Prune disease management

Dr. J.E. Adaskaveg

Department of Plant Pathology
University of California, Riverside

Cooperating: H. Forster, UC Davis, D. Thompson, UC Riverside
R. Buchner and J. Connell, UCCE Tehama and Butte, Co.

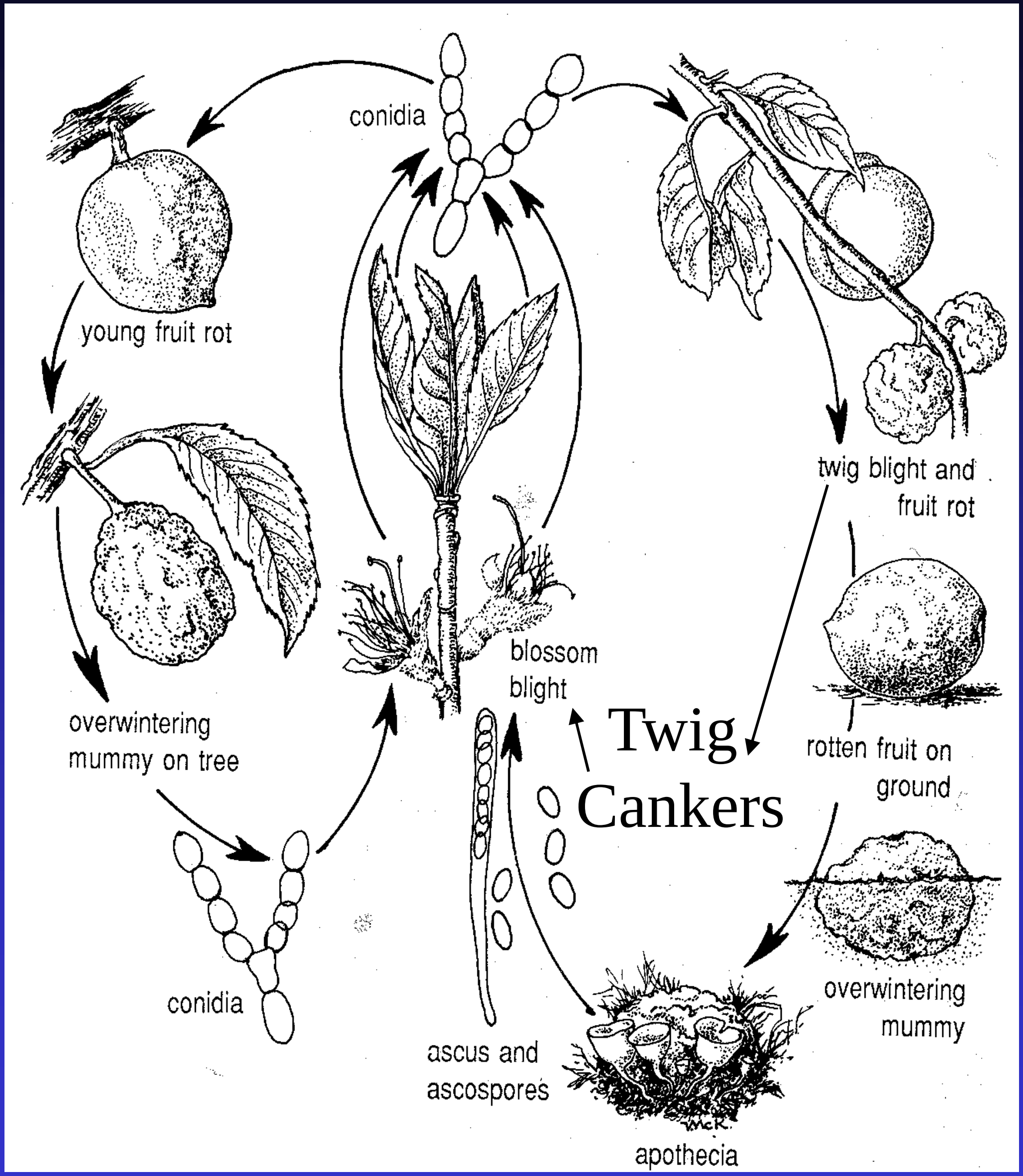
Brown rot of Prune

Monilinia laxa & M. fructicola

Blossom blight

Preharvest fruit decay

Disease cycle of *Monilinia fructicola* on prune and preharvest control measures



- The Disease Triangle of Plant Pathology -

Host

- Varietal susceptiblity,
- Planting design

Environment

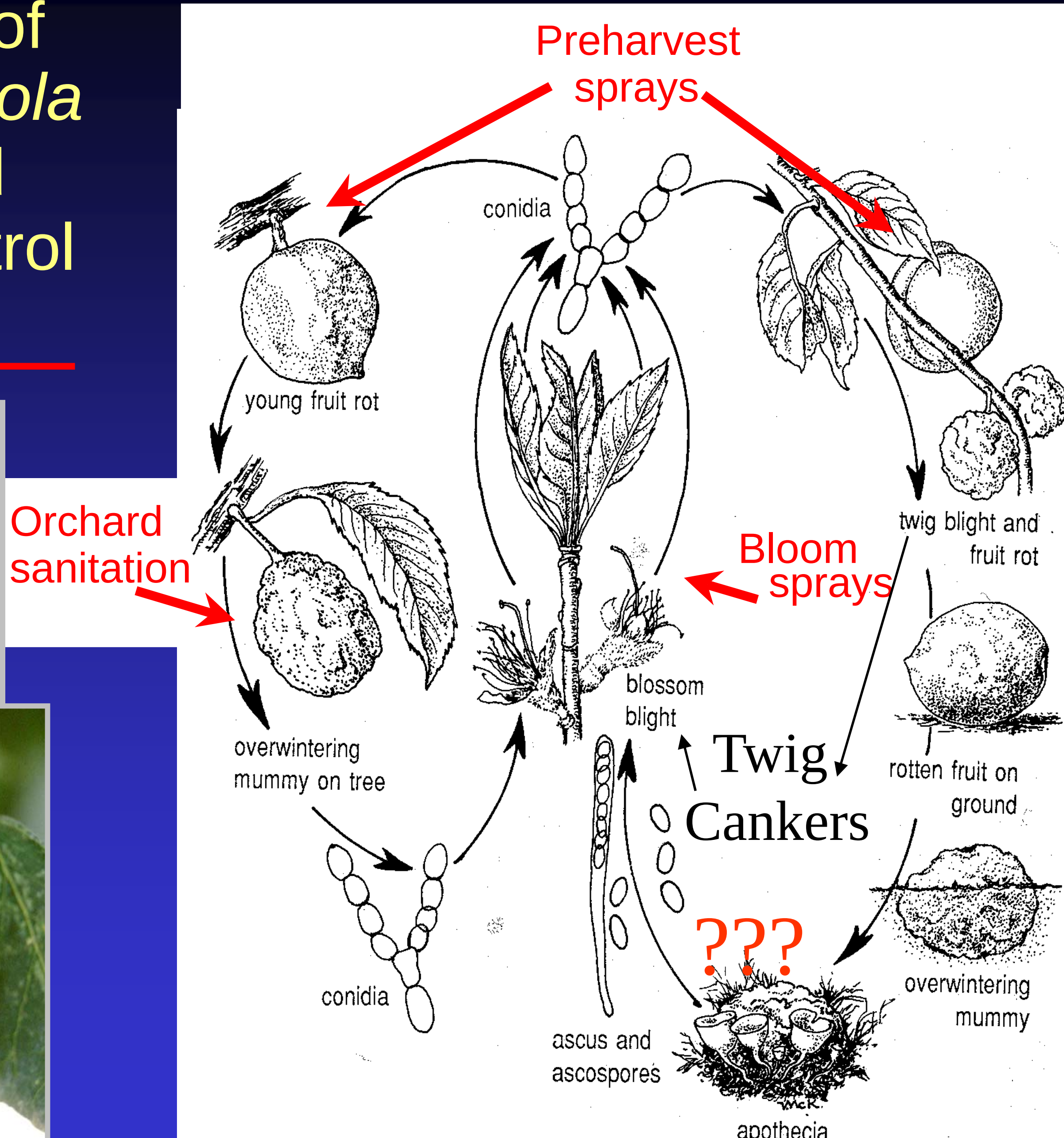
- Wetness - rainfall, irrigation
- Temperatures above 58F

Pathogen

- *M. fructicola, M. laxa*
- Inoculum potential (overwintering mummies, twig cankers)

The interactions between the components effect the amount of disease.

Disease cycle of *Monilinia fructicola* on prune and preharvest control measures



Orchard sanitation
Removal of overwintering fruit mummies

Mummies and cankers as primary inoculum sources in the spring.

Management of Brown Rot Blossom Blight

- Fungicide Maintenance Programs -

Dried Plum (prune) blossoms are susceptible at white tip through full bloom because all blossom tissues (green scales, petals, stamens, pistils) are susceptible and infection may lead to blossom blight, but the stamen and pistil tissues are the most susceptible.

EFFICACY AND TIMING OF FUNGICIDES, BACTERICIDES, AND BIOLOGICALS FOR DECIDUOUS TREE FRUIT, NUT, STRAWBERRY, AND VINE CROPS 2009

ALMOND	*PEACH*
APPLE AND PEAR	*PISTACHIO*
APRICOT	*PLUM*
CHERRY	*PRUNE*
GRAPE	*STRAWBERRY*
KIWIFRUIT	*WALNUT*

Jim Adaskaveg
Professor
University of California, Riverside

Doug Gubler
Extension Plant Pathologist
University of California Davis

Themis Michailides

Plant Pathologist

University of California, Davis/Kearney Agricultural Center

Brent Holtz
Farm Advisor
University of California Cooperative Extension, Madera County

UC Davis, Dept. of Plant Pathology
www.plpnem.ucdavis.edu

UC Kearney Agricultural Center
www.uckac.edu/plantpath

Statewide IPM Program
www.ipm.ucdavis.edu

Efficacy tables will be updated again for 2010

Fungicides Registered and in Development for Managing Prune Diseases

Single-fungicides - Inorganics and Conventional Synthetics

Inorganics
M1 – Copper, Sulfur – 1960s

Dithiocarbamates
M2 – Ziram, (Maneb) – 1940s

Phthalimides
M3 – Captan – 1950s

Isophthalonitriles
M4 – Bravo, Echo, Equus – 1960s

Aromatic Hydrocarbon
M14 – Botran – 1960s

Benzimidazoles
1 – Topsin-M, T-Methyl – 1970s

Dicarboximides
2 – Rovral, Iprodione, Nevado – 1980s

Sterol inhibitors (SBIs)
3 – Elite**, Indar, Inspire, Orbit/Bumper, Rally, Quash** – 1970s – 1980s

SDHIs
7 – Luna Privilege* – 1960s

Anilinopyrimidines
9 – Vangard, Scala – 1990s

QoIs
11 – Abound, Gem** – 1990s

Hydroxyanilides
17 – Elevate – 1990s

New 2009/10:
Elite**, Inspire, Quash**, Luna Privilege*

Multi-site mode of action | Single-site mode of action | Reduced risk fungicides

* - Not planned for registration as a single AI. ** - For fresh prune only.

Fungicides Registered and in Development for Managing Prune Diseases

Conventional Synthetic Fungicides – Pre-mixtures

- Pristine (7+11) – 2000s
- Adament* (3+11) – 2000s
- Distinguish (9+11) – **New:** 2000s
- Inspire XT (3+3) – **New:** 2010s
- Inspire Super (3+9) – **New:** 2010s
- Luna Sensation (7+11) – **New:** 2010s
- Quilt Xcel** (3+11) – **New:** 2010s
- Quadris Top (3+11) – **New:** 2010s

3 SBIs | 7 SDHIs | 9 Anilinopyrimidines | 11 QoIs

* - For fresh prune only. ** - For prune, CA registration pending.

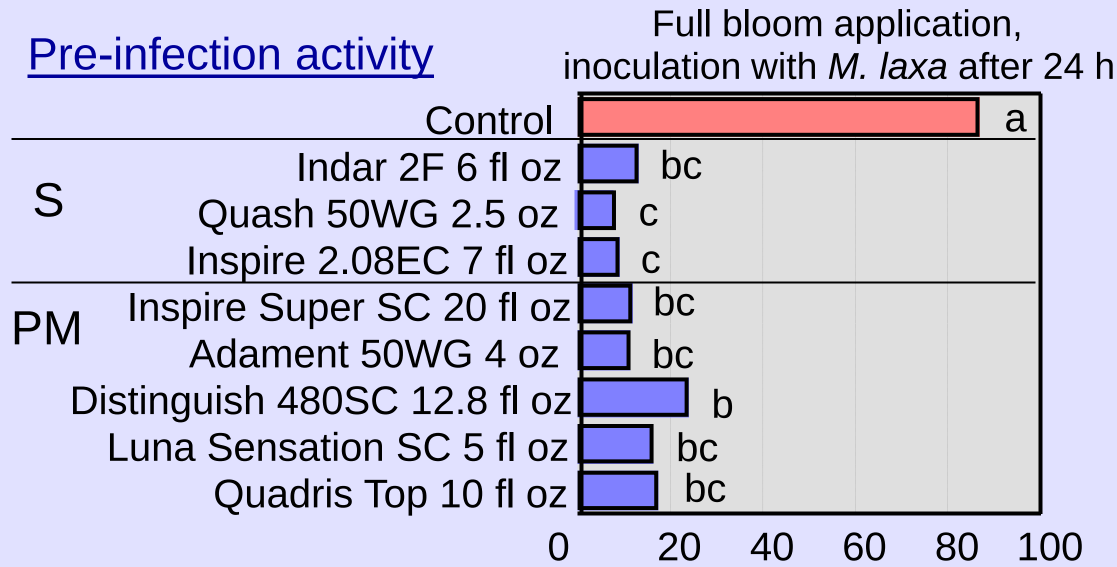
Natural Products

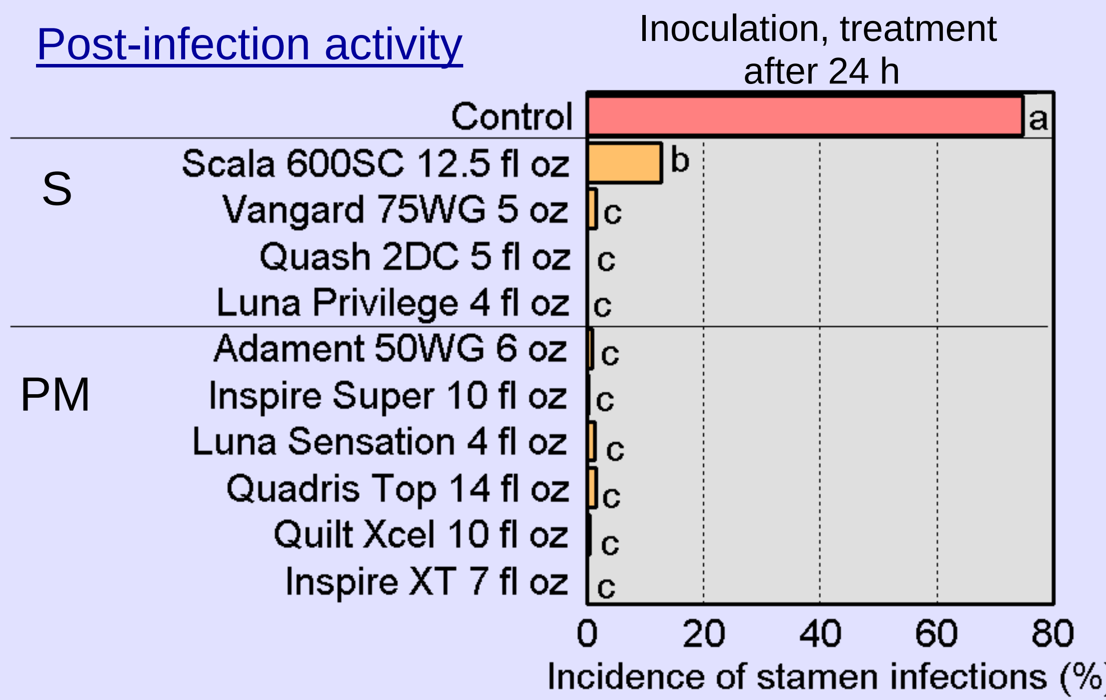
Regalia, Actinovate, Cerebrocide – 2000s

Natural products from plant extracts that potentially will be OMRI approved were evaluated for organic farming of almonds.

Pre- and post-infection treatments with selected fungicides - Blossom blight of French prune -

Control Quash





Summary: Fungicides for blossom blight control

- ***Highly effective (+++ or ++++) for blossom blight, pre- and post-infection activity:***

- Registered:
  - SBIs (3): Orbit/Bumper, Indar, Elite (fresh prune only)
  - Anilinopyrimidines (AP) (9): Vangard, Scala
  - Dicarboximides (2): Rovral (-oil)/Nevado/Iprodione
  - Hydroxyanilide (17): Elevate
  - Pre-mixtures: Pristine(7/11), Adament (3/11) (fresh prune)

- Planned Registrations:
  - SBIs (3): Quash (currently for fresh, expand to dried), Inspire
  - SDHIs (7): Luna Privilege (?)
  - Pre-mixtures: Inspire Super (3/9), Inspire XT (3/3), Luna Sensation (7/11), Quadris Top (3/11), Quilt Xcel (3/11),…

Blossom blight control with fungicides

UC guidelines

2 applications during bloom

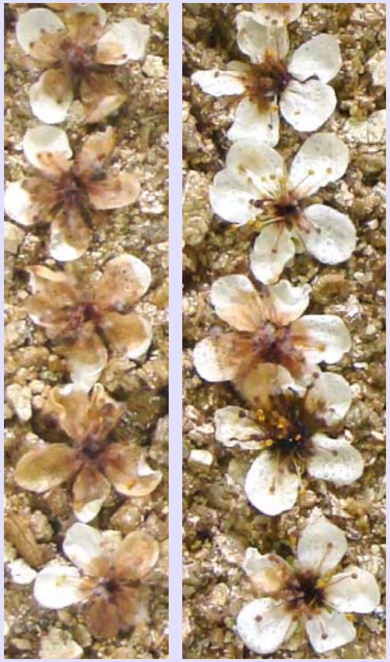
Use when environmental conditions are highly conducive (rain)

Delayed bloom application

1 application at 30-50% bloom

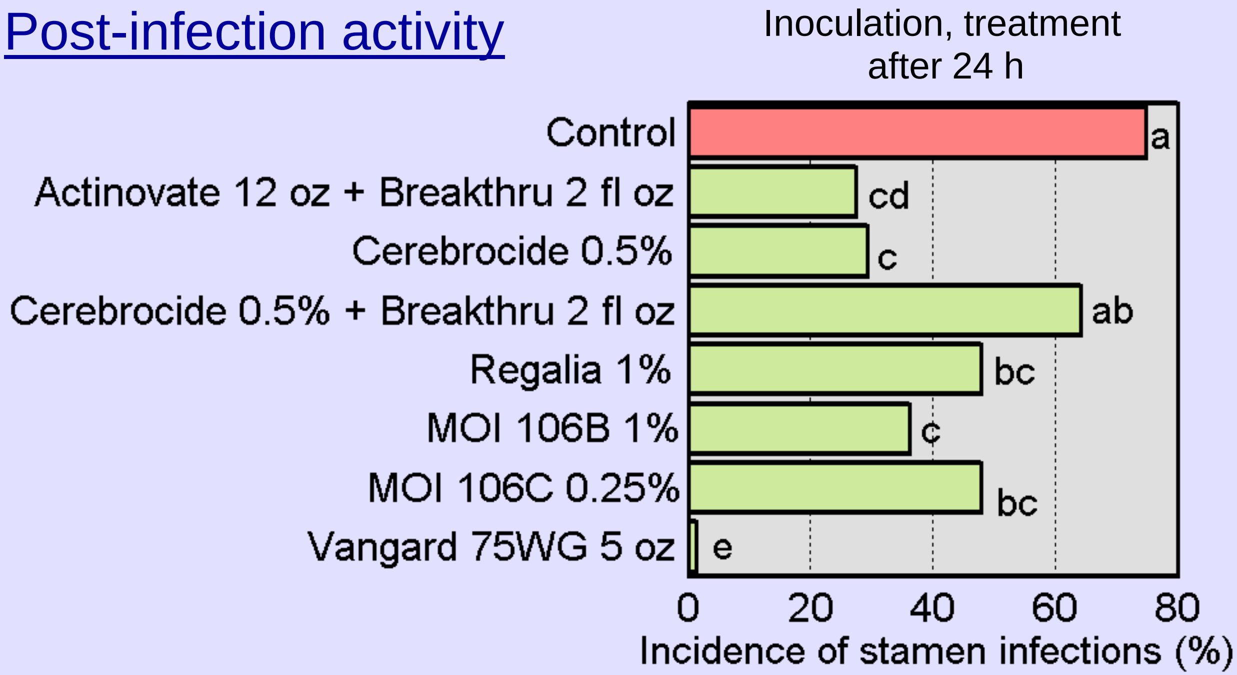
Use when environmental conditions are less favorable

Efficacy of a biocontrol and of natural products
- Blossom blight of French prune -



Control Regalia

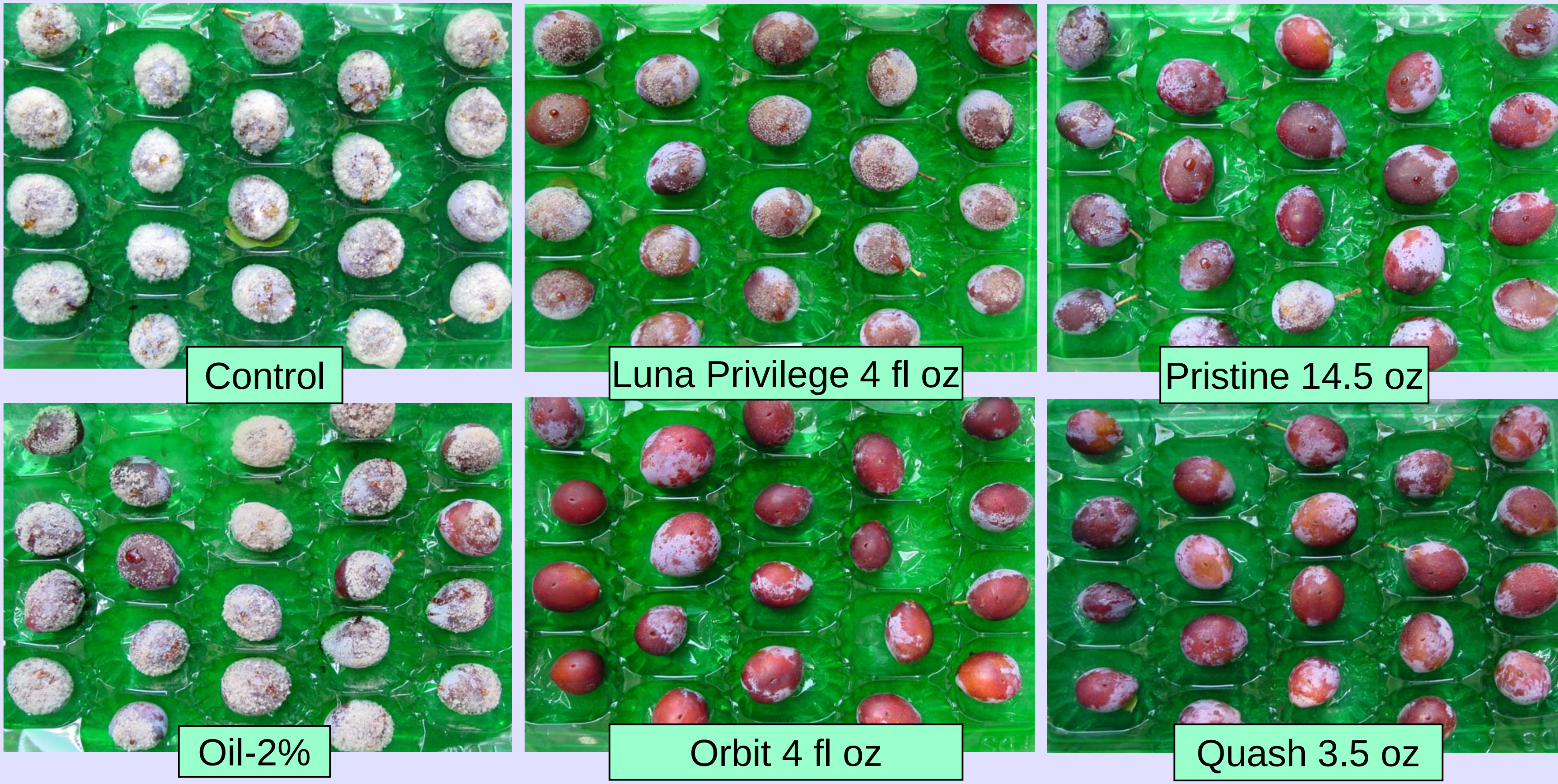
Post-infection activity



In previous years' pre-infection experiments, the activity of biologicals and natural products was low.

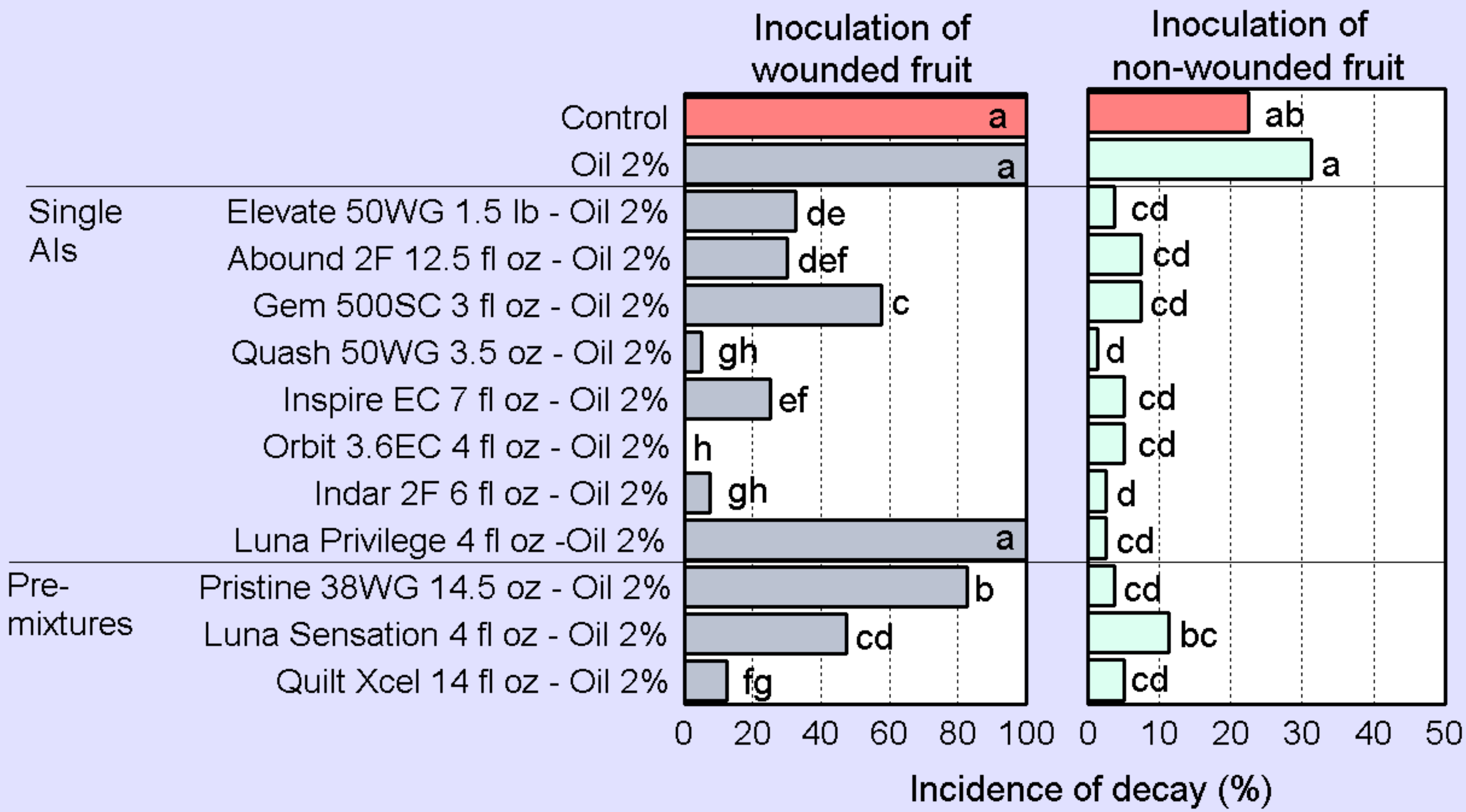
Management of brown rot fruit decay with preharvest fungicide treatments

Efficacy of 14-day PHI fungicide field treatments on the incidence of brown rot after wound inoculation



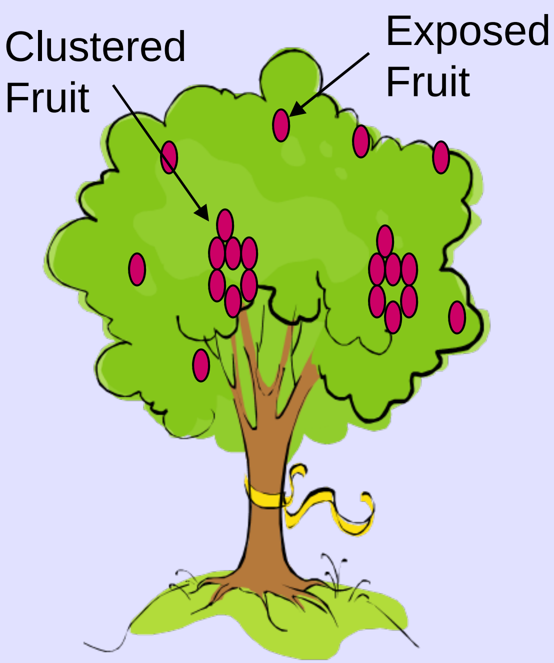
Treatments were applied in the field using an air-blast sprayer calibrated for 100 gal/A. Omni Supreme spray oil was used at 2% in all treatments. After harvest inoculated fruit were incubated for 7 days at 20C.

7-day PHI fungicide treatments for management of brown rot decay of French prune – Yuba-Sutter Co. 2009



Treatments were applied in the field on 8-4-09 using an air-blast sprayer (100 gal/A). Omni Supreme Spray oil was used. After harvest, fruit were either spray- or wound-inoculated with conidia of M. fructicola (30,000 conidia/ml). Fruit were then incubated for 7 days at 20 C.

Efficacy of high- and low-gallonage fungicide field treatments to clustered and exposed fruit on the incidence of brown rot after inoculation

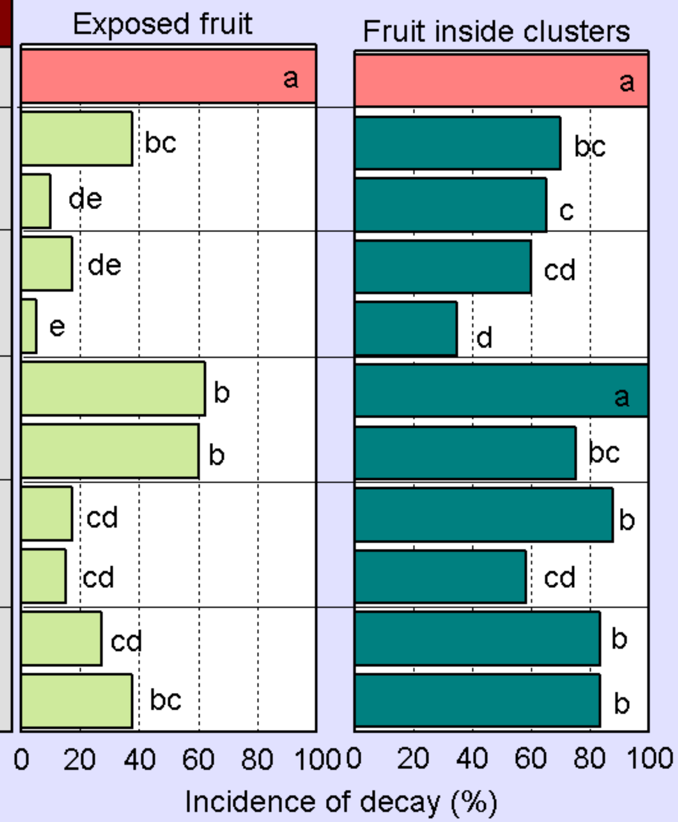


Treatments were applied on 8-14 and 8-28. All fruit were inoculated on the inside surface opposite to the perimeter.

14- and 0-day PHI treatments with natural products for management of brown rot decay of French prune - UC Davis 2009

- Evaluation of application volumes in preventing decay of exposed fruit and fruit inside clusters -

Treatment	Application volume/A
Control	---
Orbit 3.6EC 4 fl oz - Oil 1.5%	80 gal
Orbit 3.6EC 4 fl oz - Oil 1.5%	160 gal
Quash 50WG 3.5 oz - Oil 1.5%	80 gal
Quash 50WG 3.5 oz - Oil 1.5%	160 gal
Pristine 38WG 14.5 oz - Oil 1.5%	80 gal
Pristine 38WG 14.5 oz - Oil 1.5%	160 gal
Luna Sensation 7 fl oz - Oil 1.5%	80 gal
Luna Sensation 7 fl oz - Oil 1.5%	160 gal
Quadris Top 14 fl oz - Oil 1.5%	80 gal
Quadris Top 14 fl oz - Oil 1.5%	160 gal



Treatments were applied in the field on 8-14 and 8-28-09 using an air-blast sprayer at 80 or 160 gal/A. Omni Supreme Spray oil was used. At harvest, either single fruit from the tree perimeter (exposed fruit) or fruit from clusters were collected and wound-inoculated with conidia of *M. fructicola* (30,000 conidia/ml) on the unexposed side of the fruit. Fruit from inside clusters were inoculated on the inside facing side.

Summary: Fungicides for fruit brown rot control

- All fungicides significantly reduced the incidence of brown rot decay on harvested fruit after non-wound inoculation with *M. fructicola*.
- When fruit were wound-inoculated after treatment and harvest, the efficacy of most treatments was reduced as compared to the non-wound inoculations (fungicides are contact materials).
- The addition of a spray oil in general significantly increased the efficacy of the fungicides (comparative research in 2007-08).
- Biologicals and natural products were ineffective as protective treatments of fruit (research done in 2007-08).
- Application at 160 gal (as compared to 80 gal) was beneficial for protecting fruit outside and <u>inside</u> clusters from brown rot for some fungicides.

EFFICACY AND TIMING OF FUNGICIDES, BACTERICIDES, AND BIOLOGICALS FOR DECIDUOUS TREE FRUIT, NUT, STRAWBERRY, AND VINE CROPS 2009

ALMOND	*PEACH*
APPLE AND PEAR	*PISTACHIO*
APRICOT	*PLUM*
CHERRY	*PRUNE*
GRAPE	*STRAWBERRY*
KIWIFRUIT	*WALNUT*

Jim Adaskaveg
Professor
University of California, Riverside

Doug Gubler
Extension Plant Pathologist
University of California Davis

Themis Michailides
Plant Pathologist
University of California, Davis/Kearney Agricultural Center

Brent Holtz
Farm Advisor
University of California Cooperative Extension, Madera County

---

UC Davis, Dept. of Plant Pathology
www.plpnem.ucdavis.edu

UC Kearney Agricultural Center
www.uckac.edu/plantpath

Statewide IPM Program
www.ipm.ucdavis.edu

Efficacy tables will be updated again for 2010

PRUNE (OR DRIED PLUM)—FUNGICIDE EFFICACY

http://www.ipm.ucdavis.edu

Fungicide	Resistance risk	FRAC No.	Brown Rot Blossom	Brown Rot Fruit	Russet scab	Rust
Benlate	high	1	++++	++++	----	----
Distinguish (Reg. but not marketed)	medium	9/11	++++	++	----	++
Orbit, Bumper	high	3	++++	++++	----	+++
Elite (Fresh prune)	high	3	++++	++++	----	+++
Indar	high	3	++++	++++	----	+++
Adament (Fresh prune)	medium	3/11	++++	++++	----	+++
Pristine	medium	7/11	++++	++++	ND	ND
Rovral/Iprodione/Nevado w/oil	low	2	++++	NR	----	NR
Scala	high	9	++++	6	----	ND
Topsin-M/T-Methyl w/oil	high	1	++++	++++	----	----
Vangard	high	9	++++	6	----	ND
Benlate	high	1	+++	+/-	----	----
Elevate	high	17	+++	+++	ND	----
Rovral/Iprodione/Nevado	low	2	+++	NR	----	NR
Topsin-M/T-Methyl	high	1	+++	+/-	----	----
Abound	high	11	++	+	----	+++
Botran	medium	M14	++	++	ND	ND
Bravo/Chlorothalonil/Echo/Equus	low	M5	++	++	++	----
Captan	low	M4	++	++	+++	----
Gem (Fresh prune)	high	11	++	+	----	+++
Rally	high	3	++	++	----	----
Sulfur	low	M2	+/-	+/-	----	++

Fungicide treatment timing in prune (dried plum)

http://www.ipm.ucdavis.edu

Disease	Green bud	White bud	Full bloom	May	June	July
Brown rot[a]	+++	+++	++++	—	+	++
Russet scab[b]	—	—	+++	—	—	—
Rust[c]	—	—	—	+	++	+++

Rating: +++ = most effective, ++ = moderately effective, + = least effective, and — = ineffective.

Timings used will depend upon orchard history of disease, length of bloom, and weather conditions each year.

a. Flowers are susceptible beginning with the emergence of the sepals (green bud) until the petals fall, but are most susceptible when open.

b. A physiological disorder, no pathogens involved.

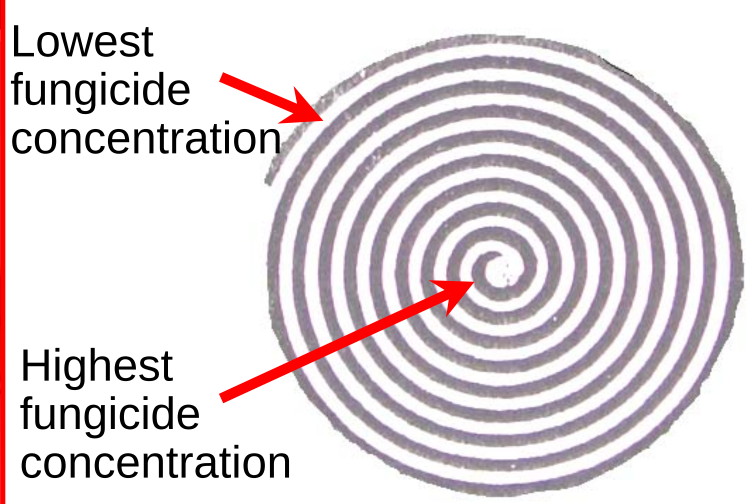
c. More severe when late spring rains occur.

Fungicide resistance in pathogens of prune

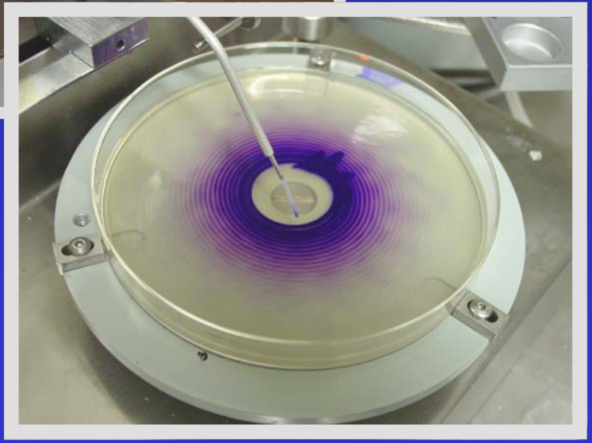
Evaluation of the in vitro toxicity of fungicides against *Monilinia* spp.

- Reported control failures after treatments with anilinopyrimidine (AP) and SBI fungicides.
- Resistance to AP fungicides in pathogens of other crops has been reported in CA.
- In 2007 we found AP resistance in one isolate of *M. fructicola* in one CA prune orchard (West Butte Co.).
- Resistance against SBI fungicides has developed in other stone fruit growing areas of the country.
- Fungal isolates obtained from decaying fruit in 2009 were evaluated for their in vitro sensitivities (central Butte Co.).

Quantification of fungicide sensitivity: The spiral gradient dilution method



After a 2-4 h incubation period a continuous gradient is formed.



Creating a radial, exponential gradient of a fungicide using a spiral plater

Forster *et al.*, Phytopathology 94:163-170, 2004.

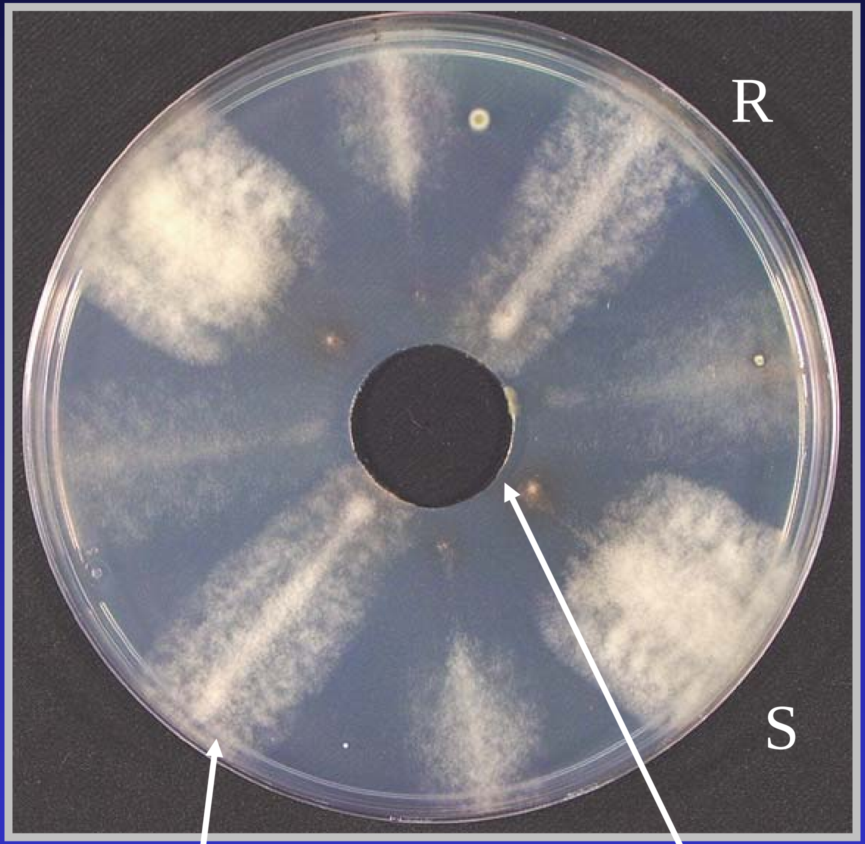
Brown rot resistance to AP fungicides in a California stone fruit orchard in 2009

- In Northern California:

  - AP resistance in the brown rot pathogen *M. fructicola* was detected in 2007.

  - AP resistance in the brown rot pathogen *M. laxa* was detected in 2009.

- All isolates were sensitive to propiconazole (Orbit) and Rovral

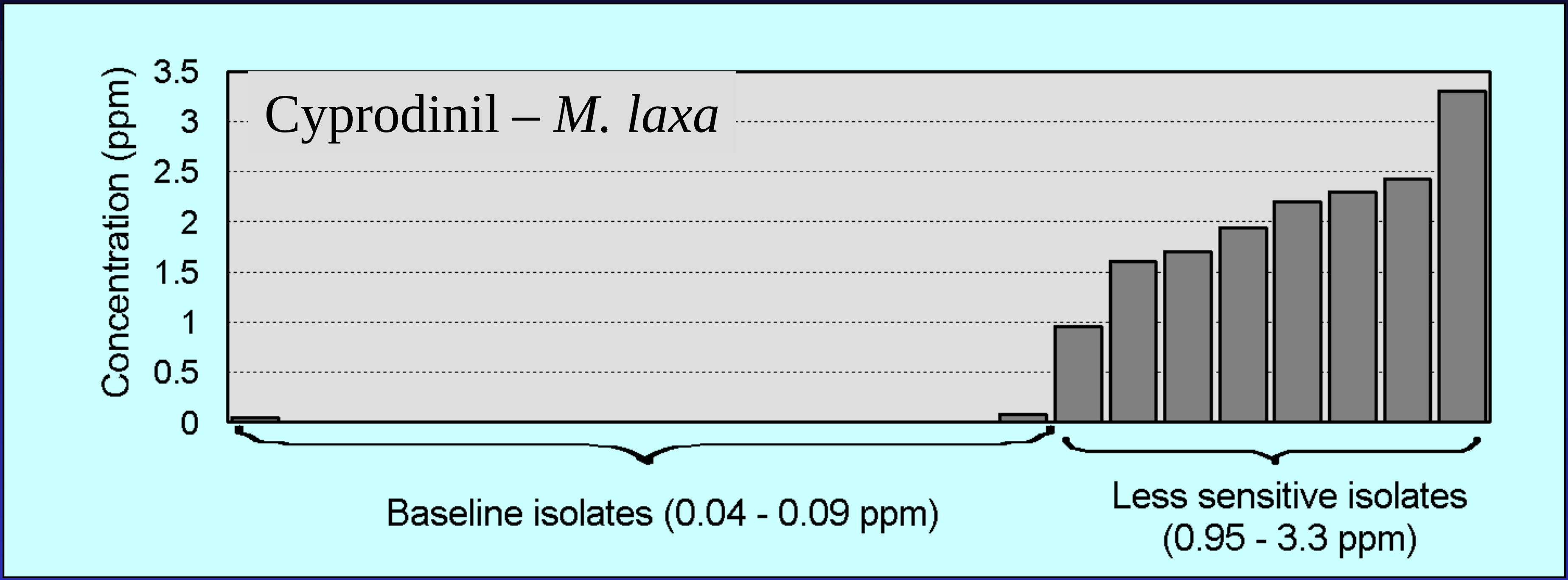


Cyprodinil

Lowest concentration

Highest concentration

In vitro toxicity of fungicides against *M. laxa - 2009*



- EC_{50} values of 8 of the 9 isolates collected in an orchard with treatment failures increased by 10 to 30 times as compared to baseline sensitive wild-type isolates.

- These isolates were highly sensitive to SBI fungicides.

Summary: In vitro toxicity of Monilinia spp. *against selected fungicides*

- One isolate of *M. fructicola* resistant to cyprodinil was found in our limited 2007 survey.
- The majority of isolates of *M. laxa* collected from one location in 2009 was resistant to AP fungicides (e.g., cyprodinil, pyrimethanil).
- Thus, resistance development is occurring. If not managed with appropriate anti-resistance strategies, resistant isolates will likely continued to be selected for. This may result in widespread treatment failures and loss of an important fungicide class.
    - Limit AP fungicides to bloom treatments (ideally 1/yr)
    - Mix with other fungicides (e.g., captan, chlorothalonil)

Prune rust caused by *Tranzschelia discolor*

Early symptoms of disease will start in late April/early May. Defoliation may occur in July and August in severe years.

The incidence of rust was very low at most locations in 2007-2009 and our studies on this disease were postponed.

Components of an integrated disease management program for brown rot of stone fruit

- Early disease detection
- Planting
  - Variety selection (host resistance)
  - Plant spacing (greater air movement, shorter drying times)
- Cultural practices
  - Avoid high-angle sprinkler irrigation
  - Provide a balanced nutrition
  - Pruning practices (improved microclimate, removal of diseased tissue)
- Sanitation
  - At harvest remove all fruit from trees
  - Remove overwintering mummies from trees and cultivate mummies into soil
- Chemical control

Identification of *Aspergillus* species associated with dried plum fruit

A. niger *A. chevalieri*

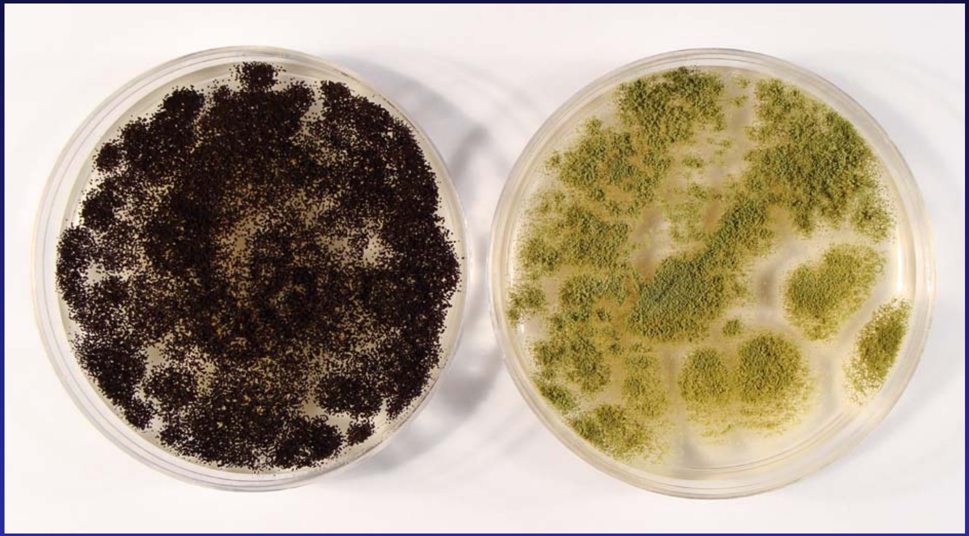
- Two morphologically distinct species obtained in 2008 were identified based on morphological characteristics: *A. niger* and *A. chevalieri.*

- No new reports on fungal growth on dried plums in 2009.
- Molecular methods based on DNA sequence data are being developed for additional identification.
- Goal: Development of a simple method to differentiate between harmless saprobes and potentially harmful mycotoxin-producing species: *A. flavus* (toxin = aflatoxins), *A. ochraceus* (toxin = ochratoxin A), *A. terreus* (toxin = citrinin).

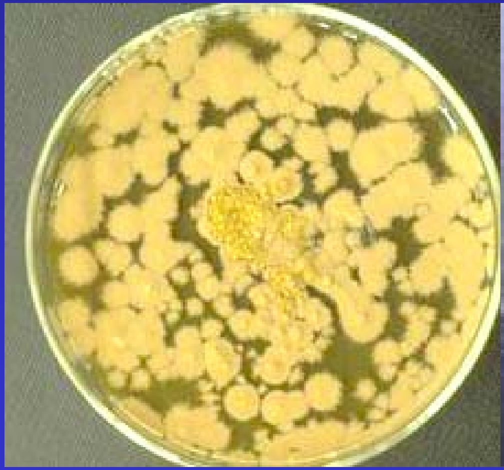
Cultures of *Aspergillus* species associated with dried plum fruit

Factors affecting cultural characteristics include agar media, age, species variability, etc.

A. niger



A. chevalieri



A. terreus

A. flavus

A. ochraceus

DNA sequence-based approach for identification of *Aspergillus* species

```
EF652070_E.interm./A. chev.       ---------AAGGATCATTACCGAGTGCGGGCC-CTCTGG---GTCCAAC
EF652047_E._herb./A. glaucus      ---------AAGGATCATTACCGAGTGCGGGCC-CTCTGG---GTCCAAC
AF138904_Aspergillus_niger        AACCTGCGGAAGGATCATTACCGAGTGCGGGTC-CTTTGG---GCCCAAC
FJ878637_Aspergillus_terreus      AACCTGCGGAAGGATCATTACCGAGTGCGGGTC-TTTATG---GCCCAAC
FJ844610_Aspergillus_fumigatus    AACCTGCGGAAGGATCATTACCGAGTGAGGGCC-CTCTGG---GTCCAAC
AY373859_Aspergillus_parasitic    AACCTGCGGAAGGATCATTACCGAGTGTAGGGT-TCCTAGCGAGCCCAAC
FJ487932_Aspergillus_flavus       AACCTGCGGAAGGATCATTACCGAGTGTAGGGT-TCCTAGCGAGCCCAAC
FJ878645_Emericella_nidulans      AACCTGCGGAAGGATCATTACCGAGTGCGGGCTGCCTCCGGGCGCCCAAC
                                           ******************  **         *   * *****

EF652070_E.interm./A. chev.       CTCCCATCCGTGTCTATCTGTACCCT-GTTGCTTCGGCGTGGCCACGGC-
EF652047_E._herb./A. glaucus      CTCCCATCCGTGTCTATCTGTACCCT-GTTGCTTCGGCGTGGCCACGGC-
AF138904_Aspergillus_niger        CTCCCATCCGTGTCTAT-TGTACCCT-GTTGCTTCGGCGGGCCCGCCGCT
FJ878637_Aspergillus_terreus      CTCCCACCCGTGACTAT-TGTACCTT-GTTGCTTCGGCGGGCCCGCCAGC
FJ844610_Aspergillus_fumigatus    CTCCCACCCGTGTCTAT-CGTACCTT-GTTGCTTCGGCGGGCCCGCCGTT
AY373859_Aspergillus_parasitic    CTCCCACCCGTGTTTAC-TGTACCTTAGTTGCTTCGGCGGGCCCGCCGTC
FJ487932_Aspergillus_flavus       CTCCCACCCGTGTTTAC-TGTACCTTAGTTGCTTCGGCGGGCCCGCCATT
FJ878645_Emericella_nidulans      CTCCCACCCGTGACTACCTA-ACACT-GTTGCTTCGGCGGGAGCCCCC
                                  ****** *****   **       **  * ************ *     *

EF652070_E.interm./A. chev.       ---------------------------------------CCGCCGGA
EF652047_E._herb./A. glaucus      ----------------------------------------CCGCCGAA
AF138904_Aspergillus_niger        TGTCGGCCGCCGGGGGGCGCCTCTGCCCCCCGGGCCCGTGCCCGCCGGA
FJ878637_Aspergillus_terreus      -GTTGCTGGCCGCCGGGGGGCGACTCGCCCCCGGGCCCGTGCCCGCCGGA
FJ844610_Aspergillus_fumigatus    -TCGACGGCCGCCGGGGAGGCCTTGCGCCCCCGGGCCCGCGCCCGCCGAA
AY373859_Aspergillus_parasitic    ----ATGGCCGCCGGGGGCG----TCAGCCCCGGGCCCGCGCCCGCCGGA
FJ487932_Aspergillus_flavus       ---CATGGCCGCCGGGGGCTC---TCAGCCCCGGGCCCGCGCCCGCCGGA
FJ878645_Emericella_nidulans      ------------AGGGGGCG--------------------AGCCGCCGGG
                                                                          ******

EF652070_E.interm./A. chev.       GACTAACATTTGAACGCTGT--CTGAA-GTT-TGCAGTCTGAGT-TTTTA
EF652047_E._herb./A. glaucus      GACTAACATTTGAACACTGT--CTGAA-GTT-TGCAGTCTGAGT-TTTTA
AF138904_Aspergillus_niger        GACCCCAACACGAACACTGT--CTGAAAGCG-TGCAGTCTGAGT-TGATT
FJ878637_Aspergillus_terreus      GACCCCAACATGAACCCTGTT-CTGAAAGCT-TGCAGTCTGAGTGTGATT
FJ844610_Aspergillus_fumigatus    GACCCCAACATGAACGCTGTT-CTGAAAGTA-TGCAGTCTGAGT-TGATT
AY373859_Aspergillus_parasitic    GACAC---CACGAACTCTGT--CTGATCTAG-TGAAGTCTGAGT-TGATT
FJ487932_Aspergillus_flavus       GACAC---CACGAACTCTGT--CTGATCTAG-TGAAGTCTGAGT-TGATT
FJ878645_Emericella_nidulans      GACCAC----TGAACTTCATGCCTGAGAGTGATGCAGTCTGAGCCTGAAT
                                  ***        ****    *  ****        ** ********   *

EF652070_E.interm./A. chev.       GT-TAAACAATCGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCAT
EF652047_E._herb./A. glaucus      GT-TAAACAATAATTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCAT
AF138904_Aspergillus_niger        GA-ATGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCAT
FJ878637_Aspergillus_terreus      CT-TTGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCAT
FJ844610_Aspergillus_fumigatus    AT-C-GTAATCAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCAT
AY373859_Aspergillus_parasitic    GTATCGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCAT
FJ487932_Aspergillus_flavus       GTATCGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCAT
FJ878645_Emericella_nidulans      AC----AAATCAGTCAAAACTTTCAACAATGGATCTCTTGGTTCCGGCAT
                                         *    * ************** *********************

EF652070_E.interm./A. chev.       CGATGAAGAACGCAGCGAAATGCGATAATTAATGTGAATTGCAGAATTCA
EF652047_E._herb./A. glaucus      CGATGAAGAACGCAGCGAAATGCGATAATTAATGTGAATTGCAGAATTCA
AF138904_Aspergillus_niger        CGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCA
FJ878637_Aspergillus_terreus      CGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCA
FJ844610_Aspergillus_fumigatus    CGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCA
AY373859_Aspergillus_parasitic    CGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCC
FJ487932_Aspergillus_flavus       CGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCC
FJ878645_Emericella_nidulans      CGATGAAGAACGCAGCGAACTGCGATAAGTAATGTGAATTGCAGAATTCA
                                  ******************* ******** ** ****************

EF652070_E.interm./A. chev.       GTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGG
EF652047_E._herb./A. glaucus      GTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGG
AF138904_Aspergillus_niger        GTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGG
FJ878637_Aspergillus_terreus      GTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGG
FJ844610_Aspergillus_fumigatus    GTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGG
AY373859_Aspergillus_parasitic    GTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGG
FJ487932_Aspergillus_flavus       GTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGG
FJ878645_Emericella_nidulans      GTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGCATTCCGGGG
                                  **************************************** *********
```

rDNA ITS 1 sequences were obtained from GenBank and the alignment was done using Clustal W.

Alignments are based on 3 isolates of *Aspergillus chevalieri* (teleomorph *Eurotium intermedium*), 3 isolates of *A. glaucus* (teleomorph *E. herbariorum*), 2 isolates of *A. niger*, 3 isolates of *A. terreus*, 3 isolates of *A. fumigatus*, 4 isolates of *A. parasiticus*, 3 isolates of *A. flavus* and 1 isolate of *A. nidulans*.

Highly variable DNA regions

DNA sequence-based approach for identification of *Aspergillus* species

- Molecular detection methods are being developed for species identification.
- Due to the high variability within the ribosomal DNA region a PCR-RFLP approach will be first pursued (easier to develop and less expensive)
  - Amplification of the rDNA region
  - Digest with restriction enzymes
  - Electrophoresis
- Alternatively or subsequently, species-specific primers will be developed for predominantly occurring species