# Laurel wilt of avocado: Basic and applied pathology research

3 November 2009
Laurel Wilt Symposium

UNIVERSITY OF FLORIDA
Institute of Food and Agricultural Sciences

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University of Florida
Homestead

# Host response

- Avocado germplasm
- Microscopic interactions

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## Disease management

- Fungicides

## Host response

- Avocado germplasm
- Microscopic interactions

## Disease management

- Fungicides

## Diagnosis

- *Raffalea lauricola* in Homestead?
- Molecular detection…Jason

# Florida Division of Plant Industry
# Gainesville quarantine facilities
**2007**

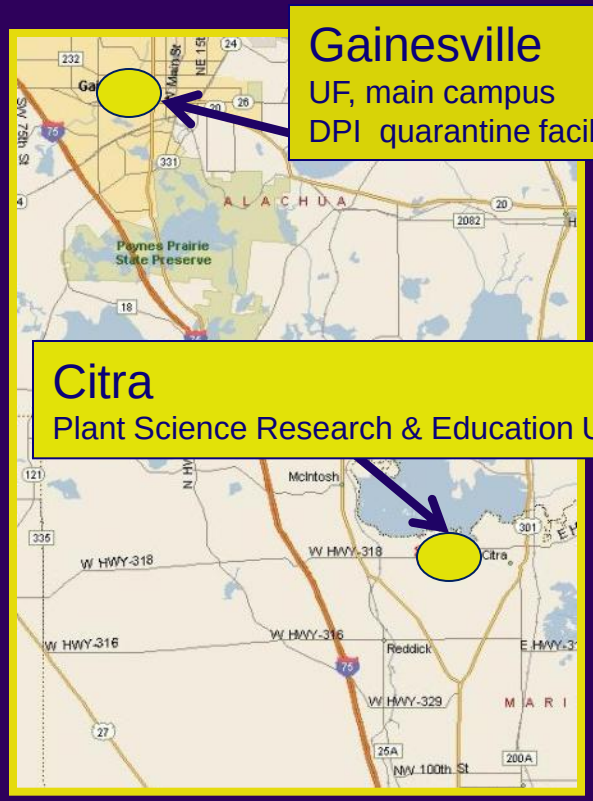

# University of Florida
# Citra




Chattahoochee
Tallahassee
Lake City
St. Augustine
Palm Coast
Gainesville
Daytona Beach
Orlando
Titusville
Spring Hill
Kissimmee
Clearwater
St. Petersburg
Tampa
Palm Bay
Sarasota
West Palm Beach
Lake Okeechobee
Fort Myers
Cape Coral
Fort Lauderdale
Miami
Coral Gables
Homestead
Tropical Research & Education Center
Florida Bay
Key West
Marathon
Gainesville
UF, main campus
DPI quarantine facility
ALACHUA
Paynes Prairie
State Preserve
Micanopy
Orange Lake
McIntosh
W HWY-318
N HWY-329
Citra
Citra
Plant Science Research & Education Unit
NW 100th St

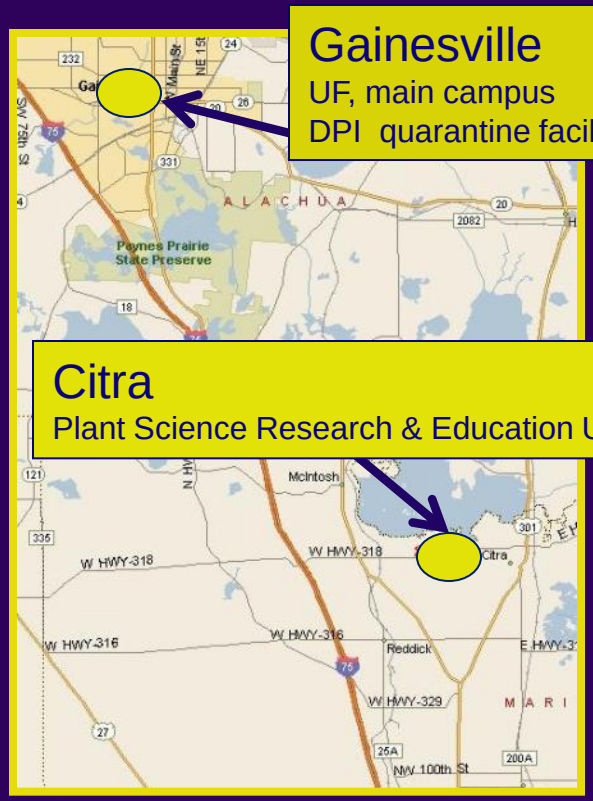
# Citra, 2008 and 2009


# Gainesville (DPI), 2007



# Citra


5 expts established, May 2009



# Citra


5 expts established, May 2009

1.Cultivar evaluation

2.Fungicide efficacy (Choquette and Simmonds)

- Trunk injection (Chemjet): Alamo, Arbotect
- Soil drench: Alamo, Arbotect, Agri-phos

# Citra


5 expts established, May 2009

1. Cultivar evaluation
2. Fungicide efficacy
3. Time course/disease development

Influence of time (1, 2, 3 and 4 wks post-inoculation), cultivar (Brogdon, Choquette, Lula, and Simmonds), and plant size (3 and 7 gal Choquette) on disease development

# Citra


5 expts established, May 2009

1. Cultivar evaluation
2. Fungicide efficacy
3. Time course/disease development

How does avocado respond to the pathogen?

Could any of these responses be used to identify resistant genotypes?

# Citra


5 expts established, May 2009

1. Cultivar evaluation
2. Fungicide efficacy
3. Time course/disease development
4. Plant size
5. Inoculum dose/threshold

Could small plants be used in disease studies?

# Citra


**Figure 1. Plot diagram for 2009 Citra laurel wilt experiments**

| Row | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | bc A | bt A | br A | bg A | ct A | ch A | dy A | dn A | et A | hl A | hs A | ll A | mg A | mn A | pl A | rd A | rs A | sm A | tn A | tr A | wl A | wm A | | bt1 A | bt5 A | hl1 A | hl5 A | ll5 B | hl5 B | bt5 B |
| 2 | ch B | bt B | tr B | bc B | pl B | rd B | sm B | wl B | mn B | rs B | dn B | tn B | ct B | wm B | hs B | br B | mg B | hl B | dy B | ll B | bg B | et B | | hs5 A | ll1 A | ll5 A | hl1 B | bt1 B | sm5 B | hs5 B |
| 3 | mn C | ll C | dn C | ct C | bt C | wm C | sm C | ch C | rd C | bg C | et C | pl C | tn C | hs C | dy C | rs C | mg C | wl C | hl C | br C | tr C | bc C | | hs1 A | sm1 A | sm5 A | | ll1 B | hs1 B | sm 1 B |
| 4 | dn D | wl D | bc D | ct D | bg D | rd D | et D | br D | tn D | hl D | wm D | sm D | dy D | mg D | rs D | tr D | mn D | hs D | bt D | pl D | ll D | ch D | | sm1 C | hs5 C | ll5 C | hs1 C | ll1 D | bt1 D | sm 5 D |
| 5 | tr E | bg E | hs E | ct E | sm E | bt E | br E | dn E | tn E | rd E | ch E | hl E | wl E | mn E | bc E | pl E | wm E | mg E | ll E | et E | dy E | rs E | | hl5 C | sm5 C | ll1 C | | sm1 D | ll5 D | hl1 D |
| 6 | wm F | et F | ct F | tr F | bc F | wl F | sm F | rs F | mg F | br F | ll F | bt F | mn F | hl F | pl F | bg F | tn F | dy F | rd F | dn F | hs F | ch F | | bt1 C | hl1 C | bt5 C | hs1 D | hl5 D | bt5 D | hs5 D |
| 7 | 4 ch A | 1 sm 15A | 1 ch A | 5 sm 15A | | 3 ch A | 2 sm 3 A | 5 ch A | 4 sm 15A | 2 ch A | 2 sm 3 A | ch3 D | ll7 D | ch7 D | ll3 D | 4 sm A | 3 sm A | 2 sm A | 1 sm A | 3 ll7 A | 1 ll7 A | 3 ll3 A | 1 ll3 A | 3 ch7 A | 1 ch7 A | 3 ch3 A | 1 ch3 A | 4 br A | 2 br A | 1 br A |
| 8 | 1 ch B | 2 ch B | 3 ch B | 4 ch B | 5 ch B | 1 sm 15B | 2 sm 15B | 3 sm 15B | 4 sm 15B | 5 sm 15B | | 2 sm 3 B | | ll3 C | ch3 C | 1 ll7 B | 1 ch7 B | 4 sm B | 1 ch3 B | 2 br B | 1 ll3 B | 3 sm B | 3 ch3 B | 1 br B | 3 ll7 B | 3 ch7 B | 2 sm B | 3 ll3 B | 4 br B | 1 sm B |
| 9 | 5 sm 15 C | 3 ch C | | 4 sm 15 C | 2 ch C | 3 sm 15 C | 5 ch C | 1 ch C | 1 sm 15 C | 2 sm 3 C | 4 ch C | 2 sm 15 C | | ll7 C | ch7 C | | | 1 sm C | 3 ch7 C | 3 ll7 C | 4 sm C | 3 ch3 C | 2 br C | 4 br C | 3 sm C | 1 ch7 C | 1 ll7 C | 1 br C | 2 sm C | 1 ch3 C |
| 10 | 5 ch D | 2 sm 3 D | 4 ch D | 1 sm 15 D | 3 ch D | 4 sm 15 D | 2 ch D | 5 sm 15 D | | 1 ch D | 2 sm 3 D | ch3 A | ch7 A | ll3 A | ll7 A | ll15 A | ch7 B | ll15 B | ch3 B | ll7 B | ll3 B | | 2 sm D | 1 ll7 D | 4 sm D | 3 sm D | 3 ch7 D | 3 ll7 D | 1 ch7 D | 1 sm D |

# 2009 experiments

**TREC**

- In vitro fungicide assays

# 2009 experiments

## TREC

- In vitro fungicide assays
- Fungicide application methods: small–medium sized trees in field and small (25 gal) potted trees



# Macro-infusion of fungicides


…and moves into the tree under pressure.

# Macro-infusion of fungicides

## Considerations

1. Time consuming (15-30 min/tree prep time and 10 min–90 min for infusion)

# Macro-infusion of fungicides

## Considerations

1. Time consuming
2. Tree size (only trees with flare roots are treatable)

# Macro-infusion of fungicides

## Considerations

1. Time consuming
2. Tree size
3. Expense – Fungicide and infusion device costs, applicator time

# Macro-infusion of fungicides

## Considerations

1. Time consuming
2. Tree size
3. Expense
4. Efficacy? – Ongoing work at Citra

# Macro-infusion of fungicides

Rainbow Treecare

**Consi**

1. Time c
2. Tree si
3. Expens
4. Efficac
5. Phytotoxicity



Arbotect 1x rate, 6 wks post-infusion

# Macro-infusion of fungicides

## Considerations

1. Time consuming
2. Tree size
3. Expense
4. Efficacy?
5. Phytotoxicity
6. Fruit residues?

Size of ‘Peterson’ fruit at time of application

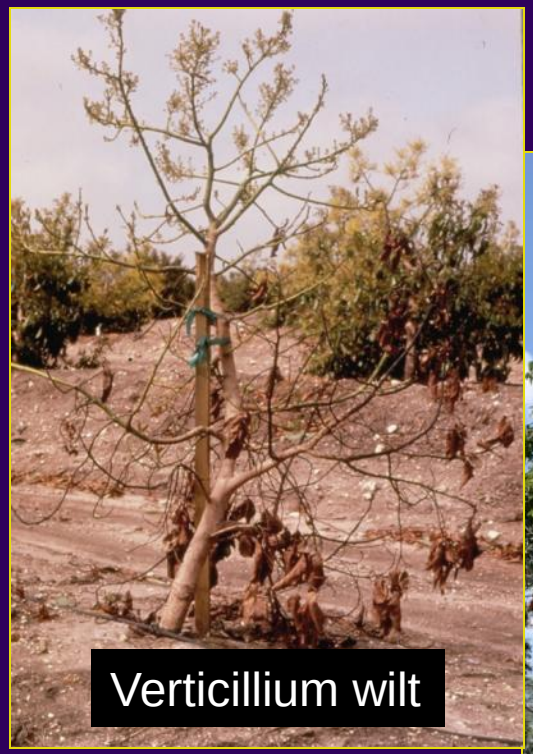
# Fungicide work

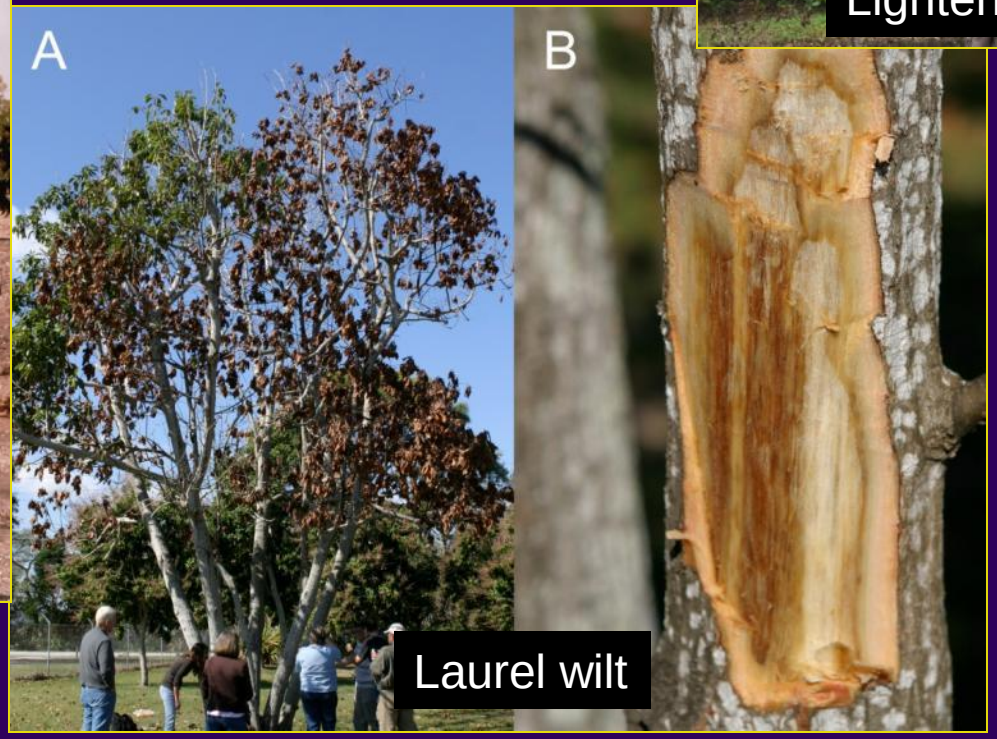
- *in vitro* growth inhibition
- tree applications
  - macro-infusions
  - soil drenches
    - TREC (‘Peterson’ ); 1x rates of Alamo and Arbotect (fruit residue)
    - Citra (‘Choquette’ and ‘Simmonds’ ); 1x rates of Alamo, Arbotect and Agri-phos (efficacy)

# Laurel wilt diagnosis

Symptoms

- Not very accurate

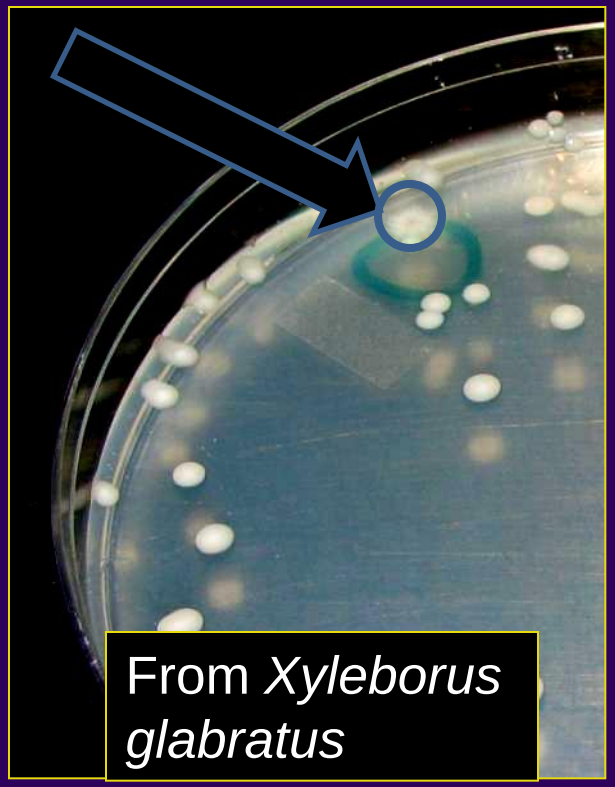



# Laurel wilt diagnosis

Isolation of *Raffaelea lauricola* on Ophiostoma semi-selective medium

- More accurate, but not specific

From diseased plants



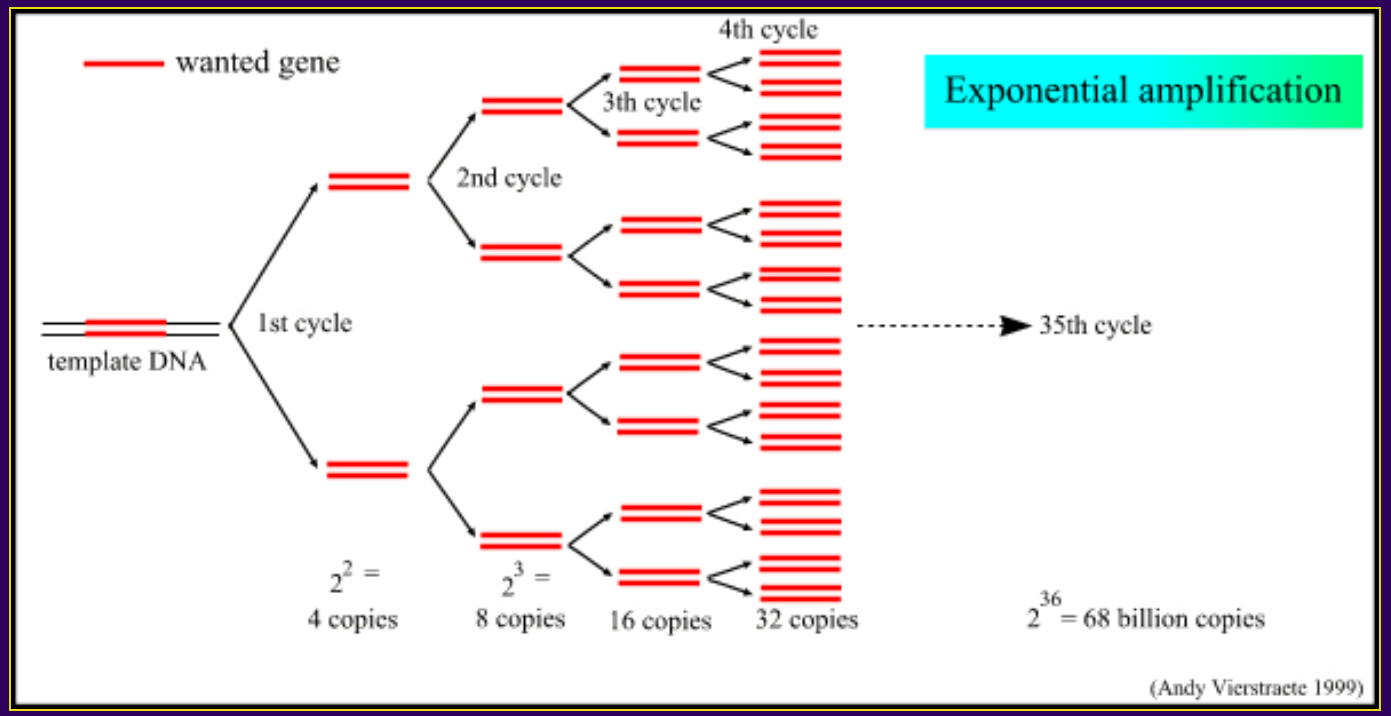
From *Xyleborus glabratus*

# Laurel wilt diagnosis

## DNA-based diagnoses

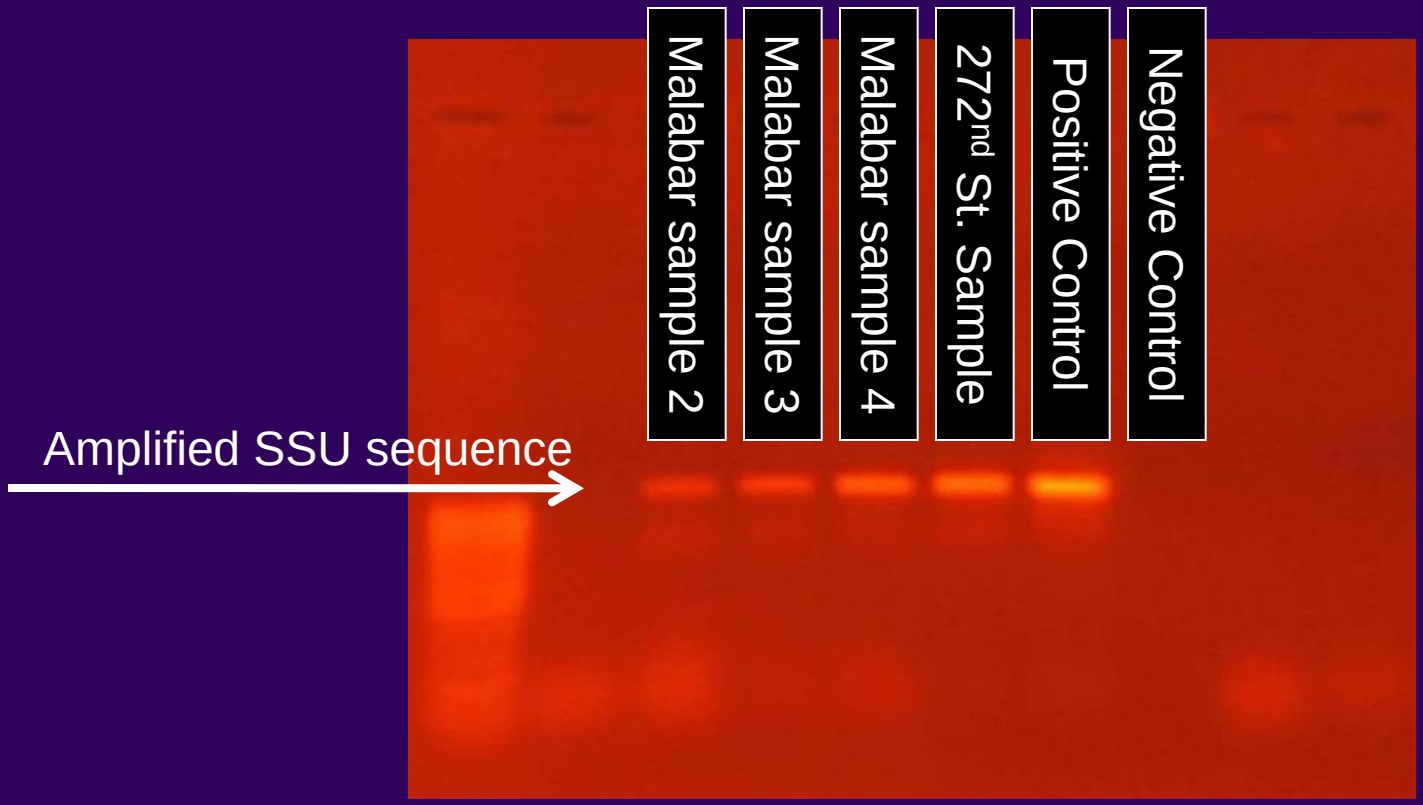
- Can be very accurate

### Polymerase Chain Reaction




http://users.ugent.be/~avierstr/principles/pcr.html

# Traditional PCR Results



# Will fungicides be management tools for laurel wilt?

- *in vitro* activity

# Fungicides evaluated against *Raffaelea lauricola*

| Fungicide | Active ingredient | Chemical class |
|---|---|---|
| *Alamo | propiconazole | azole |
| BAS 595 | triticonazole | azole |
| Baytan | triadimenol | azole |
| Eagle | myclobutanil | azole |
| Proline | prothioconazole | azole |
| | | |
| *Arbotect | thiabendazole | benzimidazole |
| Compass | trifloxystrobin | strobilurin |
| Daconil | chlorothalinil | phthalimide |
| Funginex | triforine | piperizine |
| Heritage | azoxystrobin | strobilurin |
| Manzate | mancozeb | dithiocarbamate |
| Omega 500 | fluazinam | pyrimidine |
| Stature SC | dimethomorph | morpholine |
| Terramaster | etridiazole | triadiazole |

# *In vitro* activity

| | % of growth on nonamended MEA | | | |
|---|---|---|---|---|
| **Fungicide** | **100ppm** | **1ppm** | **0.1ppm** | **0.01ppm** |
| Alamo | 100 | 100 | 100 | 78 |
| BAS 595 | 100 | nt | 93 | 46 |
| Baytan | 100 | 100 | 60 | 8 |
| Eagle | 100 | 100 | 30 | 2 |
| Proline | 100 | 73 | 26 | 11 |
| | | | | |
| Arbotect | 100 | 63 | 15 | nt |
| Compass | 94 | 67 | nt | nt |
| Daconil | 98 | 69 | nt | nt |
| Funginex | 87 | 17 | nt | nt |
| Heritage | 100 | 100 | 82 | 59 |
| Manzate | 97 | 62 | nt | nt |
| Omega 500 | 99 | 97 | nt | nt |
| Stature SC | 61 | 48 | nt | nt |
| Terramaster | 66 | 4 | nt | nt |

Will fungicides be management tools for laurel wilt?

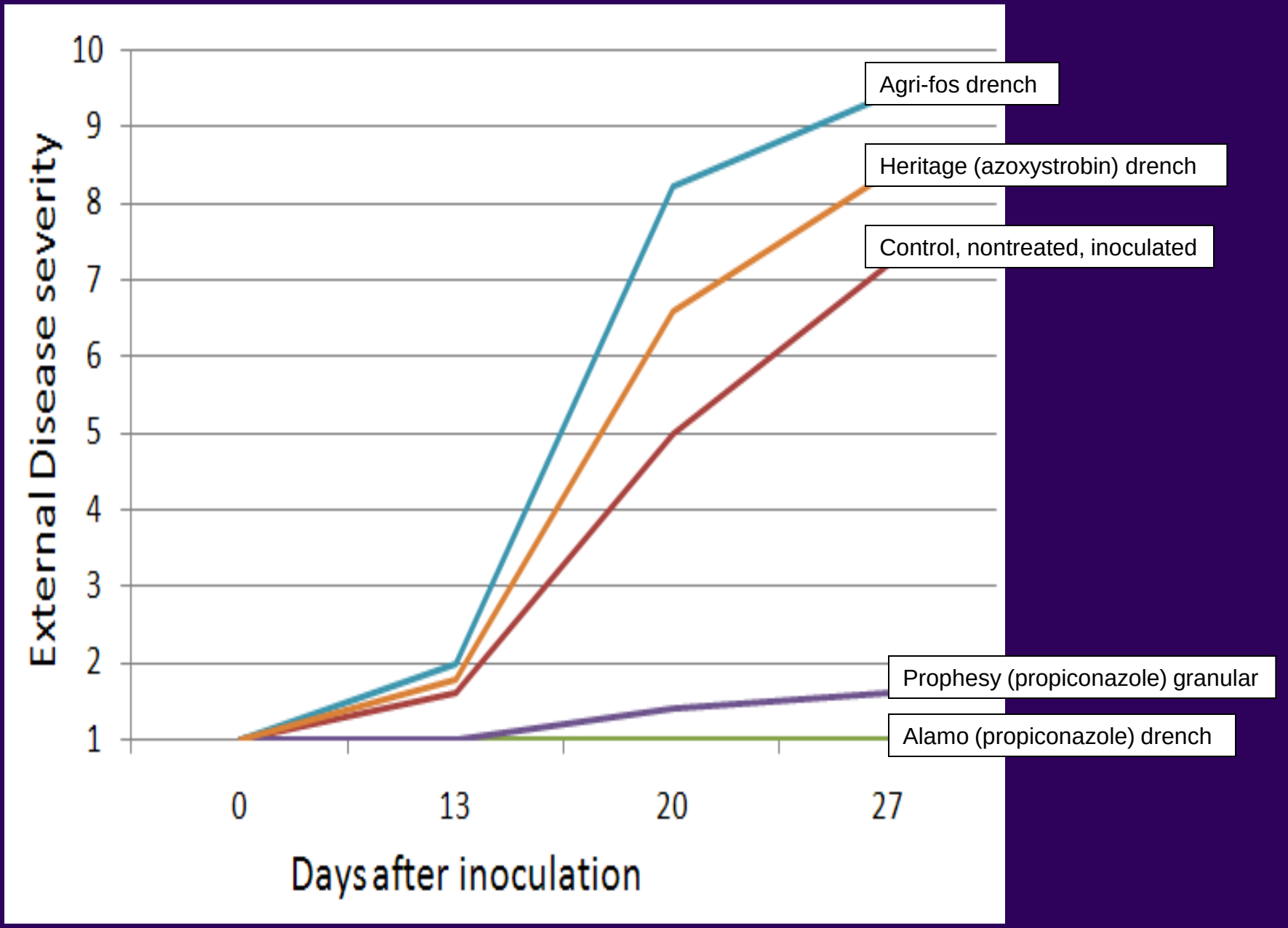
- *in vitro* activity
- *in planta* activity (efficacy)

| Treatment | Rate product |
|---|---|
| 1. Control, nontreated, noninoculated | - |
| 2. Control, nontreated, inoculated | - |
| 3. Alamo (14.3% a.i., propiconazole) drench | 20 ml / inch dia in 1 liter of water |
| 4. Prophesy 0.72G (0.72%a.i., propiconazole), | 50 g / inch dia |
| 5. Alamo (14.3% a.i., propiconazole) + Pentrabark (2%) | 20 ml / inch dia in 100 ml 2% Pentrabark |
| 6. Arbotect 20S (20% a.i., tbz) drench | 50 ml / inch dia in 1 liter of water |
| 7. Arbotect 20S (20% a.i., tbz) + Pentrabark (2%) | 50 ml / inch dia in 100 ml 2% Pentrabark |
| 8. Agri-fos (45.8% a.i.) drench | 10 ml in 1 liter of water |
| 9. Agri-fos (45.8% a.i.) + Pentrabark (2%) | 10 ml in 100 ml 2% Pentrabark |
| 10. Agri-fos (45.8% a.i.) foliar spray | 10 ml in 100 ml water |
| 11. Proline 480 SC (41.1% a.i., prothioconazole) drench | 7 ml in 1 liter of water |
| 12. Proline 480 SC (41.1% a.i., prothioconazole) + Pentrabark (2%) | 7 ml in 100 ml 2% Pentrabark |
| 13. Heritage (50% a.i., azoxystrobin) drench | 0.06 ml in 1 liter of water |
| 14. Heritage (50% a.i., azoxystrobin) + Pentrabark (2%) | 0.06 ml in 100 ml 2% Pentrabark |
| 15. Baytan 30 (30% a.i., triadimenol) drench | 0.33 ml in 1 liter of water |
| 16. Baytan 30 (30% a.i., triadimenol) + Pentrabark (2%) | 0.33 ml in 100 ml 2% Pentrabark |
| 17. Omega  500 (40% a.i., fluazinam) drench | 0.42 ml in 1 liter of water |
| 18. Omega 500 (40% a.i., fluazinam) + Pentrabark (2%), | 0.42 ml in 100 ml 2% Pentrabark |

# Fungicide efficacy, Sept-Oct 2009

| Treatment | Disease severity $y^{max}$ (after 4 weeks) External | Internal |
|---|---|---|
| Control, nontreated, noninoculated | 1.0 e | 1.0 d |
| Control, nontreated, inoculated | 7.2 ab | 7.8 ab |
| | | |
| Alamo (14.3% a.i., propiconazole) drench | 1.0 e | 2.0 d |
| Alamo (14.3% a.i., propiconazole) + Pentrabark (2%) | 1.0 e | 1.8 d |
| Prophesy 0.72G (0.72%a.i., propiconazole) | 1.0 e | 2.6 d |
| Proline 480 SC (41.1% a.i., prothioconazole) drench | 1.0 e | 1.0 d |
| Proline 480 SC (41.1% a.i., prothioconazole) + Pentrabark (2%) | 2.6 cde | 2.6 d |
| Baytan 30 (30% a.i., triadimenol) drench | 1.0 e | 2.2 d |
| Baytan 30 (30% a.i., triadimenol) + Pentrabark (2%) | 1.0 e | 1.0 d |
| | | |
| Arbotect 20S (20% a.i., tbz) drench | 1.8 de | 2.8 d |
| Arbotect 20S (20% a.i., tbz) + Pentrabark (2%) | 6.8 ab | 8.8 ab |
| | | |
| Agri-fos (45.8% a.i.) drench | 9.4 a | 9.4 a |
| Agri-fos (45.8% a.i.) + Pentrabark (2%) | 8.8 a | 9.0 a |
| Agri-fos (45.8% a.i.) foliar spray | 5.0 bc | 5.4 c |
| | | |
| Heritage (50% a.i., azoxystrobin) drench | 8.4 a | 10.0 a |
| Heritage (50% a.i., azoxystrobin) + Pentrabark (2%) | 4.6 bcd | 6.4 bc |
| | | |
| Omega 500 (40% a.i., fluazinam) drench | 7.2 ab | 8.2 ab |
| Omega 500 (40% a.i., fluazinam) + Pentrabark (2%) | 7.2 ab | 9.0 a |

Subjective 1-10, DMRT, $P<0.05$


External Disease severity
10
9
8
7
6
5
4
3
2
1
0
13
20
27
Days after inoculation
Agri-fos drench
Heritage (azoxystrobin) drench
Control, nontreated, inoculated
Prophesy (propiconazole) granular
Alamo (propiconazole) drench

Will fungicides be management tools for laurel wilt?

- *in vitro* activity
- *in planta* activity (efficacy)
- fruit residue?

# Macro-infusion of fungicides

# Fruit residue analyses (ppm). Syngenta. Sept 2009

| | Sample | Thiabendazole | Propiconazole |
|---|---|---|---|
| 8 | 2B ALAMO 1X INFUSE L | <0.01 | 0.20 |
| 10 | 2B ALAMO 1X INFUSE M | <0.01 | 0.28 |
| 11 | 2B ALAMO 1X INFUSE H | <0.01 | 0.18 |
| 12 | 3A ALAMO 0.1X INFUSE L | <0.01 | 0.11 |
| 13 | 3A ALAMO 0.1X INFUSE M | <0.01 | 0.16 |
| 14 | 3A ALAMO 0.1X INFUSE H | <0.01 | 0.17 |
| 20 | 4B ALAMO 1X DRENCH M | <0.01 | <0.05 |
| 21 | 4B ALAMO 1X DRENCH H | <0.01 | <0.05 |
| | | | |
| 22 | 5A ARBOTECT 1X INFUSE L | <0.01 | N/A |
| 23 | 5A ARBOTECT 1X INFUSE M | 0.54 | N/A |
| 24 | 5A ARBOTECT 1X INFUSE H | 0.92 | N/A |
| 28 | 6A ARBOTECT 0.1X INFUSE L | <0.01 | N/A |
| 29 | 6A ARBOTECT 0.1X INFUSE M | <0.01 | N/A |
| 30 | 6A ARBOTECT 0.1X INFUSE H | 0.012 | N/A |
| 33 | 7A ARBOTECT 1X DRENCH L | <0.01 | N/A |
| 34 | 7A ARBOTECT 1X DRENCH M | <0.01 | N/A |
| 35 | 7A ARBOTECT 1X DRENCH H | <0.01 | N/A |

# Thank you for your support


- Florida Avocado Committee
- University of Florida, IFAS Vice President
- Pine Island Nursery
- Zill High Performance Plants, Inc.
- USDA, T-STAR Special Grants
- Miami-Dade County Commission
- USDA APHIS-PPQ
- USDA, CSREES, SCRI