

Update on Verticillium wilt, New Species and their Disease Potential



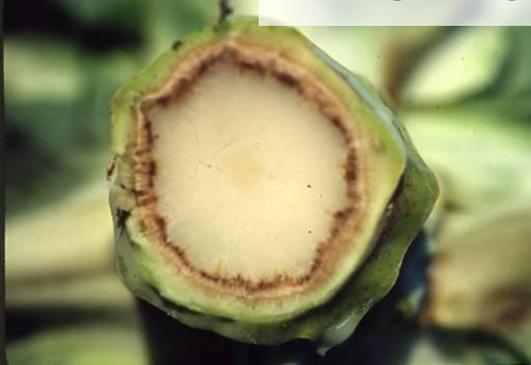
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Prior to 1995, lettuce was not considered a host of *V. dahliae*; even when grown in rotation with other diseased crops.



VERTICILLIUM WILT

Foliar Symptoms:

- Angular Chlorosis
- Necrosis
- Wilting
- Darkening of Leaf Veins
- Acropetal Progression
- Collapse of Head





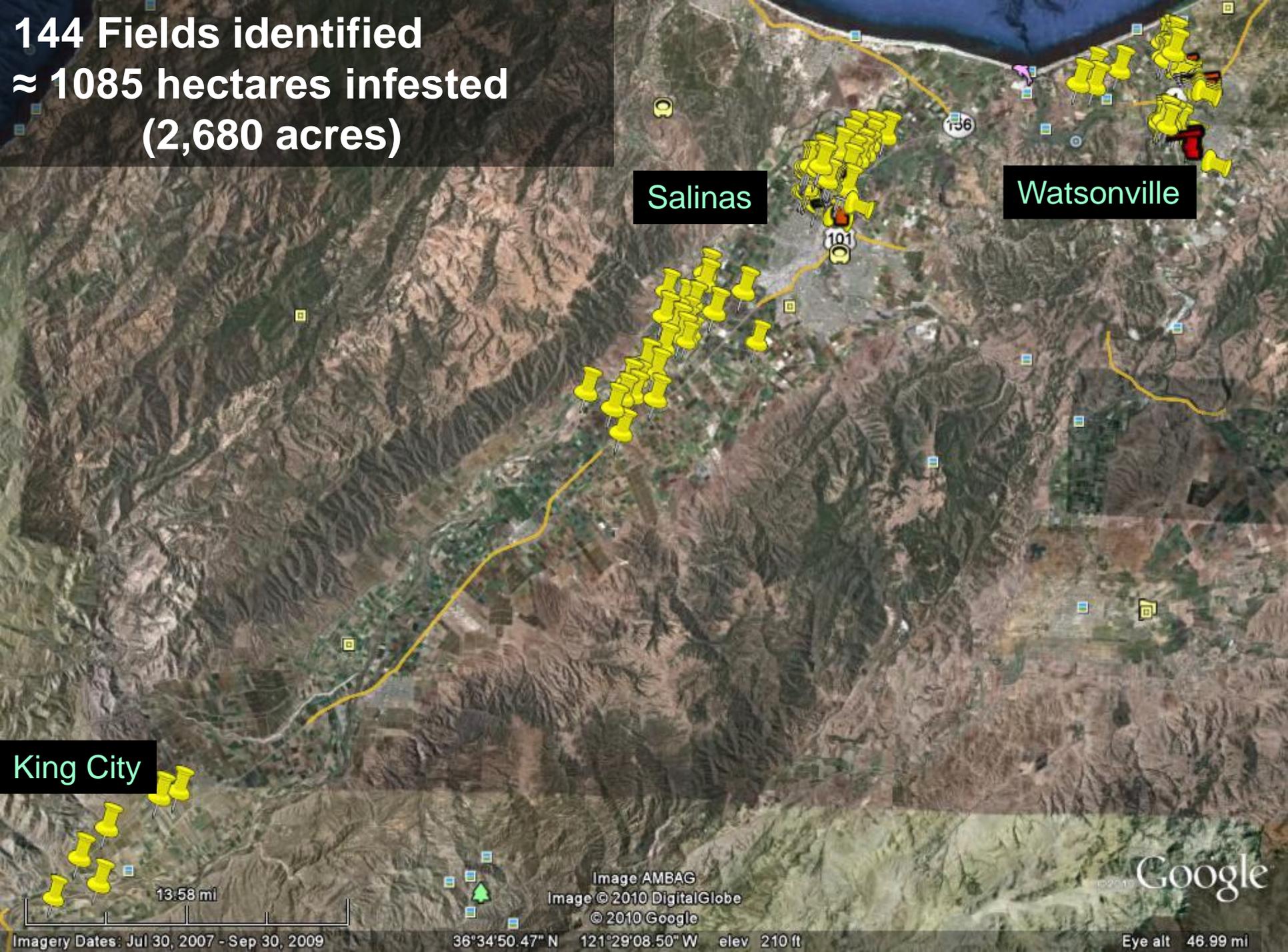
VERTICILLIUM WILT

Root Symptoms:

- Vascular Discoloration
- Occur ~2 wk before foliar symptoms



**144 Fields identified
≈ 1085 hectares infested
(2,680 acres)**



Salinas

Watsonville

King City

13.58 mi

Image AMBAG
Image © 2010 DigitalGlobe
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Google

Imagery Dates: Jul 30, 2007 - Sep 30, 2009

36°34'50.47" N 121°29'08.50" W elev 210 ft

Eye alt 46.99 mi

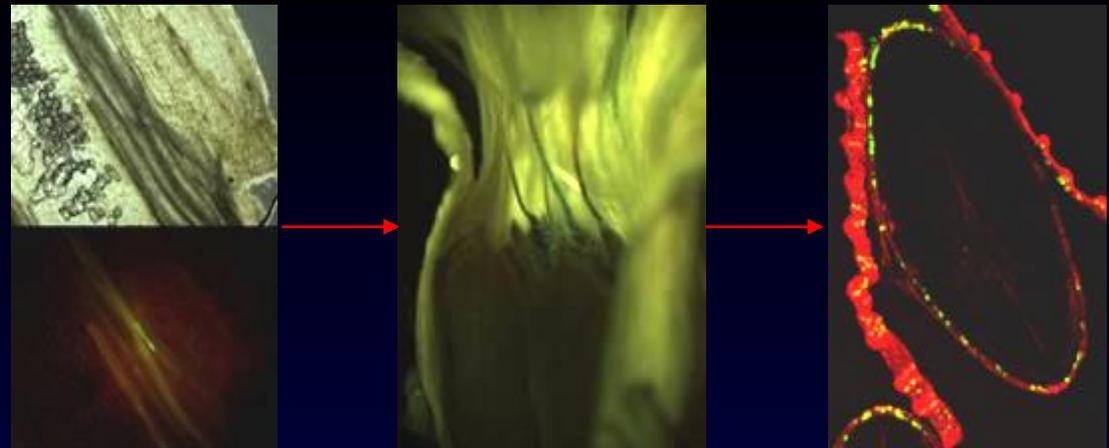
Verticillium wilt of lettuce

- First report in 1995
 - Extensive damage in Watsonville, CA
- Detected in 175 fields from 1995 to 2010
- ~75 fields in 2010 alone
- Incidence 70-100%
- Random distribution of fields
- 2-3 crops following fumigation
- Often first observed by field edge
- Complete plant death
- Pathogen is seedborne

- Assess seed lots of lettuce types for *V. dahliae* infestation.



FACTS



- *Verticillium dahliae* is seedborne in lettuce and other crops and weeds.
- It is both externally seedborne and internally seedborne.
- Infested seed germinates, plants grow and develop wilt.
- Seed harvested from infected plants are infested with *V. dahliae*.

Lettuce types (2008-10)

Type	Seed lots	Infested	Range
Baby Leaf	34	0	-
Crisphead	84	13	0.1-4
Romaine	38	8	0.5-2
Red Romaine	7	1	3
Green Leaf	31	2	0.5-2
Red Leaf	22	2	0.5
Butterhead	5	0	-
Other/unknown	44	22	0.5-5
Total	265	48	

Where produced (2008-10)

Country	Seed lots	Infested	Range
USA	195	33	0.1-5
United Kingdom	3	2	0.0-4
China	22	8	0.5-3
Chile	33	5	0.5-2
The Netherlands	5	0	-
Australia	7	0	-
Total	265	48	

Verticillium wilt of lettuce

- Exceedingly high production of microsclerotia
 - Calculated ~3 million microsclerotia/plant
- Two races
 - Race 1 currently ~75% of population
 - Resistance for race 1 available

Why did lettuce succumb to *Verticillium dahliae*?

- What is the source of these “apparently” novel isolates?
 - Endemic; Parasexual cycle (mitotic recombination)?
 - No teleomorph ever observed.
 - Exotic; Just another invasive in CA?
 - How diverse is the *V. dahliae* population in the Salinas Valley?
 - Did these isolates wash in from elsewhere during the 1995 floods?
- Big implications for disease management & resistance durability.

- Determine whether or not nitrate fertilizers used in strawberries following fumigation render soil Verticillium wilt-conducive relative to ammonium fertilizers.

Effect of different form of Nitrogen on Verticillium wilt of lettuce

Treatments

Ammonium sulfate
Ammonium nitrate
Calcium nitrate
(60 lb N per acre)

Other essential nutrients

Hoagland solution without nitrogen was prepared and applied three times per week

Pathogen

Verticillium dahliae

Time of sampling for N analysis

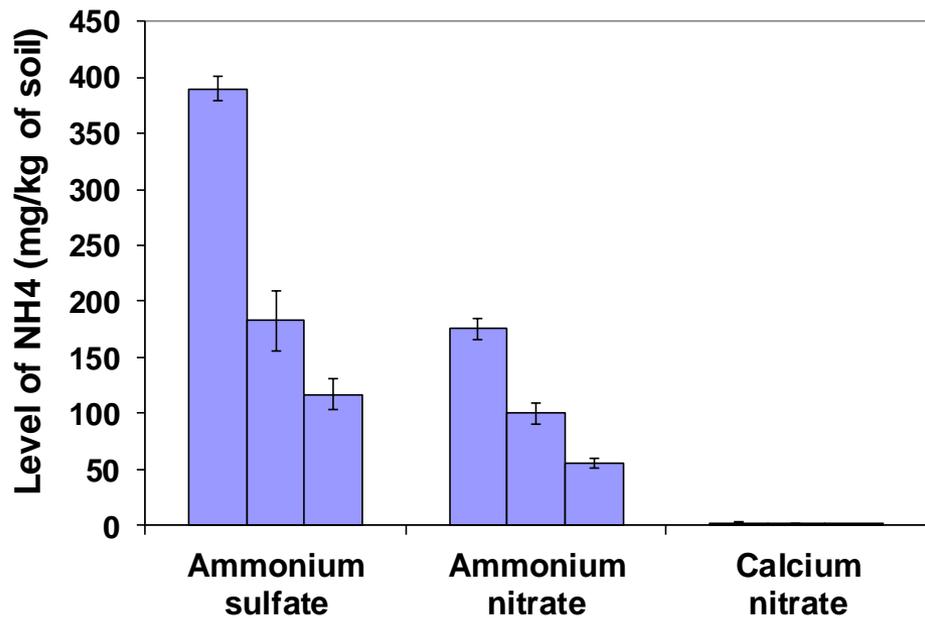
0 days after fertigation
3 days after fertigation
7 days after fertigation

Data

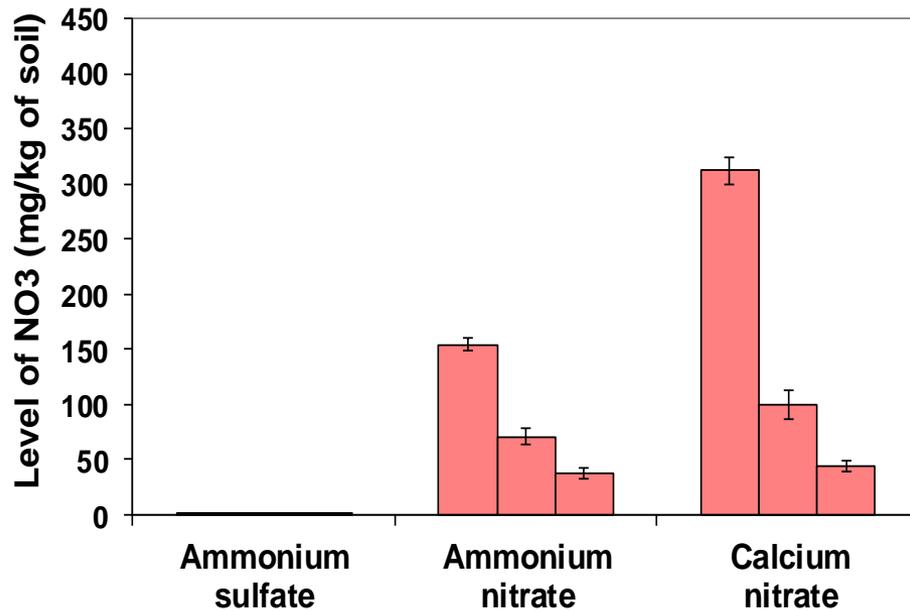
Disease incidence
Disease severity

Levels of Ammonium and Nitrate in Sand

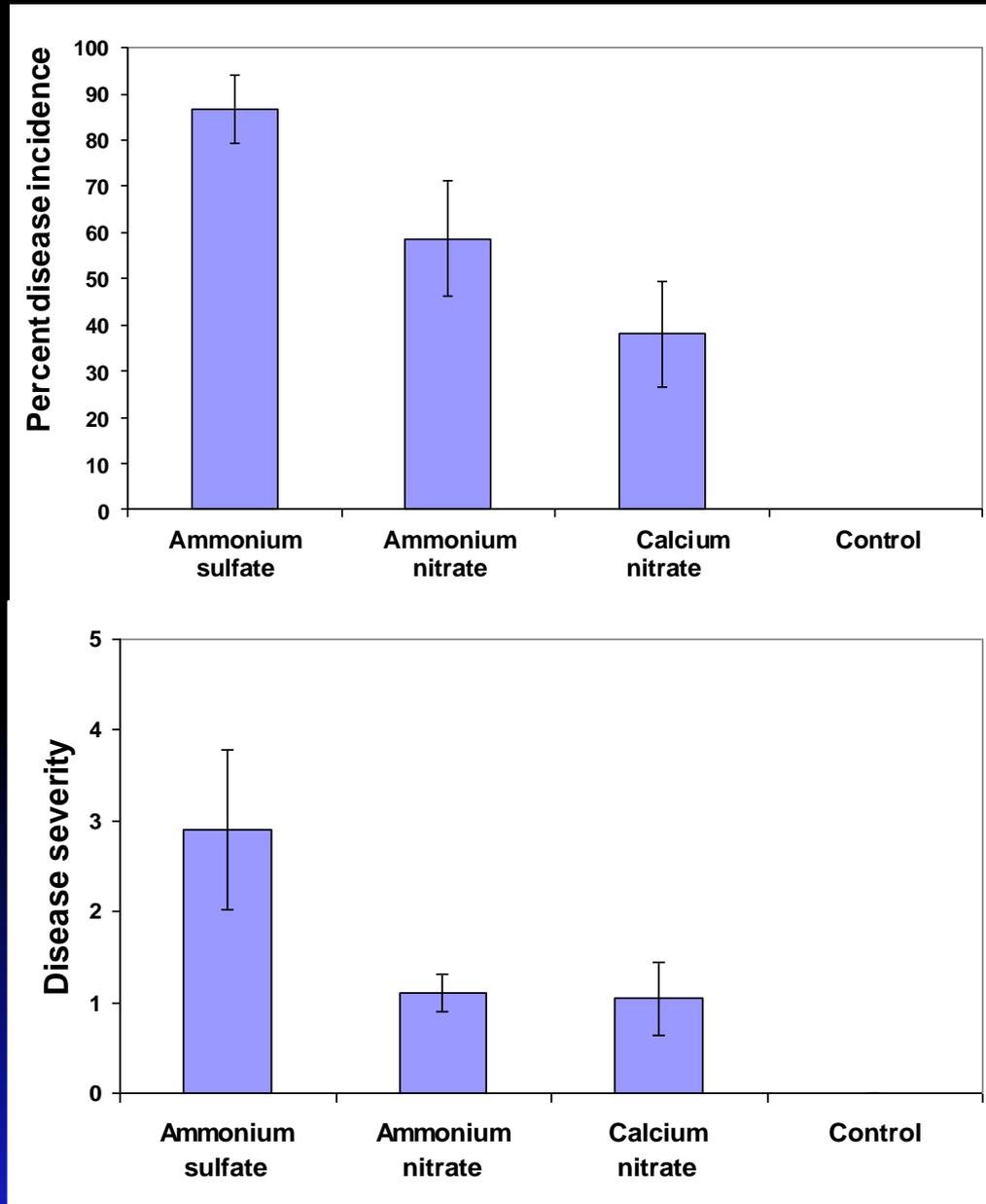
NH_4



NO_3

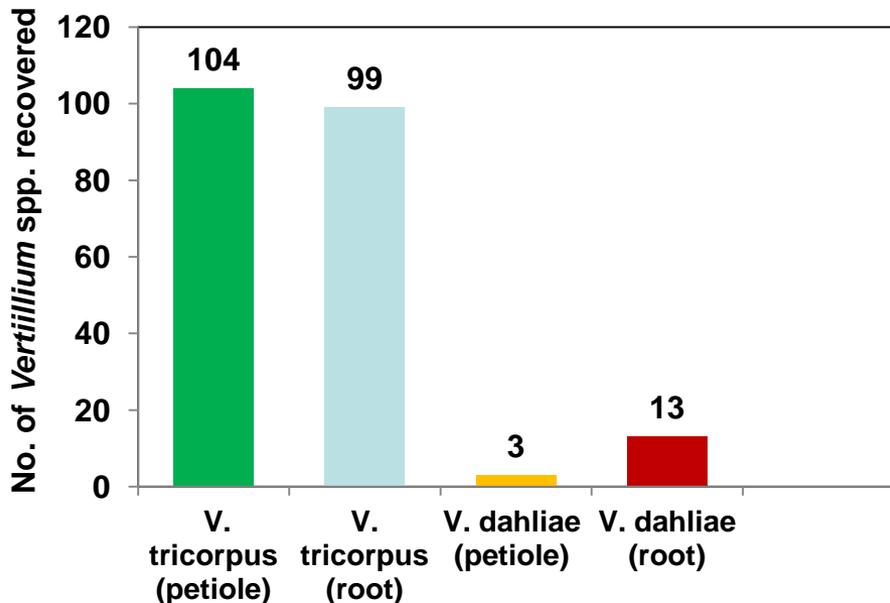


Effects of Nitrogen form on Verticillium wilt

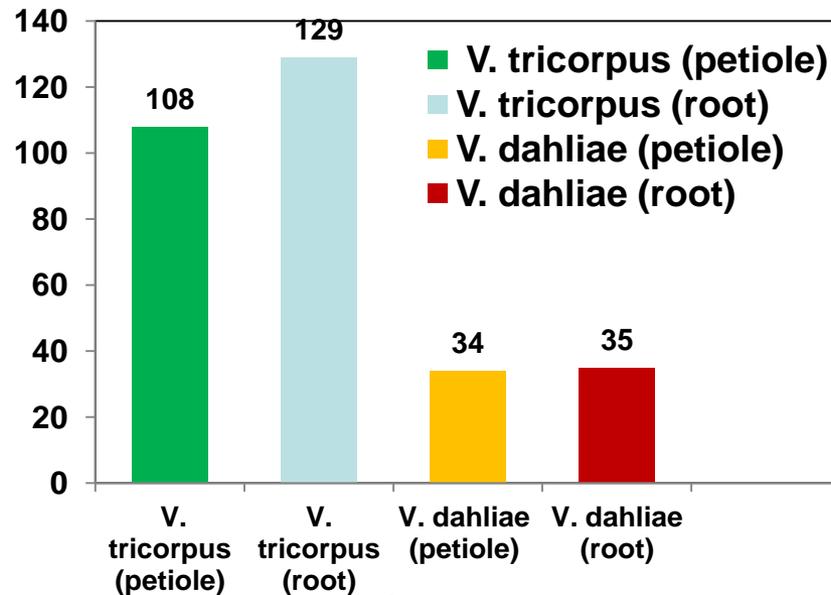


- Sample spinach seedlings in Monterey, San Benito, and Santa Barbara counties and assay for *Verticillium dahliae*.

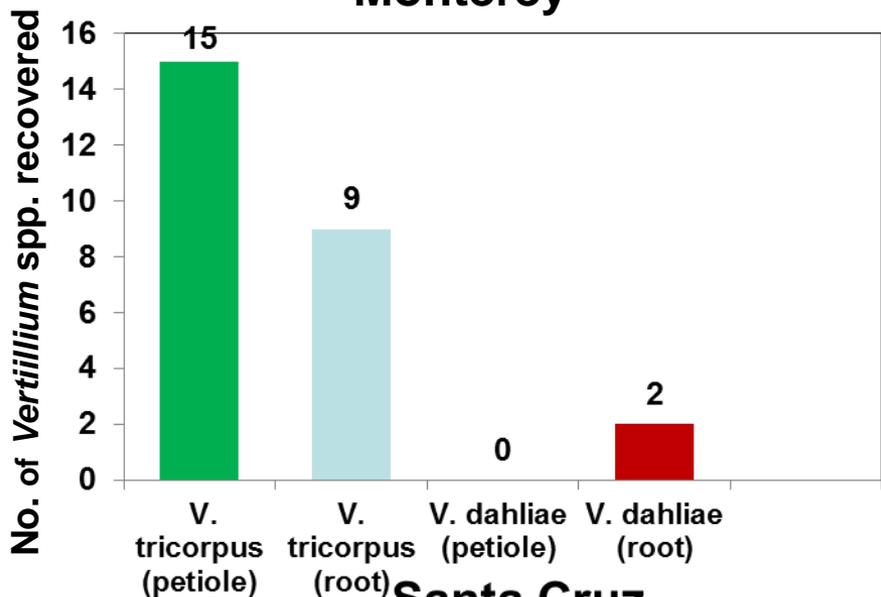
Number of fields Surveyed: Monterey= 23; Santa Barbara = 25; Santa Cruz = 6; and San Benito = 15



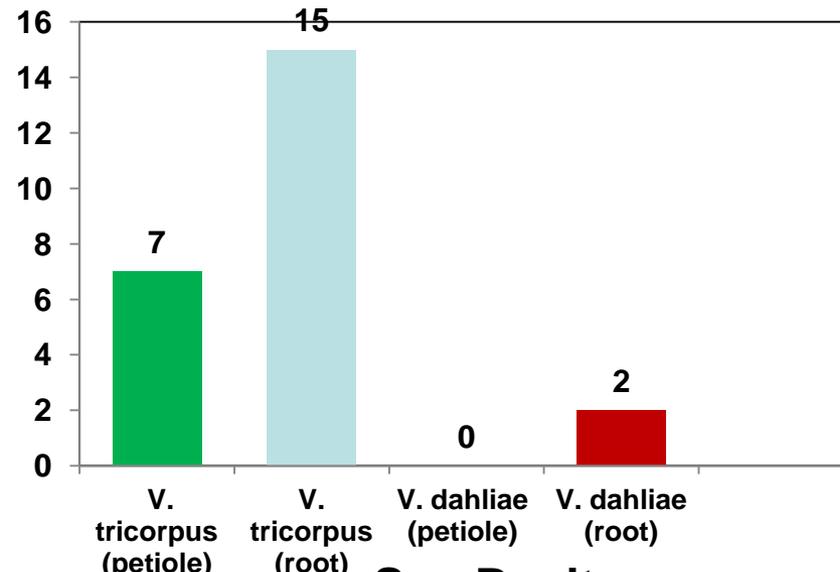
Monterey



Santa Barbara

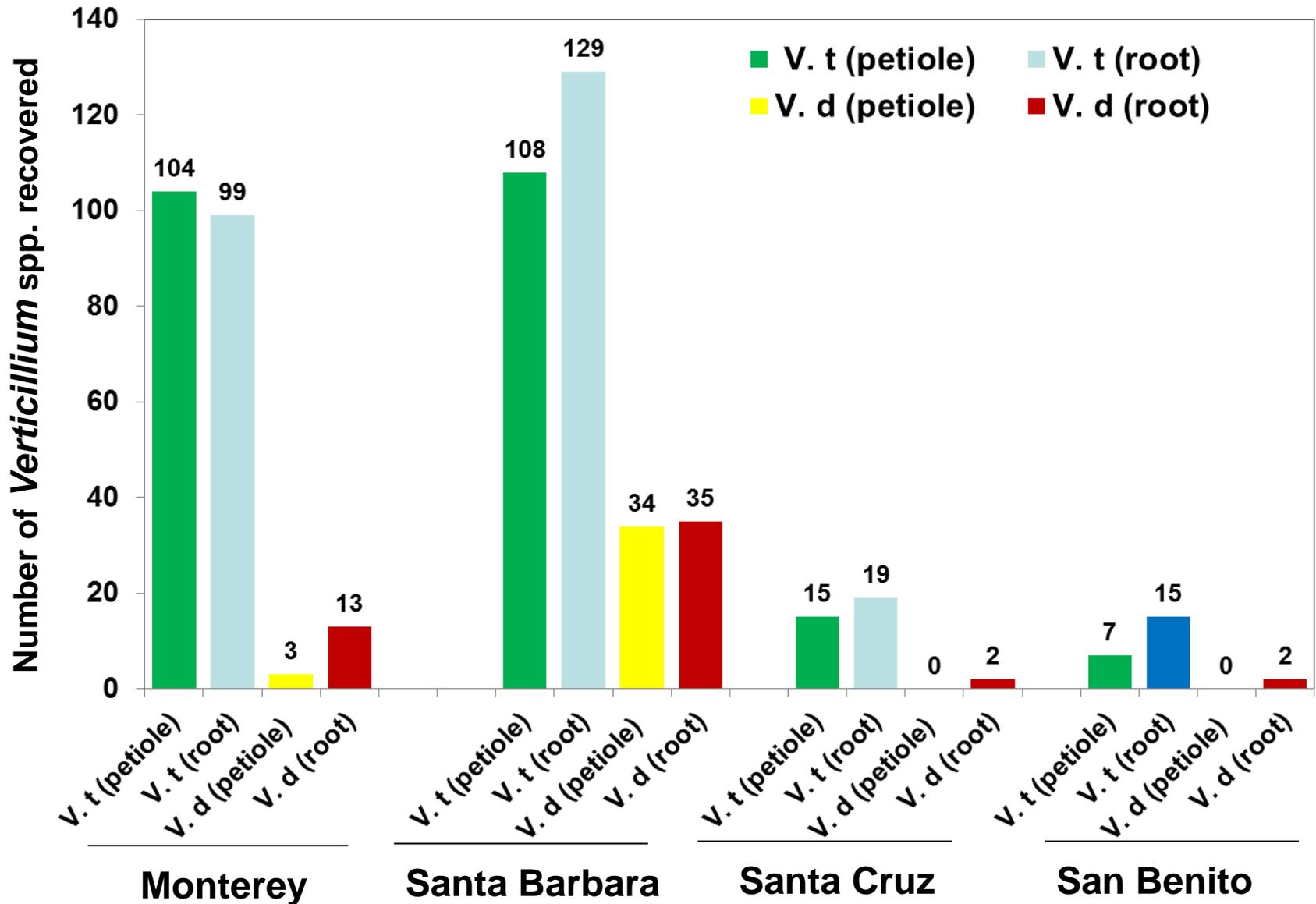


Santa Cruz

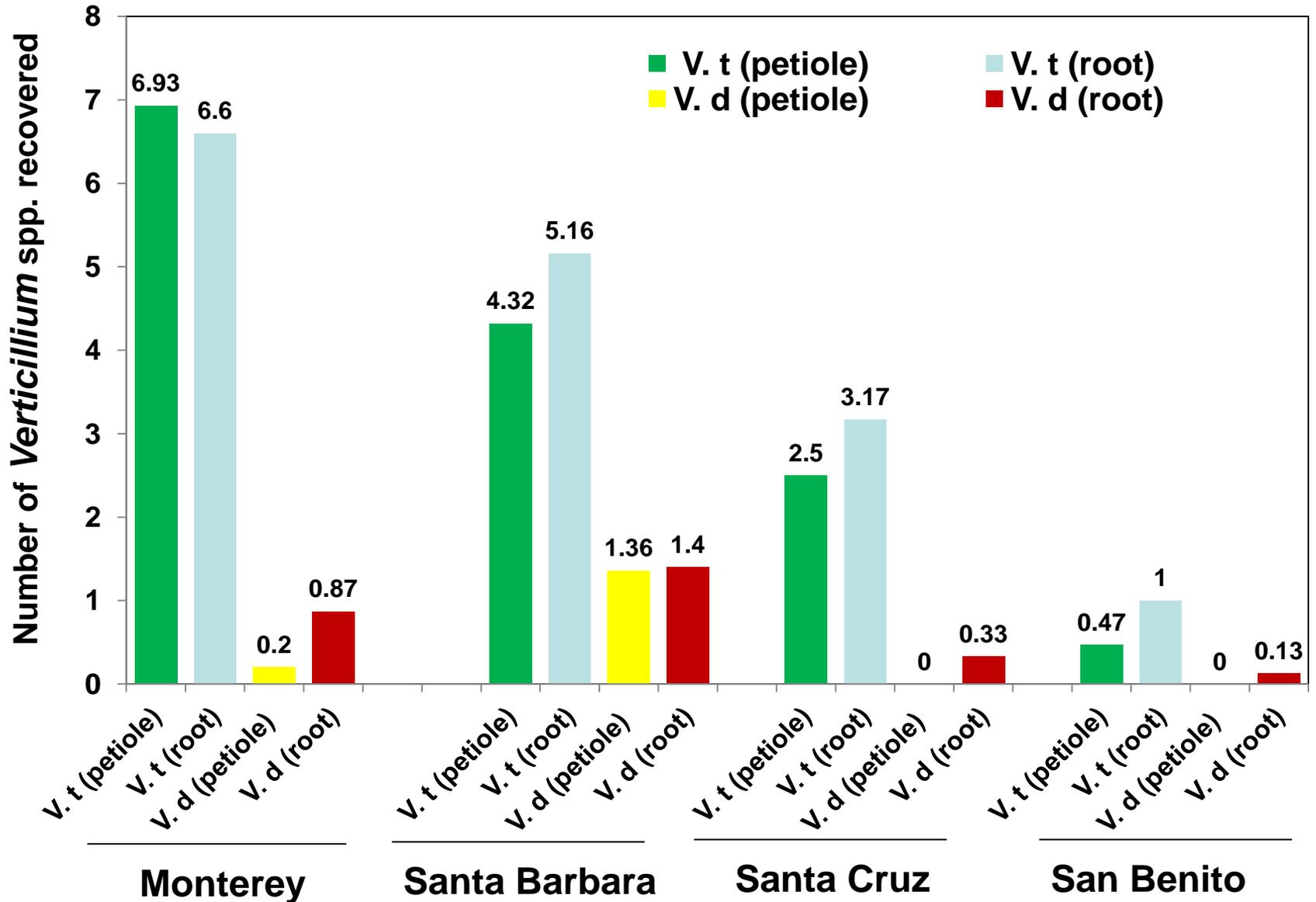


San Benito

Distribution of *Verticillium* spp. on spinach in four California counties



Verticillium spp. recovered per field



Acres of Spinach Grown in Monterey and Santa Barbara Counties from 1993

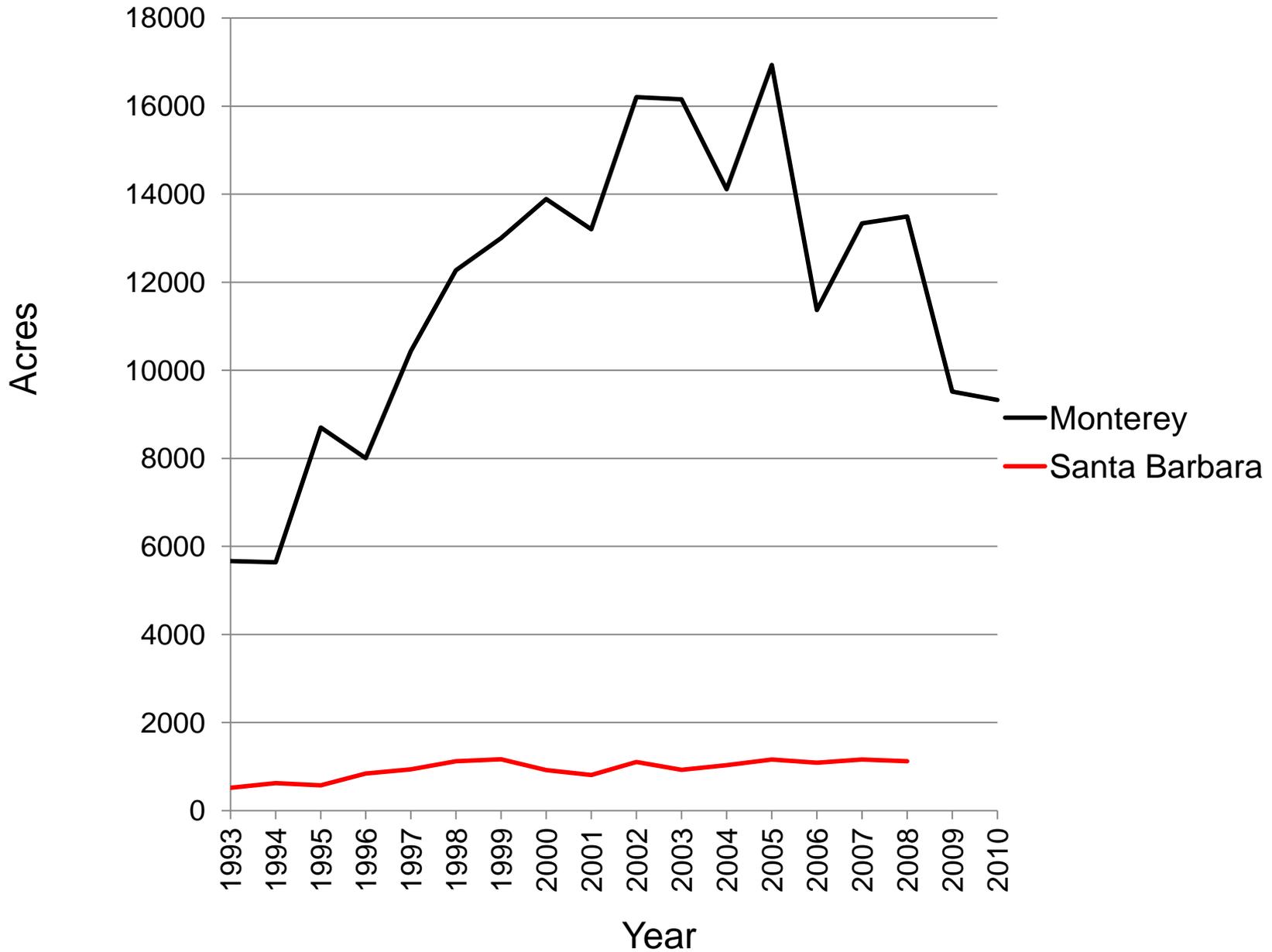
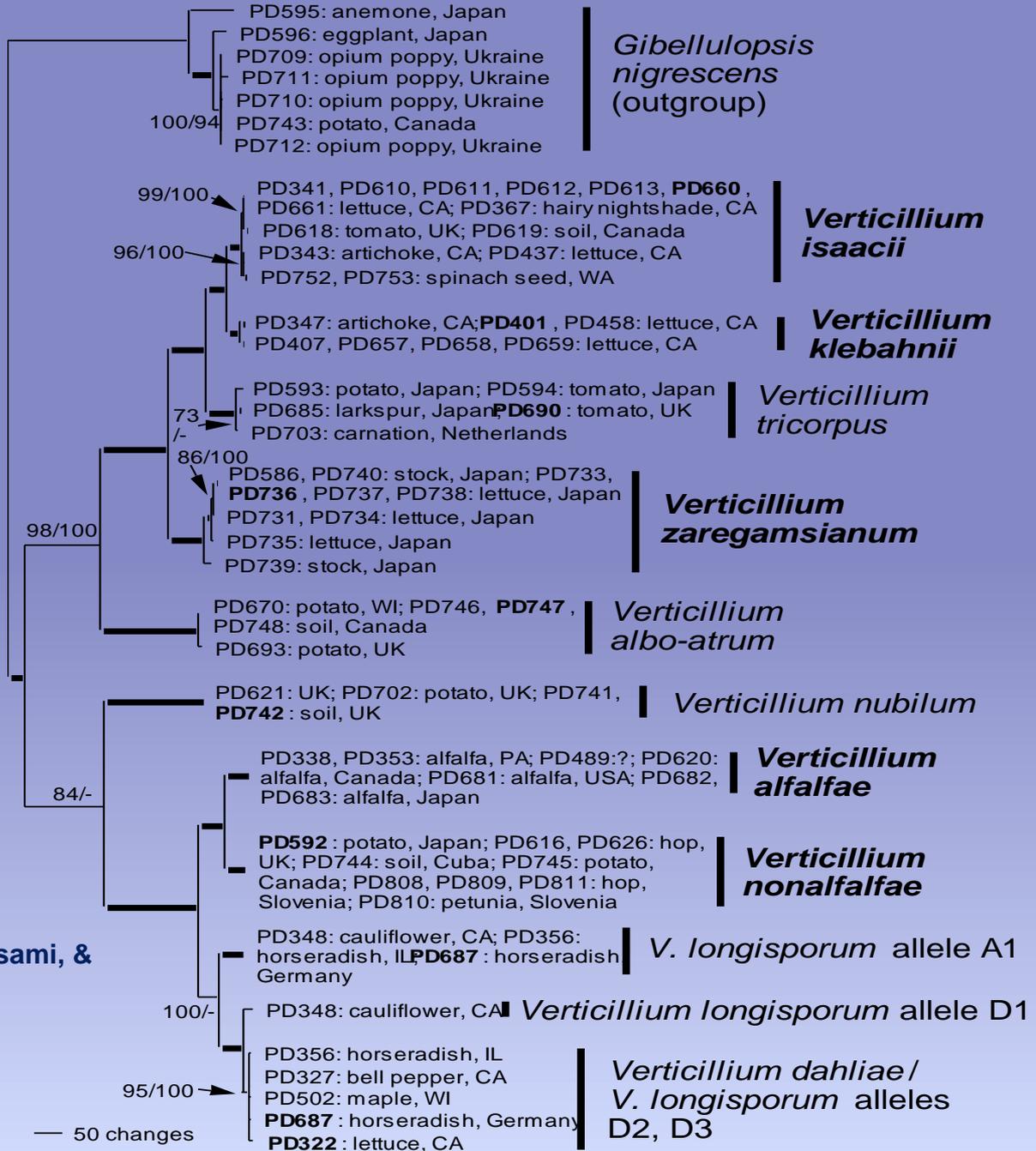


Figure. Phylogenetic relationships of the ten *Verticillium* species based on the combined *ACT*, *EF*, *GPD* and *TS* dataset of 2658 characters and 77 taxa, with *Gibellulopsis nigrescens* as outgroup. One of the most parsimonious trees is shown. Species in bold are newly described in this study, strain identifiers in bold represent ex-type strains. Numbers by the branches are parsimony and Bayesian and likelihood support values above 70.



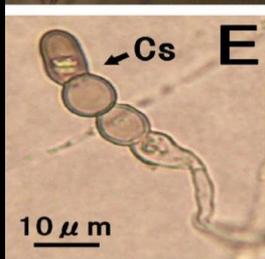
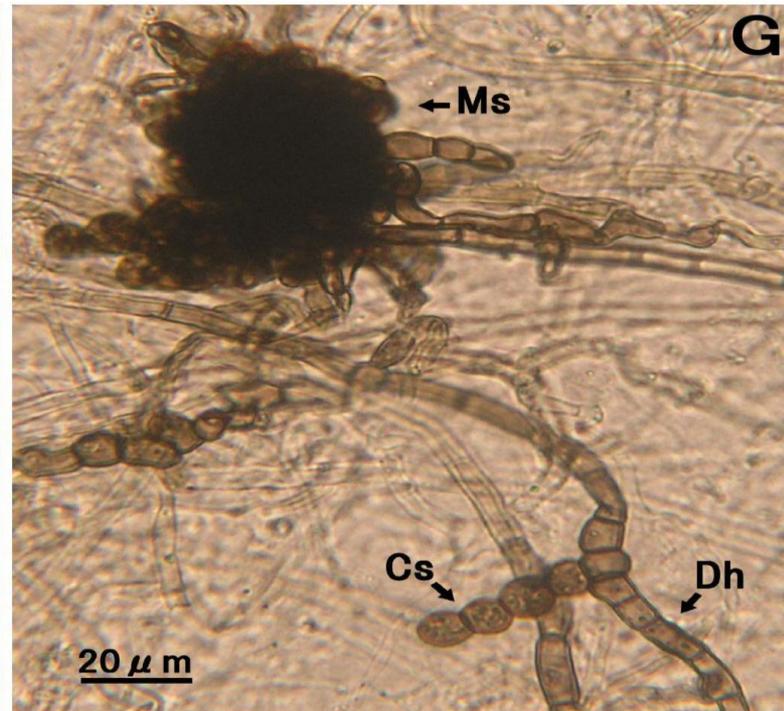
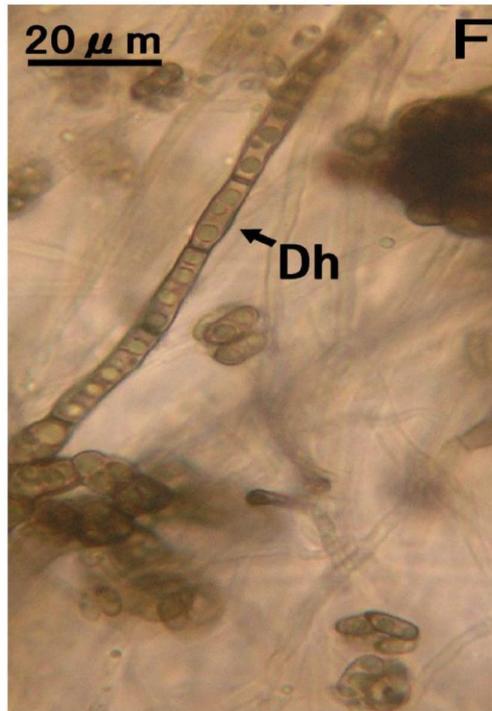
Inderbitzin, Bostock, Davis, Platt, Usami, & Subbarao. 2011. PLoS One: e28341

Clade Flavexudans
Clade Flavonexudans

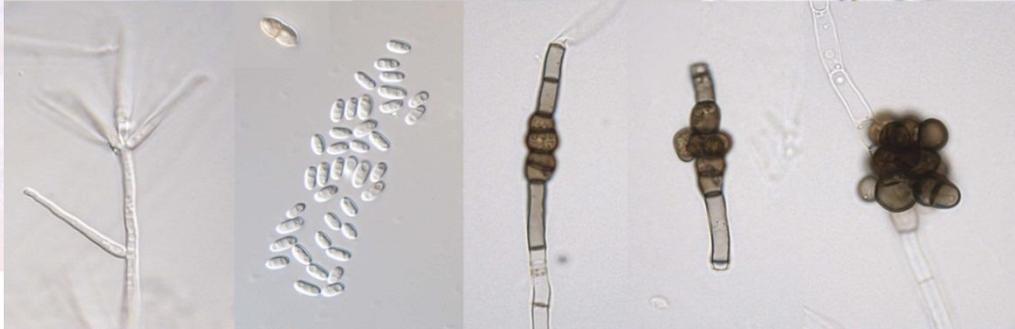
Verticillium tricorpus: Is now three species, *V. tricorpus*, *V. isaacii* and *V. klebahnii*, that are morphologically indistinguishable. And they may differ in pathogenicity and virulence. Which of these are present in CA?

Also of interest, *V. zaregamsianum*, a lettuce pathogen in Japan. It looks similar to *V. dahliae*, forms mostly microsclerotia, only a few brown-pigmented hyphae, and has yellow-pigmented hyphae. However, the pigmented hyphae may not be formed in culture, and thus *V. zaregamsianum* may be confused with *V. dahliae*. Or if judged solely by the yellow pigmentation, it could be confused with species in the *V. tricorpus* group. Is *V. zaregamsianum* present in CA?

All these species can be differentiated by DNA sequencing, or more easily, by PCR.



Verticillium tricorpus



Verticillium zaregamsianum

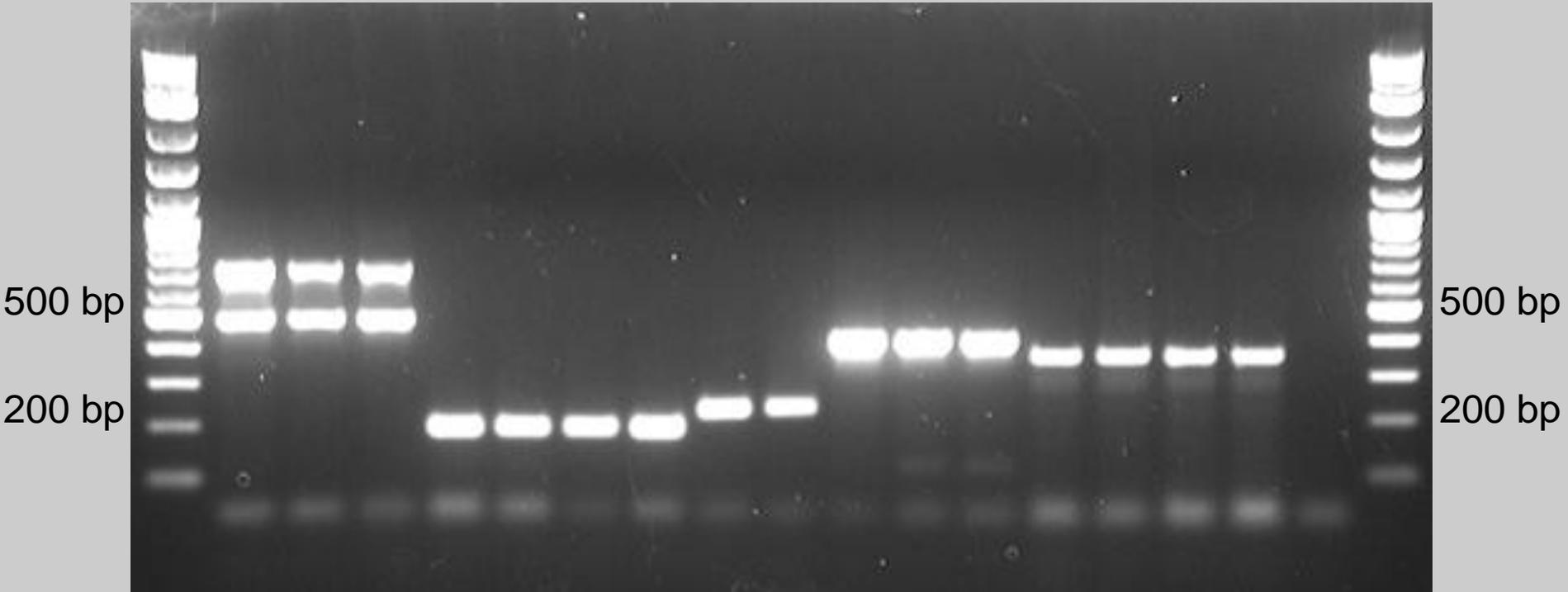
Verticillium klebahnii



Verticillium isaacii



V. isaacii *V. tricorpus*
V. dahliae *V. klebahnii* *V. zaregamsianum*



Actin
ITS

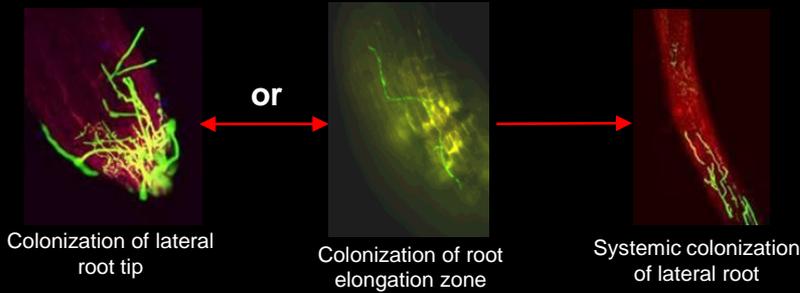
EF-1 α

EF-1 α

Actin

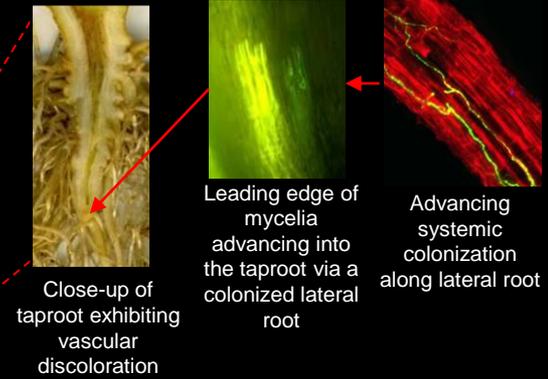
GPD

By 2 weeks PI

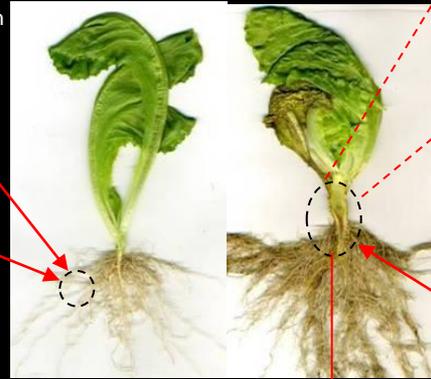
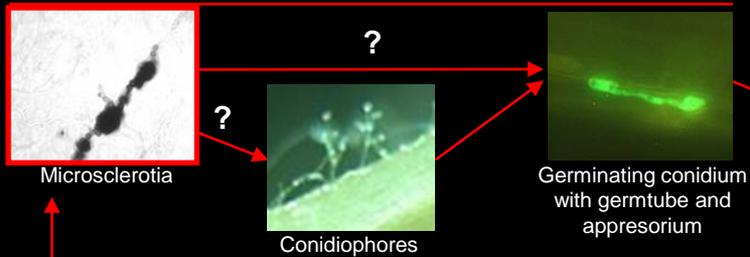


Determinative Phase

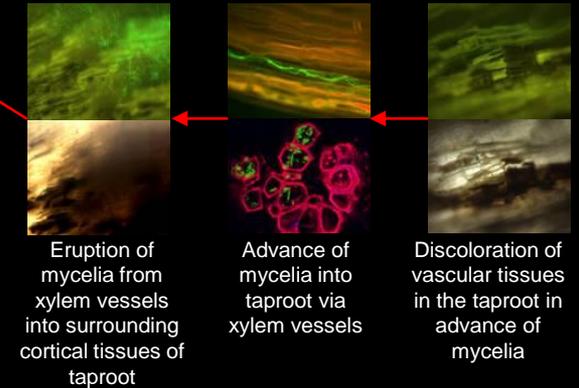
6 to 8 weeks PI



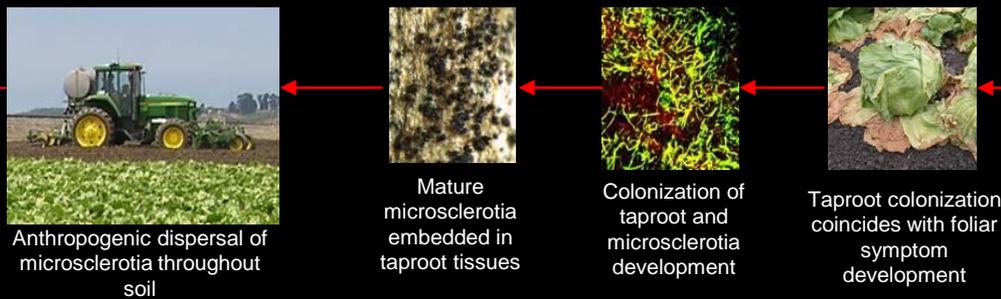
Initial 48 hours PI



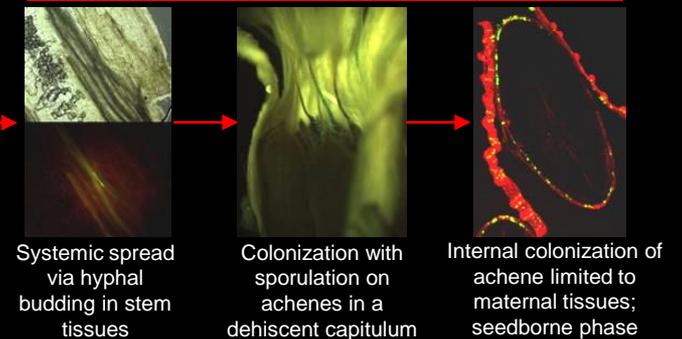
8 to 10 weeks PI



10+ weeks PI



14+ weeks PI



Lettuce - *Verticillium dahliae* Disease Cycle