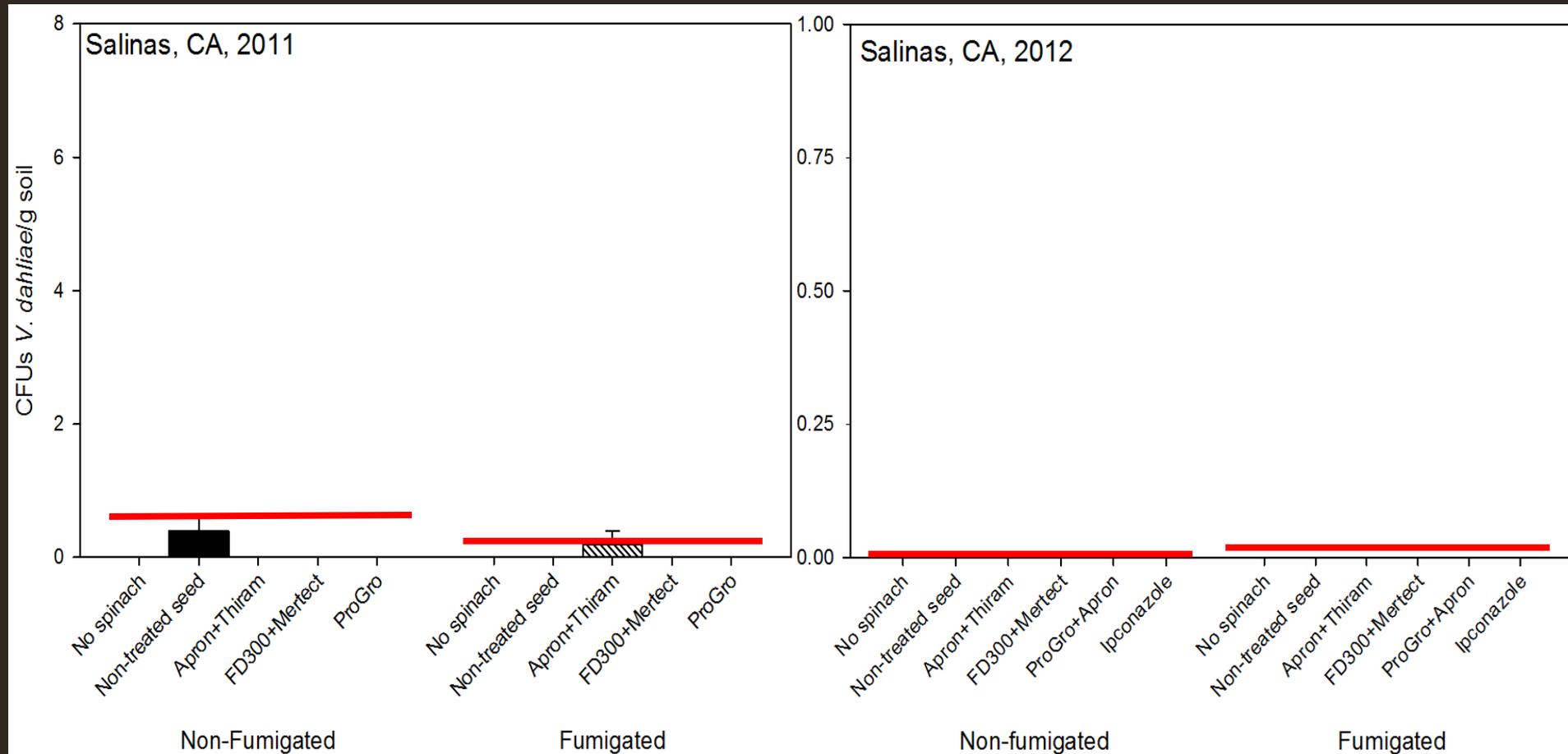
## CFU of *V. dahliae* in soil at lettuce planting



No significant fumigation or seed treatment effects

# Salinas, CA: 2010, 2011

CFUs of *V. dahliae* in soil 2-5 weeks after harvest of lettuce & incorporation of lettuce residues



No significant fumigation or seed treatment effects

# Summary

- Soil fumigation increased colonization of spinach plants by *V. dahliae* 7-fold in 2010 in WA & 3-fold in 2012 in CA (not in 2011 in WA or CA sites)
- Seed treatments reduced seed transmission effectively in all 4 trials
- Best seed treatments = not planting spinach seed
- Mertect 340F: federal registration spring 2014
- Rancona Spinach (ipconazole): registered Jan. 2013
- Topsin M 70WP: SLN spinach stock seed in WA
- Planting infested spinach seed did not result in Verticillium wilt in head lettuce in all 4 trials, & had no significant effect on soilborne *V. dahliae* detected 2-5 weeks after incorporating lettuce residues

# Characterization of *Verticillium dahliae* from spinach seed, and screening for resistance to *Verticillium* wilt in spinach.

Villarroel-Zeballos, M.I., Feng, C., Iglesias, A., du Toit, L.J., & Correll, J.C. 2012.  
*HortScience* 47:1297-1303.

# Pathogenicity, virulence, & vegetative compatibility grouping of *Verticillium* isolates from spinach seed

Iglesias-Garcia, A.M., Villarroel-Zeballos, M.I., Feng, C., du Toit, L.J., & Correll, J.C. 201\_.

*Plant Disease: submitted January 2013.*

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WA State Comm. for Pesticide Registration  
CA Leafy Greens Research Board  
Alfred Christianson Endowment

Participating seed & chemical companies  
International Seed Health Initiative - Root, Bulb & Leafy Vegetable  
International Technical Group

Mike Derie, Barbara Holmes,  
Emily Gatch, Ana Vida Alcala, Ron Dralle, others ...  
Angela Iglesias-Garcia (MS), Maria Villarroel-Zeballos (MS)



# Wilt incidence

