Fungicide resistance in stone fruits

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Stone Fruits

- Members of the genus *Prunus*, family Rosaceae
- Fruit is a drupe (one-seeded fruit with a hard, woody endocarp surrounding the seed)

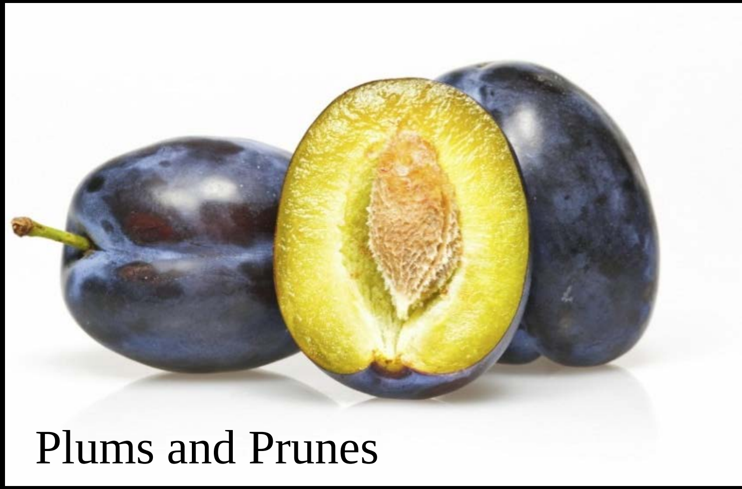
Stone Fruits

Almonds

Apricots

Peaches and Nectarines

Cherries



Plums and Prunes

Class Name	MOA Code	FRAC Group Code	Pathogen	Common Name	Crop	Reference	Remarks
MBC	B1	1	*Cladosporium carpophilum*	Scab	Peach, Nectarine	Chandler *et al*. 1978	Field
MBC	B1	1	*Coccomyces hiemalis*	Cherry leaf spot	Cherry	Jones & Ehret 1980	Field
MBC	B1	1	*Monilinia fructicola*	Brown rot	Stone fruits	Whan J H 1976	Field
MBC	B1	1	*Sphaerotheca pannosa*	Powdery mildew	Peach	Jarvis & Slingsby 1975	Field
QoI	C3	11	*Fusicladium carpophilum*	Leaf spot	Almond	Foerster *et al*. 2009	Field
QoI	C3	11	*Monilinia laxa*, *M. fructigena*, *M. fructicola*	Brown rots	Stone fruits	Meissner & Stammler 2010	Lab
Dicarboxomides	E3	2	*Monilinia fructicola*	Brown rot	Stone fruits	Penrose *et al*. 1985, Elmer and Gaunt 1994	Field
DMI	G1	3	*Blumeriella jaapii*	Leaf spot	Cherry	Proffer *et al*. 2006	Field
DMI	G1	3	*Monilinia fructicola*	Brown rot	Stone fruits	Nuninger-Ney *et al*. 1989, Elmer *et al*. 1992	Field
DMI	G1	3	*Sphaerotheca pannosa*	Powdery mildew	Nectarine	Reuveni 2001	Field
SDHI	C2	7	*Monilinia fructicola*	Brown rot	Peach	Amiri *et al*. 2010	Field

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DMI	G1	3	*Monilinia fructicola*	Brown rot	Stone fruits	Nuninger-Ney *et al*. 1989, Elmer *et al*. 1992	Field
SDHI	C2	7	*Monilinia fructicola*	Brown rot	Peach	Amiri *et al*. 2010	Field

Brown Rot of Stone Fruits

- *Monilinia fructicola*
- *Monilinia fructigena*
- *Monilinia laxa*

Brown rot of peach is an ideal candidate for resistance development.

- Large populations and rapid multiplication of target pathogen
- Extensive and concentrated areas of use
- Use of repetitive or sustained treatments


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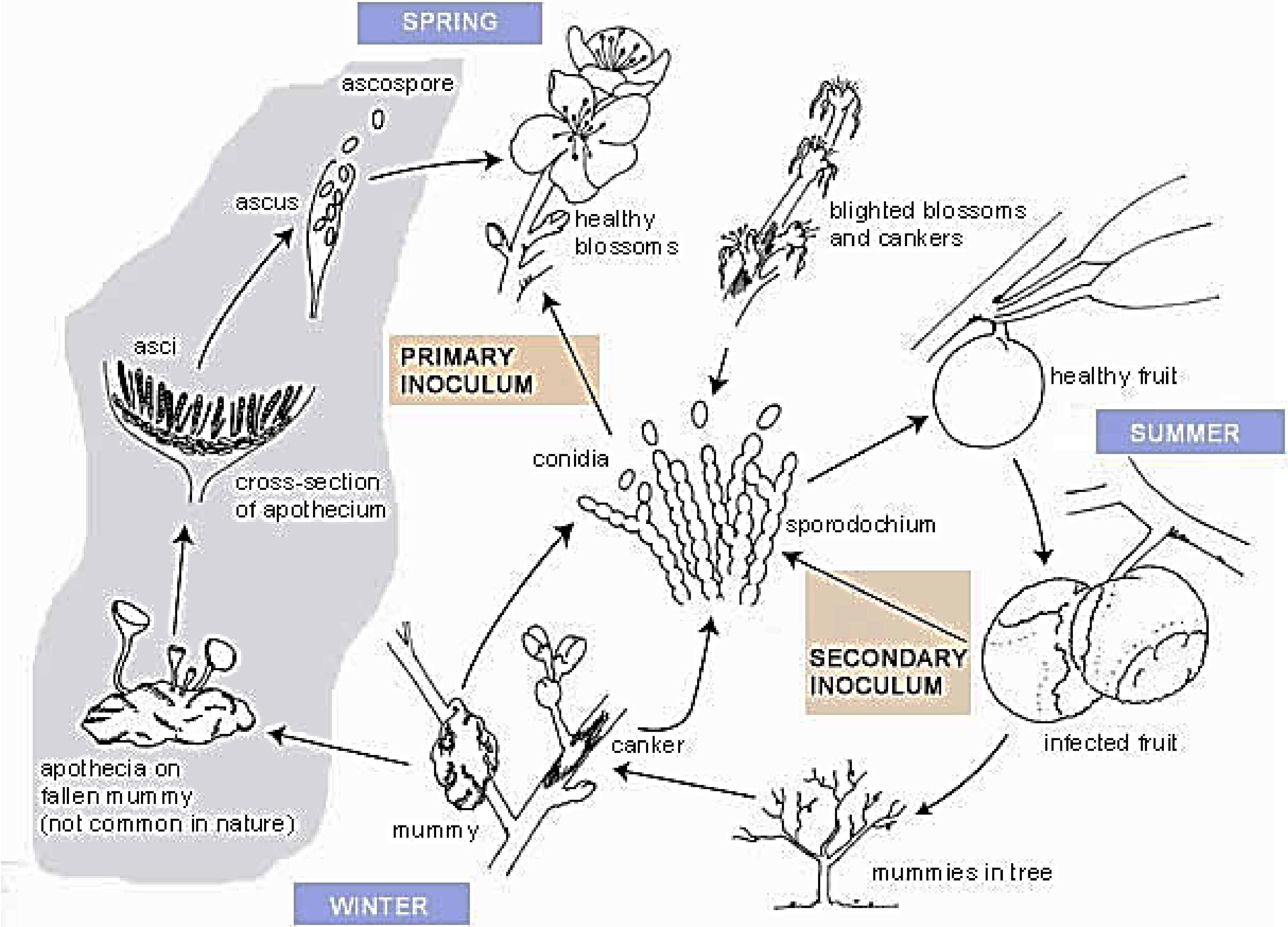


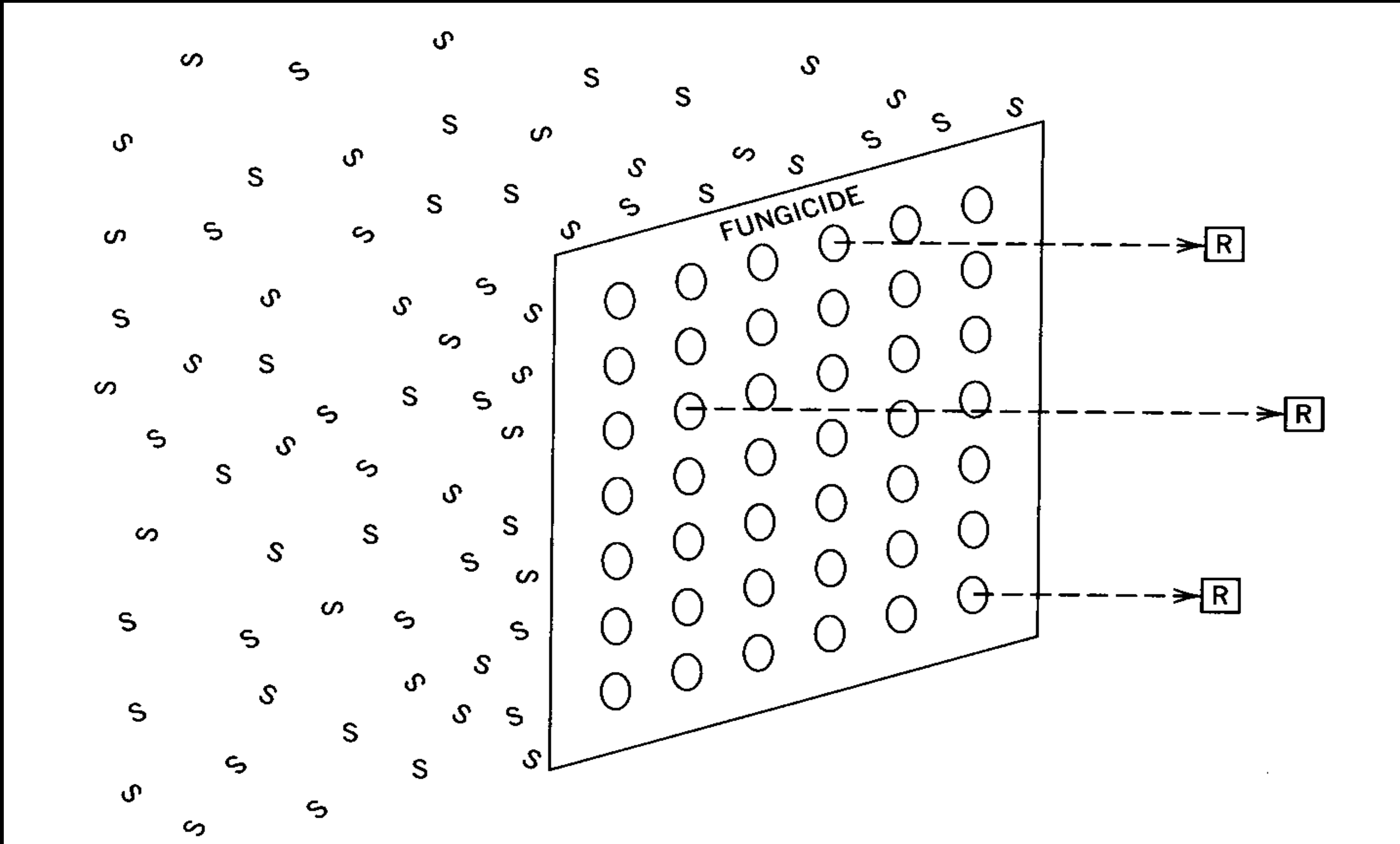
Drawing courtesy Vickie Brewster

Products for blossom blight and brown rot control

- Bravo, Equus (chlorothalonil; blossom blight only)
- Vangard (cyprodinil; blossom blight only)
- Scala (pyrimethanil)
- Captan, Captec
- Abound (azoxystrobin)
- Pristine (boscalid + pyraclostrobin)
- Orbit, Elite, Nova, Indar (DMI’s)
- Rovral (iprodione;blossom blight only)
- Topsin (thiophanate-methyl)
- Elevate (fenhexamid)

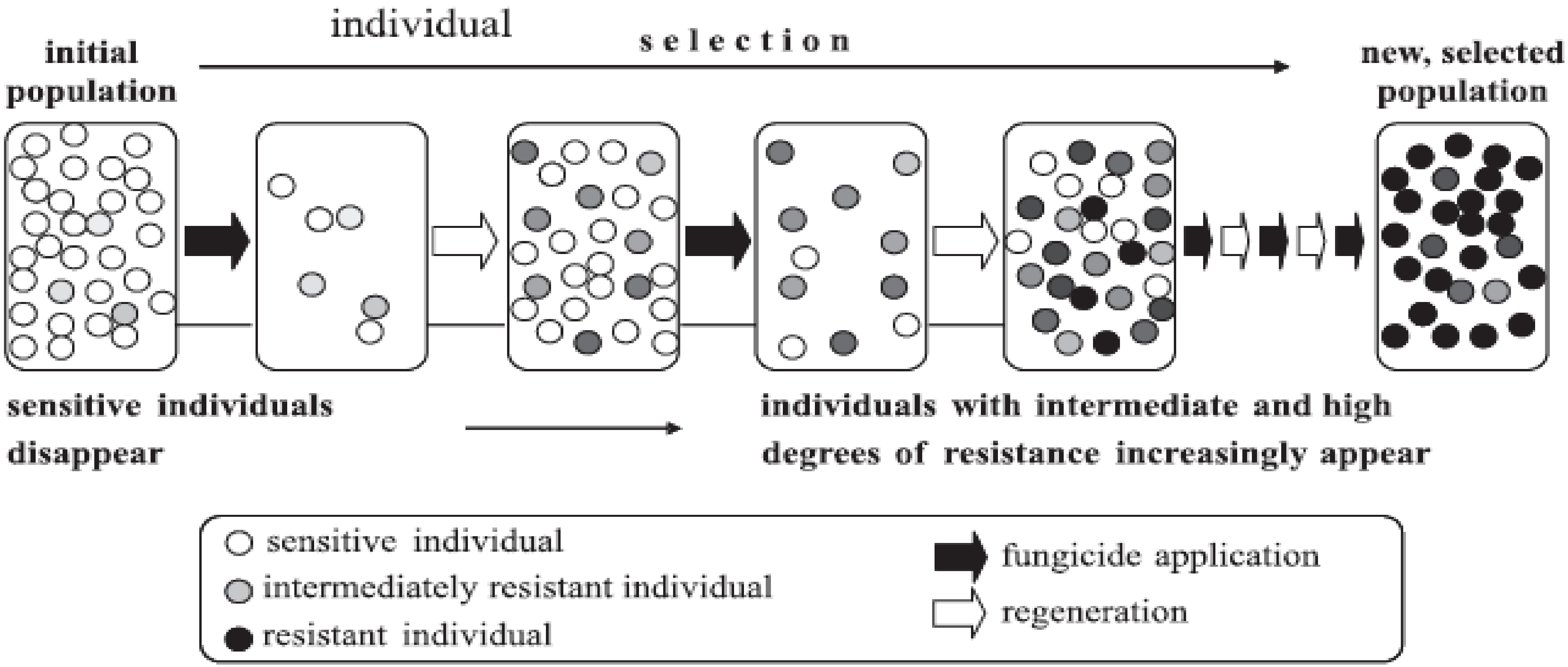
Combined risk: 1 = low, 2-6 = medium, 9 = high

benzimidazoles dicarboximides phenylamides	high (3)	3	6	9
carboxanilides DMIs phosphorothiolates anilinopyrimidines phenylpyrroles strobilurins	medium (2)	2	4	6
coppers dithiocarbamates melanin inhibitors phthalimides sulphur SAR-inducers	low (1)	1	2	3
basic fungicide risk ↑ basic disease risk →		low (1)	medium (2)	high (3)
		seed-borne (eg. *Pyrenophora, Ustilago*) soil-borne (eg. *Phytophthora*), cereal eyespot cereal rust rice sheath blight	barley *Rhynchosporium* wheat *Septoria*	apple scab banana *Sigatoka*, cereal powdery mildew, grape *Botrytis*, potato blight, citrus *Penicillium*, rice blast



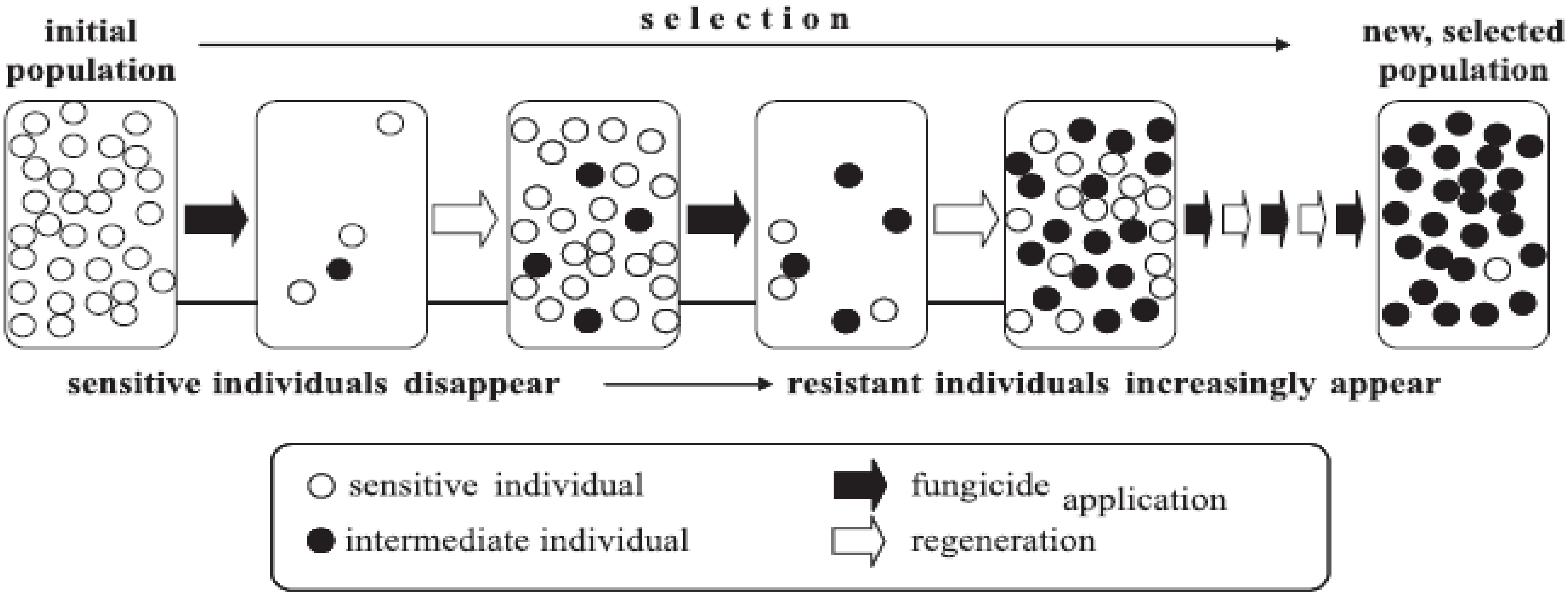
R = resistant S = sensitive

Evolution of fungicide resistance: quantitative resistance

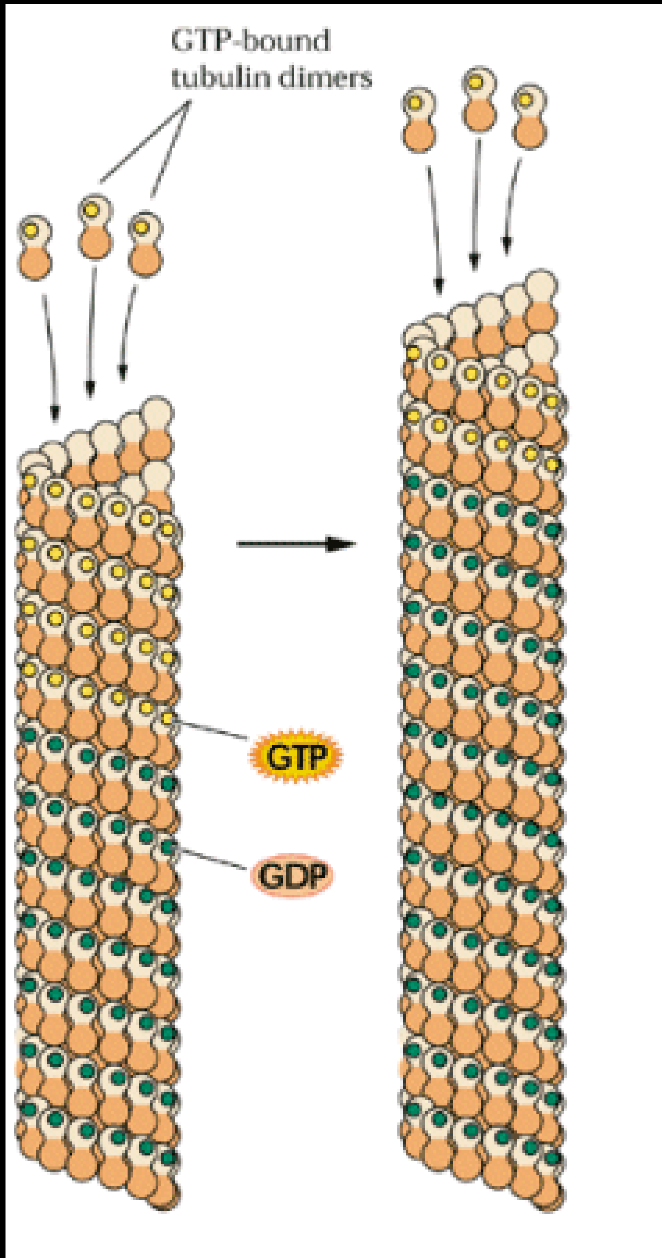


Deising H et al. Brazilian Journal of Microbiology (2008) 39:286-295

Evolution of fungicide resistance: qualitative resistance



Deising H et al. Brazilian Journal of Microbiology (2008) 39:286-295



Benzimidazoles

- Benzimidazole high-level resistance is due to a point mutation in the beta-tubulin gene. An E198A mutation in the beta-tubulin gene has been observed in SC, MI, and CA. This is widespread in the Southeast.
- A low-level resistance due to a Histidine to Tyrosine at 6 has been reported in CA only.

Benzimidazole Resistance in Peach

- Resistance was first reported in S.C. orchards in 1976.
- Wide-spread losses were observed in 1982.
- Strains may persist for long periods of time.
- "Careful, well-informed management may permit the effective use of benzimidazole fungicides in some localities, . . ., but assays for resistance should be done prior to orchard use."

E. Zehr, (1991) Plant Disease 75:590-593.

Dicarboxamides

- Resistance has been reported in South Carolina and California.

- The resistance mechanism is presumed to be the same as that observed in other systems (histidine kinase inhibition – osmoregulatory system).

Table 1. Range of sensitivity of isolates of *Monilinia fructicola* to propiconazole before and after repeated propiconazole applications in South Carolina peach orchards[a]

Source of isolates	Isolates (no.)	EC_{50} (µg/ml)[b]			Mean r^2
		Range	Median	Mean	
Musser Farm, initial[c]	44	0.02-0.15	0.03	0.04	92.0
Musser Farm, 1993	52	0.01-0.19	0.03	0.04	91.4
Musser Farm, 1994	89	0.01-0.25	0.04	0.05	90.3
Musser Farm, 1995	51	0.02-2.16	0.06	0.18	82.1
Anderson County, 1995	58	0.01-2.62	0.02	0.12	84.4
Anderson County, 1996	61	0.01-2.00	0.04	0.12	89.5
Commercial peach, 1995	47	0.01-1.82	0.085	0.15	86.2
Commercial peach, 1996	29	0.02-1.09	0.04	0.13	84.2

[a] Musser Farm site received no demethylation-inhibiting (DMI) fungicides prior to 1993 but was sprayed 29 times with propiconazole from 1993 to 1995. Anderson County site received 27 DMI fungicide applications interspersed with 11 dicarboximide applications from 1989 to 1995. In other commercial orchards, DMI fungicides were the principal fungicides used.

[b] Concentration of propiconazole in potato dextrose agar required to suppress radial growth of mycelium 50%.

[c] Isolates collected before the first application of propiconazole.

Zehr et al. Plant Disease (1999) 83(10):913-916

Table 2. Characteristics of *Monilinia fructicola* isolates from South Carolina and Georgia and their sensitivity to propiconazole

Origin of isolates[x]			Year of isolation	No. of isolates	EC_{50} values (μg/ml)		Sensitivity factor[z]
State	County	Orchard			Range	Mean[y]	
GA	Crawford	DF	2002	12	0.012-0.913	0.216 b	76.1
GA	Hall	JO	2002	12	0.011-0.035	0.027 a	3.2
GA	Macon	AP	2003	8	0.007-0.435	0.224 b	62.1
GA	Peach	DL03	2003	18	0.003-0.950	0.021 a	316.7
GA	Peach	LO	2002	11	0.019-0.217	0.081 a	11.1
SC	Anderson	DL	2001	33	0.012-0.054	0.025 a	4.5
SC	Anderson	EZ	2001	9	0.003-0.014	0.010 a	4.7
SC	Edgefield	SY	2001	15	0.003-0.027	0.013 a	9.0
SC	Saluda	CC02	2001	13	0.002-0.034	0.014 a	17.0
SC	Saluda	CC03	2003	21	0.001-0.074	0.036 a	74.0
SC	York	BS	2003	31	0.005-0.049	0.022 a	9.8
SC	York	MC	2003	14	0.015-0.175	0.047 a	11.7

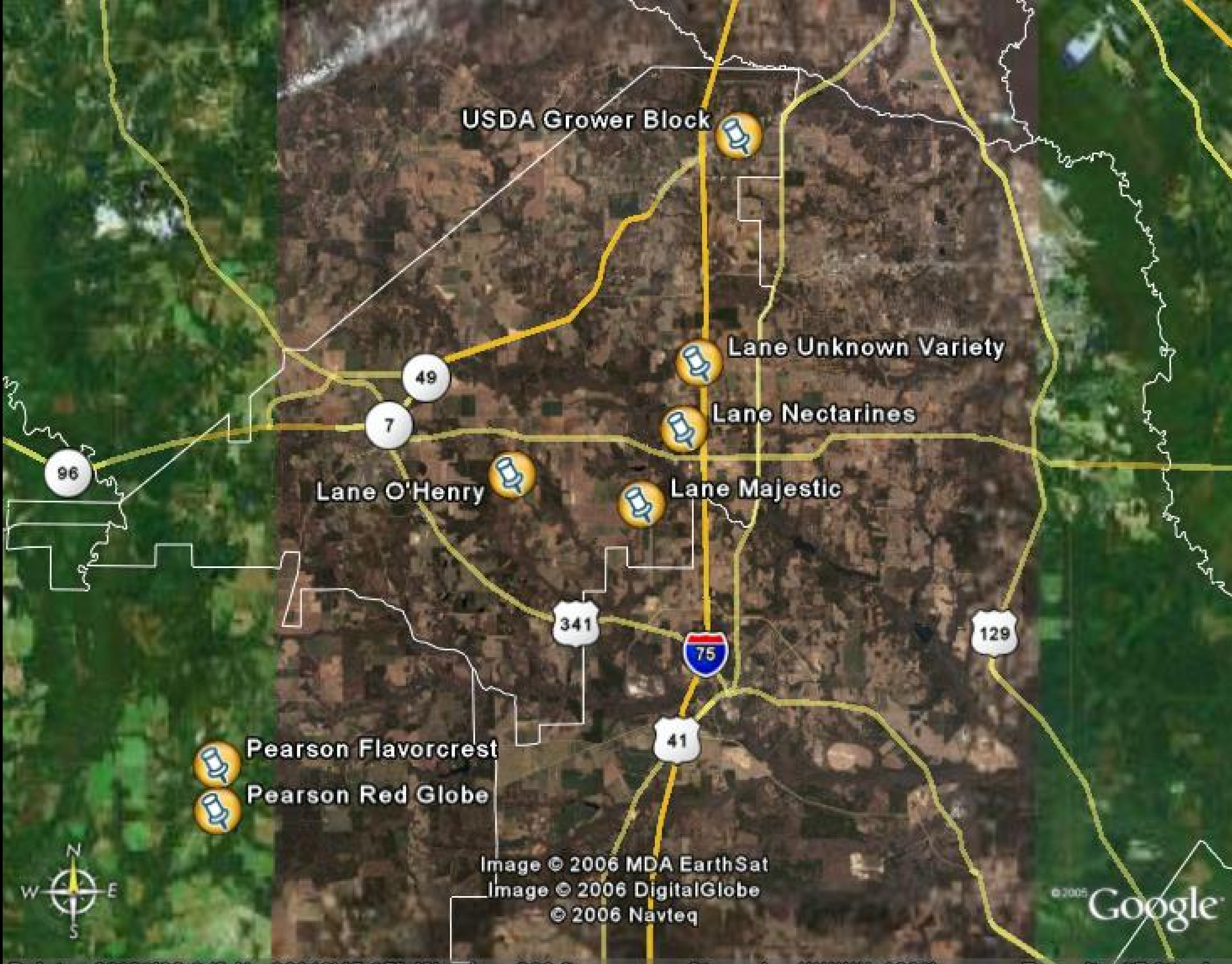
Table 3. Effect of propiconazole treatments on brown rot disease incidence on peach fruit

		Relative disease incidence (%)[y]					
		Propiconazole (liters/ha), protective treatment			Propiconazole (liters/ha), curative treatment		
Isolate	EC_{50} value[z]	0	0.15	0.3	0	0.15	0.3
DL71	0.02	100.0	54.5 a	42.2 a	100.0	21.0 a	14.3 a
DL72	0.02	100.0	58.7 a	42.4 a	100.0	25.2 a	15.0 a
AP5	0.42	100.0	85.4 a	72.9 ab	100.0	60.4 b	32.7 ab
AP6	0.43	100.0	89.3 a	86.6 b	100.0	83.7 b	42.2 b

[y] Values within a column followed by the same letter are not significantly different based on an ANOVA followed by Fisher's protected LSD ($P < 0.05$). Values are means of three independent experiments and are shown as percentage of the control.

[z] The 50% effective dose (EC_{50}) values were determined in mycelial growth tests and represent means of three different experiments.

Schnabel et al. Plant Disease (2004) 88(9):1000-1004


USDA Grower Block
Lane Unknown Variety
Lane Nectarines
Lane O'Henry
Lane Majestic
Pearson Flavorcrest
Pearson Red Globe
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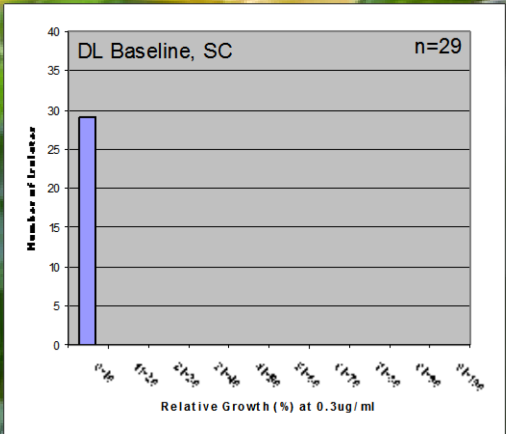
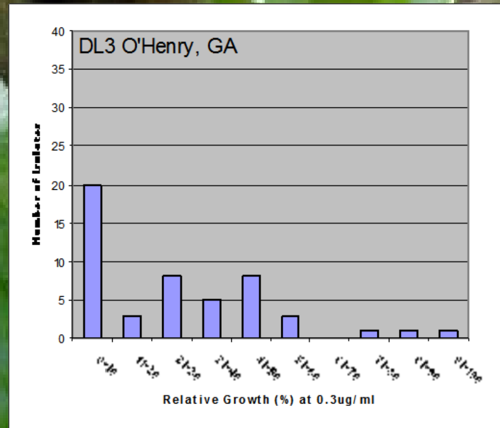
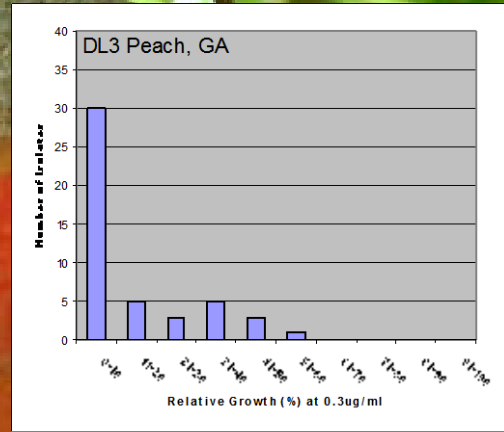
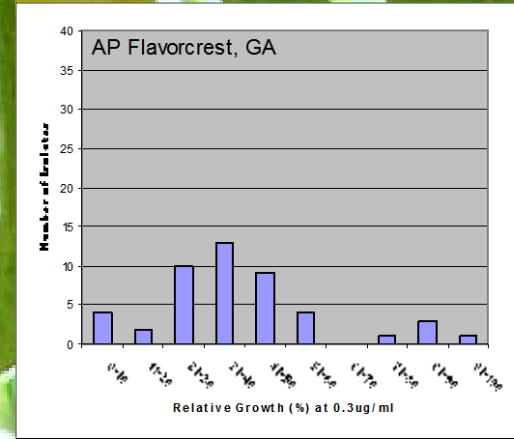
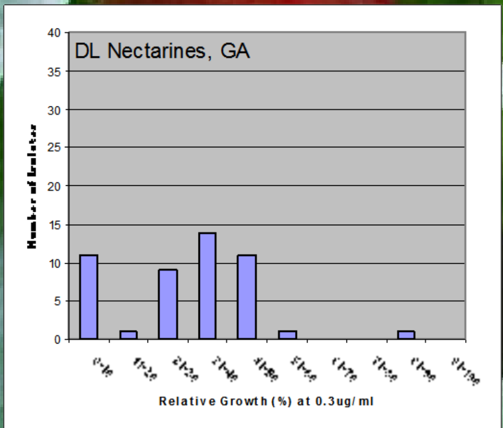
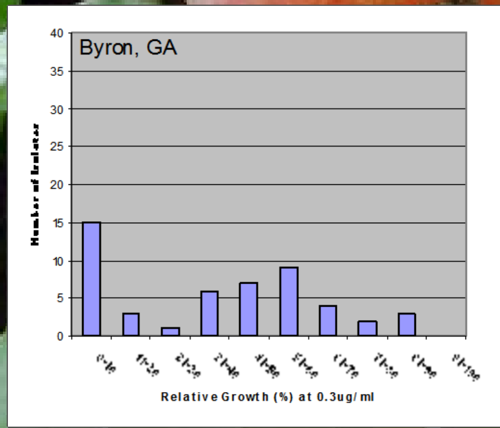


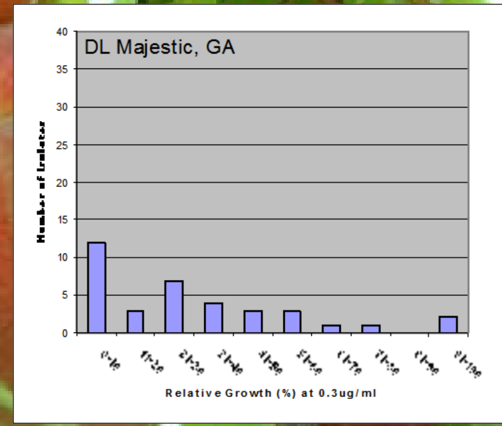


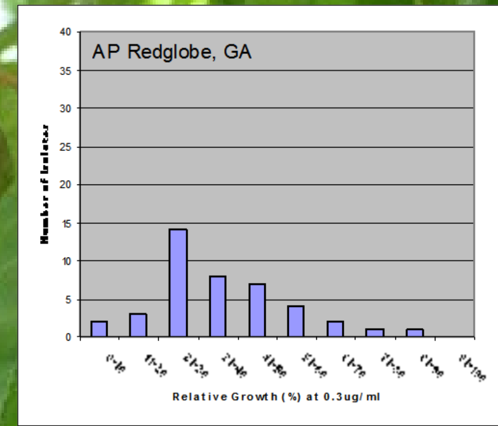












DMI Resistance Snapshot – Georgia – 2004


Byron, GA
40
35
30
25
20
15
10
5
0
Relative Growth (%) at 0.3ug/ml
49
96
© 2006 Navteq
Image © 2006 DigitalGlobe
Google
Pointer 32°40'01.20" N 83°43'40.79" W elev 499 ft Streaming |||||||||| 100% Eye alt 3780 ft
Pearson Red Globe
Image © 2006 MDA EarthSat
Image © 2006 DigitalGlobe
© 2006 Navteq
Google

2005 Peach Data

Treatment and rate/A	Brown rot incidenc 4 days after harvest
Untreated Control	46.2 a
PropiMax 3.6EC 4 fl oz (two applications)	41.7 ab
Abound 2.08F 15.4 fl oz (first spray) PropiMax 3.6EC 4 fl oz (second spray)	26.3 bcd
Pristine 38WG 14.5 oz (first spray) PropiMax 3.6EC 4 fl oz (second spray)	7.7 d
CaptEvate 68WDG 5.25 lb (first spray) PropiMax 3.6EC 4 fl oz (second spray)	34.0 abc
Pristine 38WG 14.5 oz (two applications)................................	17.3 cd
Topsin M 70WP 1.5 lb + Captan 50W 6.0 lb (first spray) PropiMax 3.6EC 4 fl oz (second spray)	14.1 d
LSD (α = 0.05)	19.1

* Means followed by the same letter within each column are not significantly different accor
test.

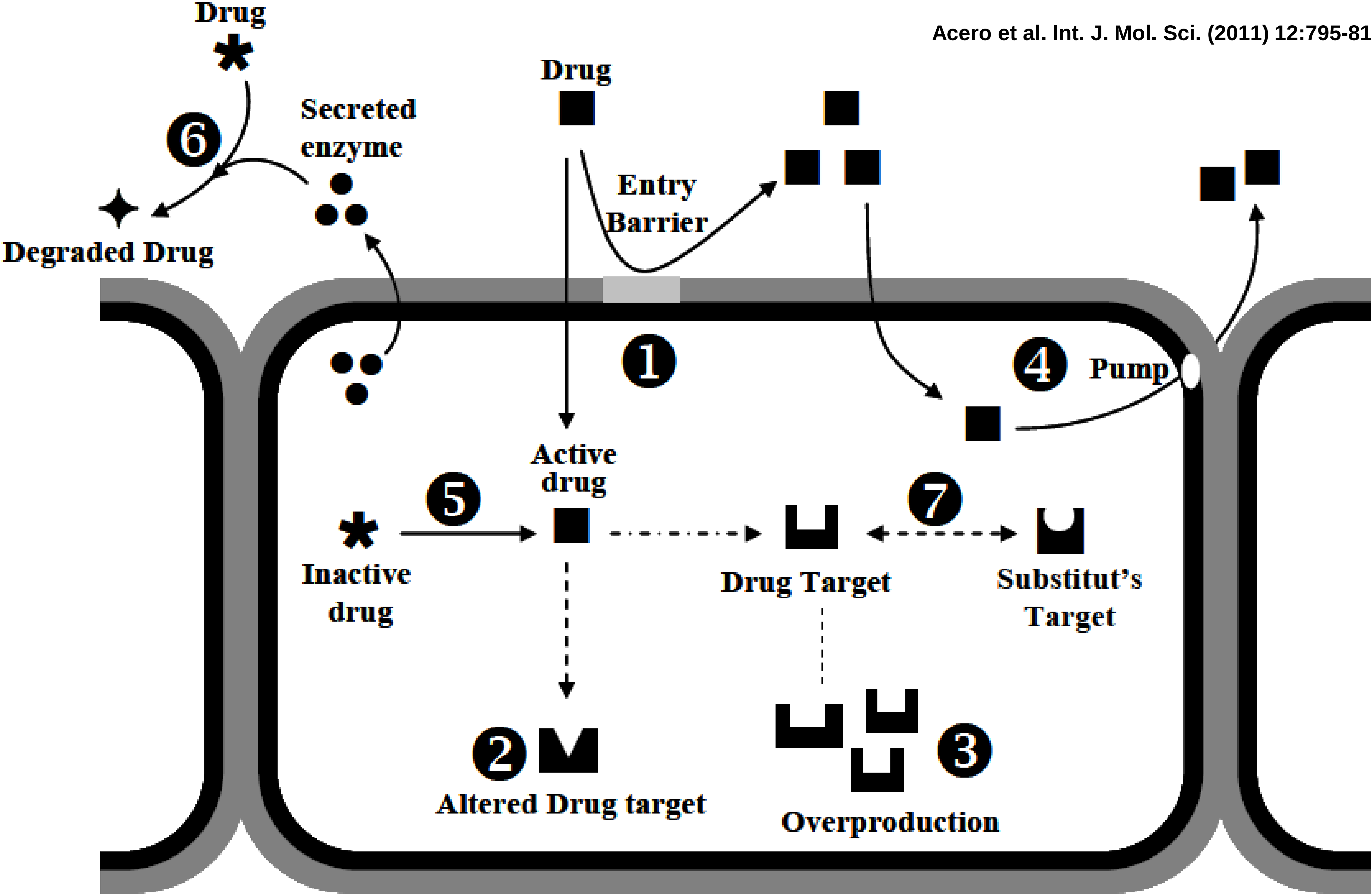
M. fructicola has developed resistance to DMI fungicides.

Alternation or mixtures might overcome DMI resistance.

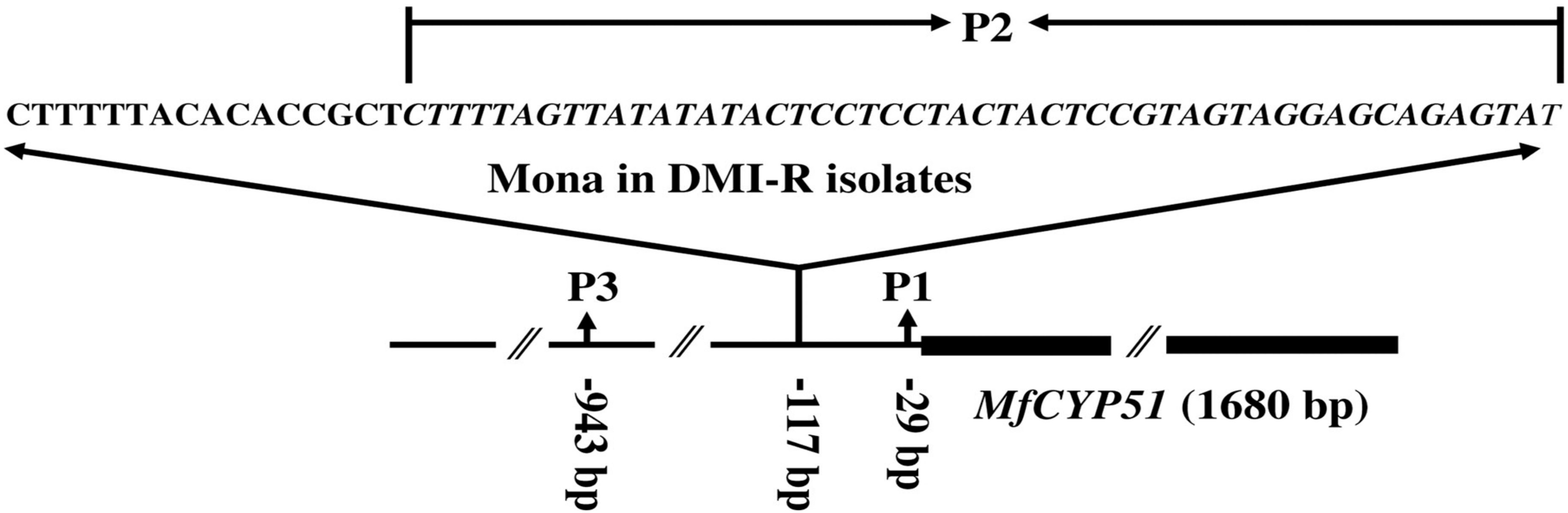
Pristine (boscalid) is a critical component of resistance management programs.

Brannen et al. F&N Test Reports (2006) 61:STF001

Acero et al. Int. J. Mol. Sci. (2011) 12:795-816



Simplistic model of the upstream region of the MfCYP51 gene in M. fructicola isolates.



*A few DMI-R isolates from NY (Cox et al.), PA (peach, unpublished), MD (peach and cherry, unpublished) did not contain Mona.

Luo C , Schnabel G Appl. Environ. Microbiol. (2008) 74:359-366

2006 Peach Data

Treatment and rate/A	Brown rot incidence* (% symptomatic fruit)	
	4 days after harvest	7 days after harvest
Untreated Control	34.6 a	79.9 a
Indar 75WSP 2 oz (two applications)	13.7 ab	55.6 ab
Indar 75WSP 3 oz (two applications)	16.7 ab	35.5 bc
Indar 75WSP 4 oz (two applications)	1.3 b	14.1 c
Pristine 38WG 14.5 oz (two applications)	1.3 b	9.8 c

* Means followed by the same letter within each column are not significantly different according to Fisher's protected LSD test ($\alpha = 0.05$). Analysis is based upon square-root-transformed data, but back-transformed data are shown for better interpretation.

Brannen et al. PDMR (2007) 1:STF003

Increased rates of DMI fungicides, such as fenbuconazole found in Indar, will overcome DMI resistance.

Supplemental Labeling

Dow AgroSciences

Dow AgroSciences LLC 9330 Zionsville Road Indianapolis, IN 46268-1054 USA

Indar® 75WSP

EPA Reg. No. 62719-421

EPA 24(c) Special Local Need Registration SLN GA-060006
For Distribution and Use Only in the State of Georgia

Control of Blossom Blight and Fruit Brown Rot in Peaches and Nectarines

ATTENTION

- It is a violation of Federal law to use this product in a manner inconsistent with its labeling.
- This labeling must be in the possession of the user at the time of application.
- Read the label affixed to the container for Indar® 75WSP fungicide before applying. Carefully follow all precautionary statements and applicable use directions.
- Use of Indar 75WSP according to this supplemental labeling is subject to all use precautions and limitations imposed by the label affixed to the container for Indar 75WSP.

Directions for Use

Refer to product label for Mixing, Handling and Application instructions.

Indar 75WSP is a protectant fungicide. Best disease control is achieved when a protectant application schedule is followed.

Use one to two 2-oz pouches of Indar 75WSP per acre in a minimum of 50 gallons by ground or 10 gallons by air. Indar 75WSP may be applied up to the day of harvest. A wetting agent or non-polymer containing spray adjuvant approved for use in registered pesticides products should be added to spray solutions according to manufacturers' use instructions to achieve optimum disease control.

To control blossom blight in peaches and nectarines, begin applications at early red bud stage before infection occurs. If conditions are favorable for disease development, apply again at full bloom and at petal fall.

To control fruit brown rot in peaches and nectarines, begin applications 2 to 3 weeks before harvest using a 7- to 10-day spray interval.

To control scab in peaches, begin applications at shuck split. Make 2 to 3 subsequent thorough coverage applications at 10- to 14-day intervals.

Specific Use Restrictions:

- Do not apply more than 1 lb of Indar 75WSP (0.75 lb active ingredient) per acre per season.
- Do not graze livestock in treated areas or feed cover crops grown in treated areas to livestock.
- Chemigation: Do not apply this product through any type of irrigation system.

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R545-074
Accepted: 10/06/06
Initial printing.

Tommy Irvin
Commissioner

Georgia Department of Agriculture

Pesticide Division, Room 550 - 19 M.L.K. Jr. Dr., • Atlanta, GA 30334
(404)-656-4958, Fax: (404)-657-8378

October 6, 2006

Mr. Jim Baxter
Dow AgroSciences LLC
9330 Zionsville Rd.
Indianapolis, IN 46268-1054

Dear Mr. Baxter:

This is to advise you that we have approved your request for a special local need (SLN) registration for Indar 75WSP (EPA Reg. # 62719-421) in Georgia. This SLN will allow the afore mentioned product to be used to control blossom blight and fruit brown rot in Georgia peaches and nectarines. It is our understanding that the University of Georgia Cooperative Extension Service supports this SLN and that data from field trials showed positive results.

The assigned SLN number is EPA SLN GA-060006. Please send two copies of the final printed label for our files. If you would prefer to e-mail a copy of the label to me, my e-mail address is scole@agr.state.ga.us. If you have any questions concerning our approval please feel free to contact us at (404) 656-4958.

Sincerely,

Stephen E. Cole

Stephen E. Cole
Agriculture Manager
Pesticide Division

CC: Mr. Doug Jones
Dr. Phillip Brannen
Dr. Paul Guillebeau

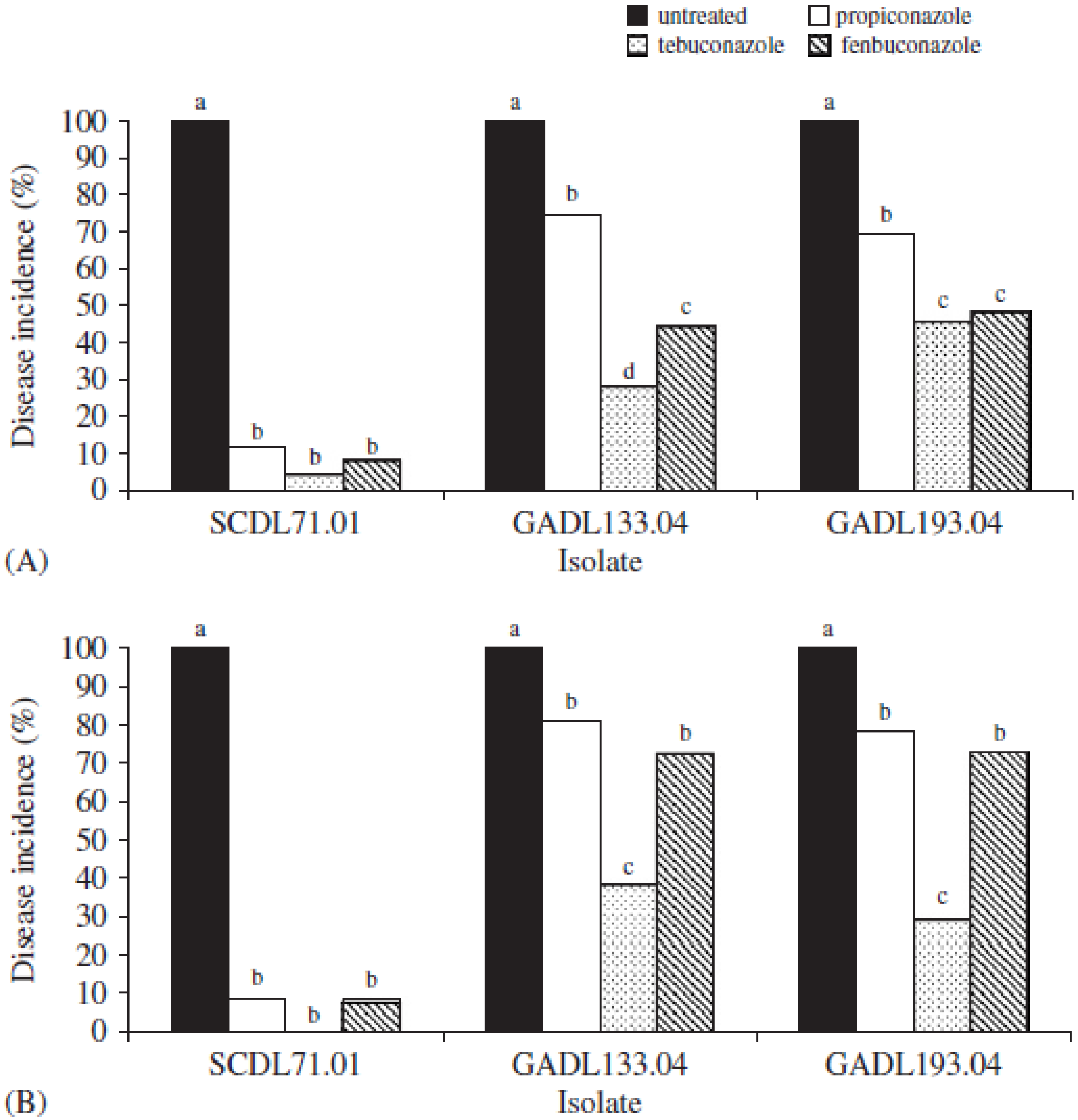


Fig. 2. Effect of protective (A) and curative (B) applications of fungicide treatments on brown rot incidence (%) caused by *Monilinia fructicola* isolates in detached fruit experiments. Different letters above bars indicate significant differences at $P = 0.05$, using a least significant difference test.

Holb I, Schnabel G Crop Protection (2007) 26:753-759

Table 2. Effect of protective and curative applications of fungicide treatments on brown rot incidence caused on detached fruit by a sensitive (DL71) and two propiconazole-resistant isolates (GADL133.04 and GADL193.04) of *Monilinia fructicola*. Treatment effects were evaluated on injured and uninjured fruit of cv. 'Coronet'

	Brown rot incidence (%)[a]									
	Application timing									
	Protective					Curative				
	Fungicide treatment[b]					Fungicide treatment[b]				
	MWett-S	Prop	MWett-S + Prop	Untreated	$LSD_{0.05}$	MWett-S	Prop	MWett-S + Prop	Untreated	$LSD_{0.05}$
Isolate DL71										
Injured	88.3 a	0.0 b	0.0 b	100.0 a	17.3	100.0 a	5.1 b	0.0 b	100.0 a	10.3
Uninjured	83.3 b	0.0 c	0.0 c	97.2 a	13.8	100.0 a	0.0 b	0.0 b	100.0 a	2.1
Isolate GADL133.04										
Injured	93.6 a	72.3 b	57.9 c	100.0 a	8.9	98.1 a	75.9 b	68.3 b	100.0 a	10.3
Uninjured	91.7 a	72.2 b	45.8 c	100.0 a	14.9	97.2 a	63.8 b	61.1 b	100.0 a	12.3
Isolate GADL193.04										
Injured	94.4 a	66.7 b	44.7 c	100.0 a	10.2	100.0 a	63.7 b	59.9 b	100.0 a	10.2
Uninjured	92.1 a	60.4 b	41.1 c	100.0 a	12.4	100.0 a	61.8 b	57.2 b	100.0 a	9.4

[a] Means are from three repeats combined. Means in each fungicide treatment were compared using least significant difference (LSD) tests at $P = 0.05$ for each isolate and fruit injury treatment. Values within rows and timing of application followed by different letters are ignificantly different.
[b] Fungicide treatments were micronized wettable sulfur (MWett-S), propiconazole (Prop), propiconazole combined with micronized wettable sulfur (Prop + MWett-S) and untreated control (untreated). Fungicides were applied protectively (24 h before inoculation) and curatively (24 h after inoculation).

Holb I, Schnabel G Pest Manag Sci (2008) 64:156-164

2007 Peach Data

Treatment and rate/A	Application timing*	Brown rot incidence** (% infected fruit) 4 days after harvest	7 days after harvest
Untreated Control		25.0 a	76.3 a
PropiMax 3.6EC 4 fl oz	7-8	26.9 a	78.2 a
Pristine 38WDG 14.5 oz	7-8	7.7 b	19.2 c
Messenger STS WDG 6.0 oz	5		
PropiMax 3.6EC 4 fl oz	7-8	15.4 ab	63.5 ab
Calcium chloride 0.4 oz	1-4, 6, 9		
PropiMax 3.6EC 4 fl oz	7-8	14.7 ab	57.7 b
PropiMax 3.6EC 4 fl oz + Sulfur 90WP 9 lb	7-8	21.8 ab	69.9 ab
PropiMax 3.6EC 4 fl oz + Captan 50W 8 lb	7-8	12.8 ab	62.2 ab
LSD ($\alpha = 0.05$)		14.8	16.0

*Treatment dates: 1 = 2 May; 2 = 16 May; 3 = 30 May; 4 = 14 Jun; 5 = 22 Jun; 6 = 27 Jun; 7 = 2 Jul; 8 = 9 Jul; 9 = 11 Jul.

** Brown rot incidence was recorded on fruit stored at ambient temperature. Means followed by the same letters are not significantly different according to Fisher's protected LSD test.

Brannen et al. PDMR (2008) 2:STF002

2007 Peach Data

Treatment and rate/A	Brown rot incidence* (% infected fruit)	
	4 days after harvest	7 days after harvest
Untreated Control	35.9 a	76.3 a
PropiMax 3.6EC 4 fl oz (two applications)	10.3 bc	48.1 b
Pristine 38WDG 14.5 oz (two applications)	3.8 c	30.1 c
Pristine 38WDG 14.5 oz (first spray)	28.8 a	65.4 ab
Pristine 38WDG 14.5 oz (first spray) PropiMax 3.6EC 4 fl oz (second spray)	6.4 bc	27.6 c
Topsin M 4.5FL 30 fl oz (two applications)	23.1 ab	74.4 a
LSD (α = 0.05)	17.8	17.7

* Means followed by the same letter within each column are not significantly different according to Fisher's protected LSD test.

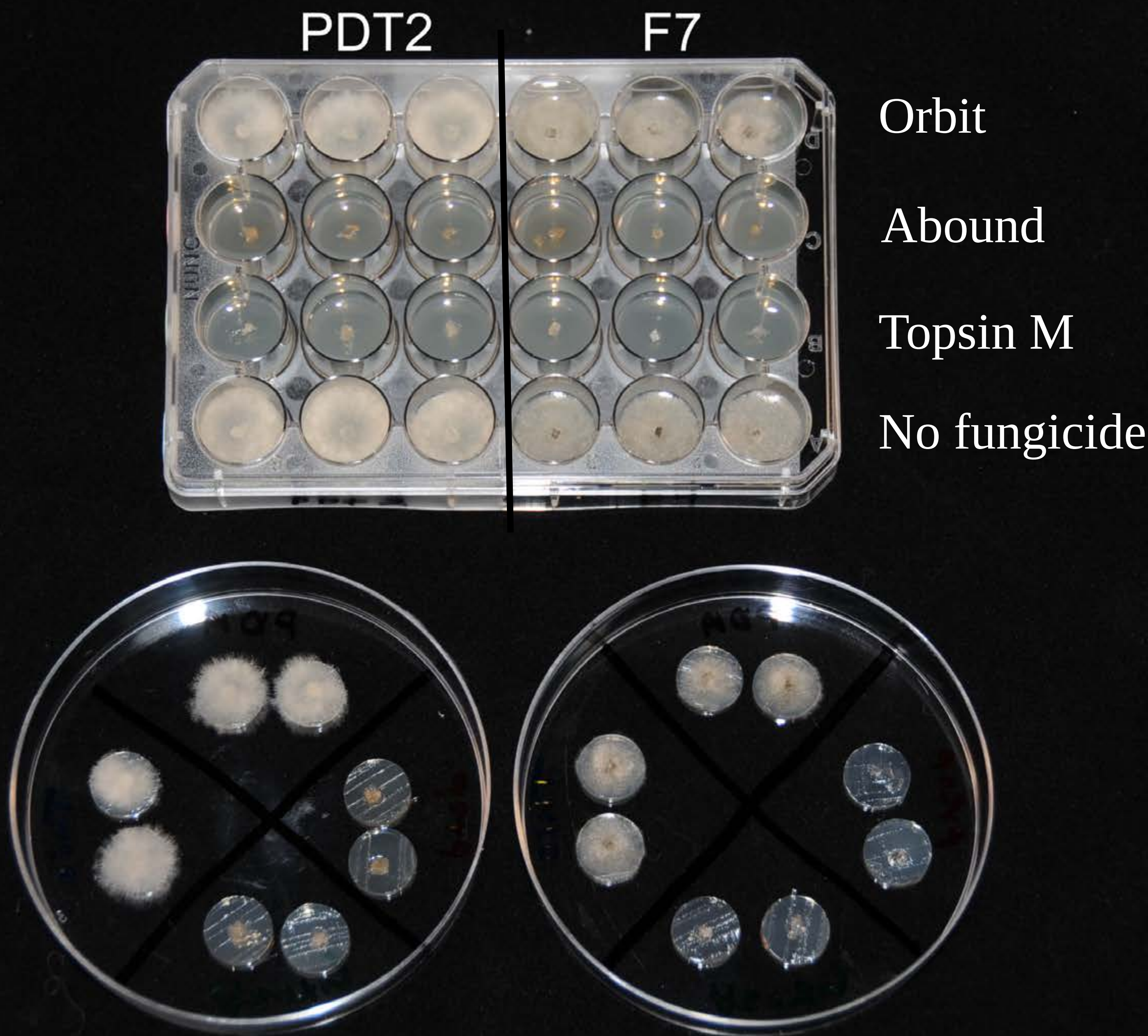
Brannen et al. PDMR (2008) 2:STF003

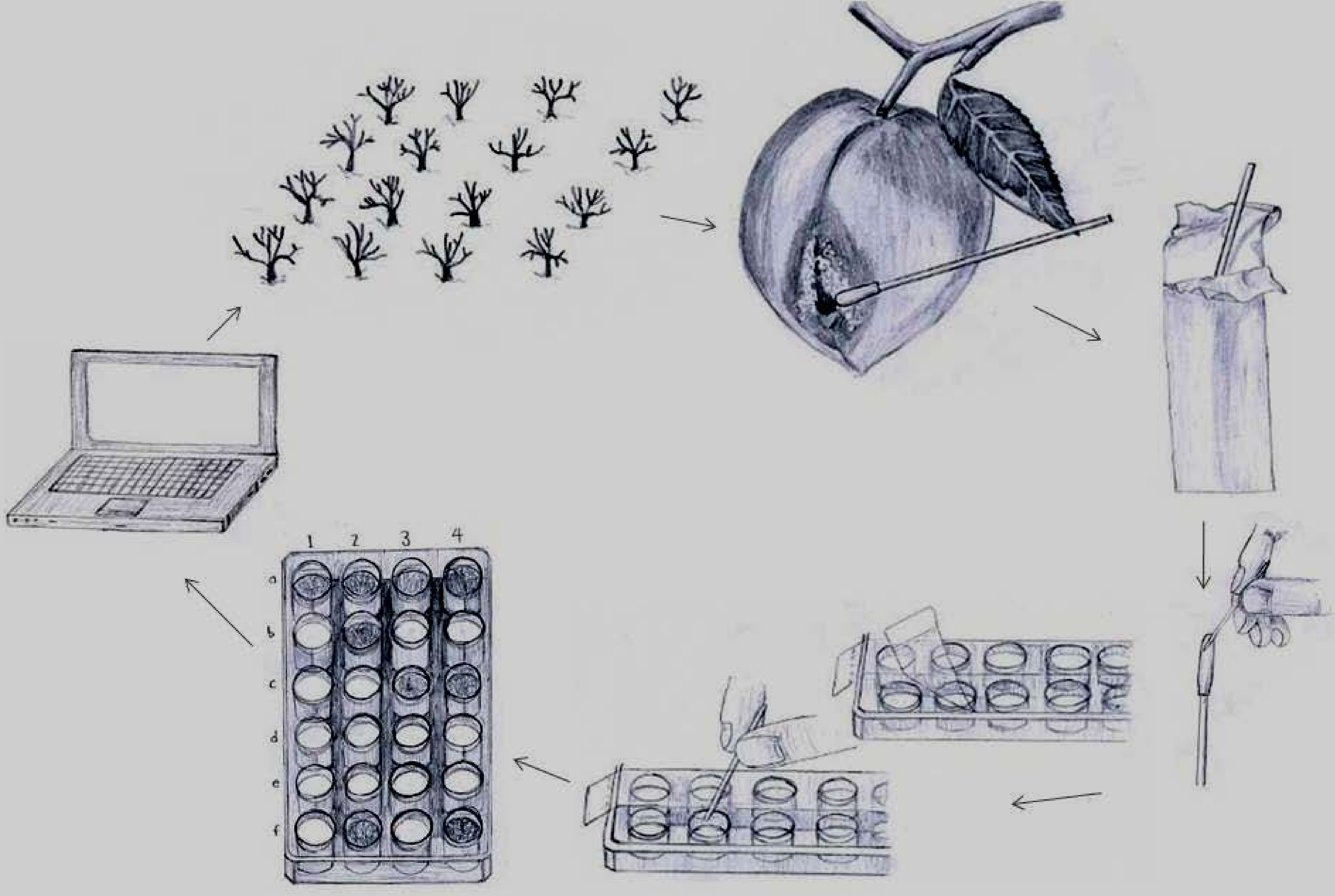
Summary of 2008-2012 Trials

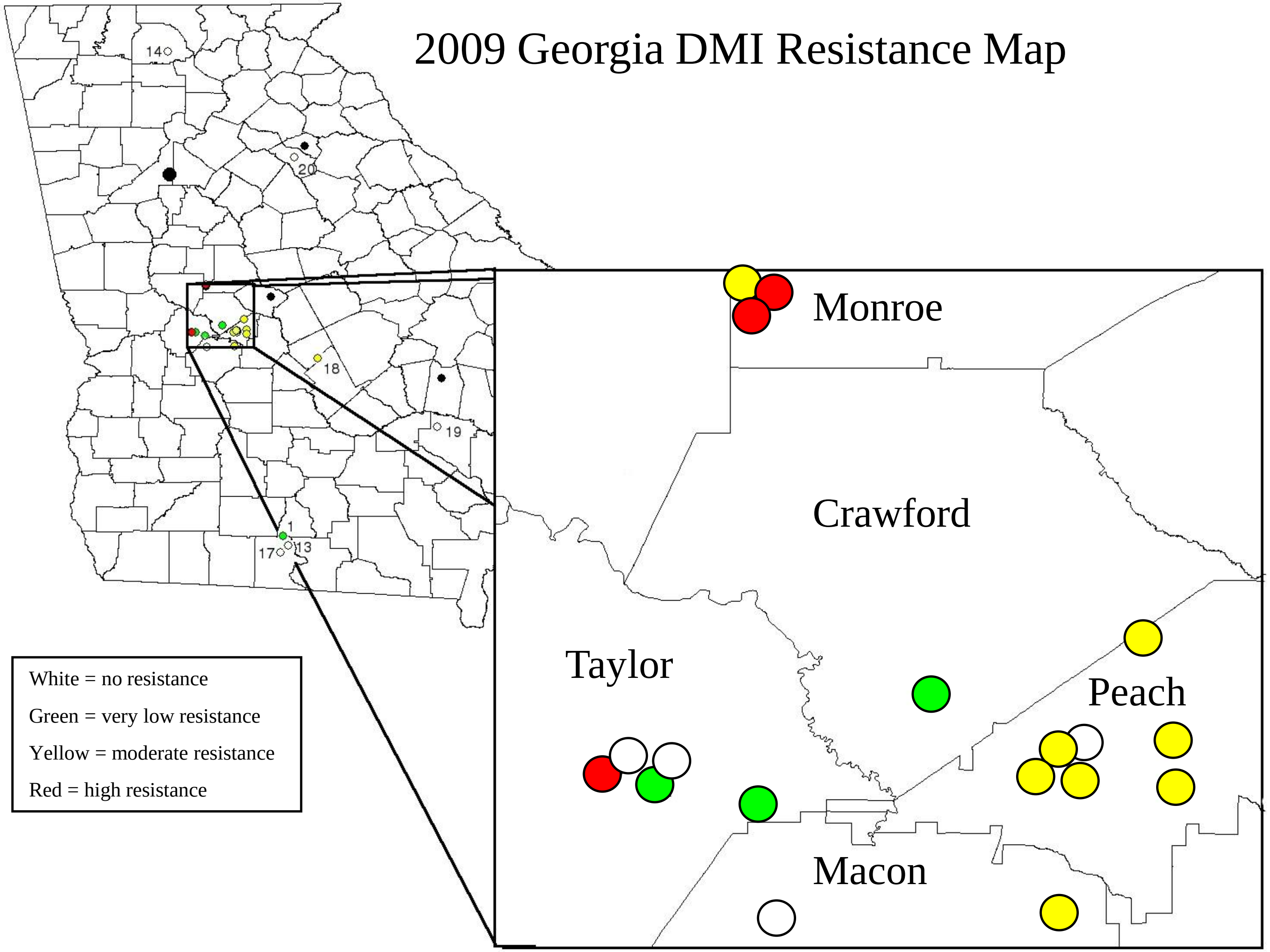
- Cross-resistance is observed with the DMIs (i.e. metconazole [Quash]), so each DMI would have to be tested for efficacy in the presence of resistant strains.
- Mixture products, such as those containing a DMI and a strobilurin (i.e. tebuconazole + trifloxystrobin [Adament]), have performed poorly in the presence of DMI resistance – not recommended.
- Increased DMI rates and varied DMI actives will allow use in the presence of DMI resistance.

PROFILE

Resistance Management Program


PDT2
F7
Orbit
Abound
Topsin M
No fungicide


1
2
3
4
a
b
c
d
e
f

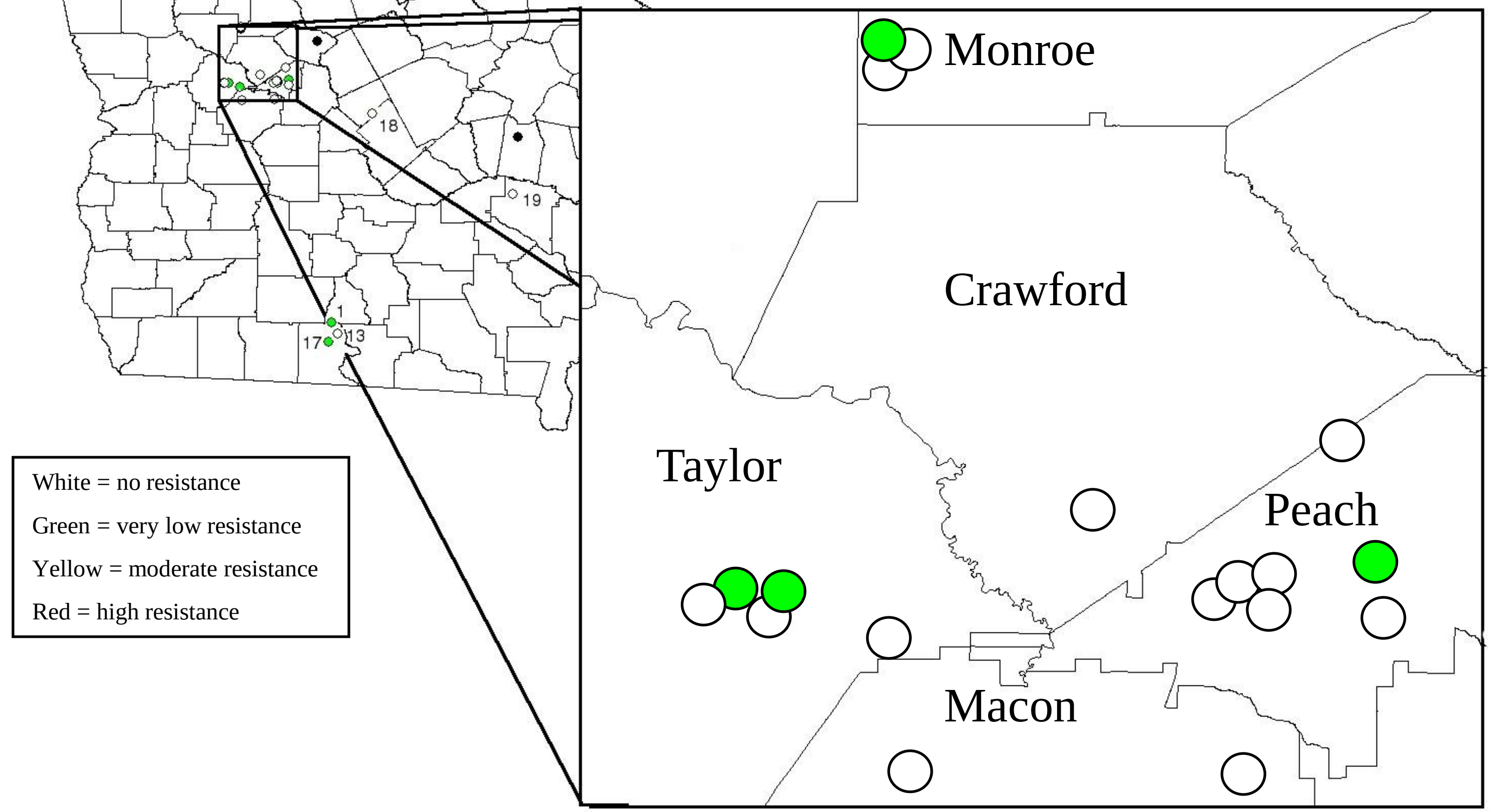


(1) DMI resistance is widespread throughout the mid-Georgia peach area.

(2) There is a moderate shift towards resistance in the south-Georgia peach area.

(3) Other areas have not shown resistance to date.

2009 Georgia QoI Resistance Map

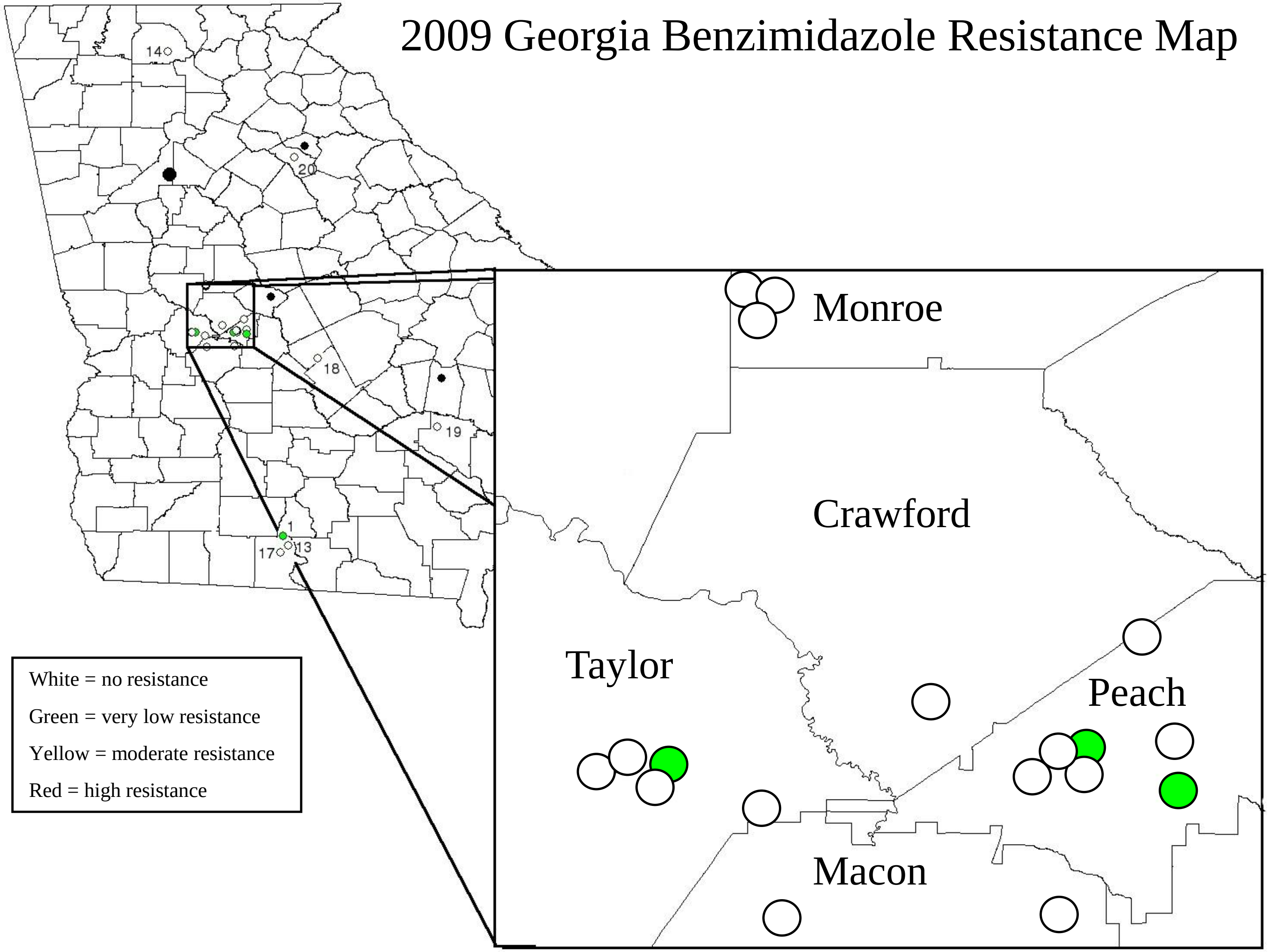


(1) QoI resistance is minimal (possibly background).

(2) There may be a moderate shift towards resistance where QoIs have been utilized.

(3) QoIs should be utilized sparingly for late-season, preharvest applications only. Rotation with other MOA fungicides is highly recommended.

2009 Georgia Benzimidazole Resistance Map

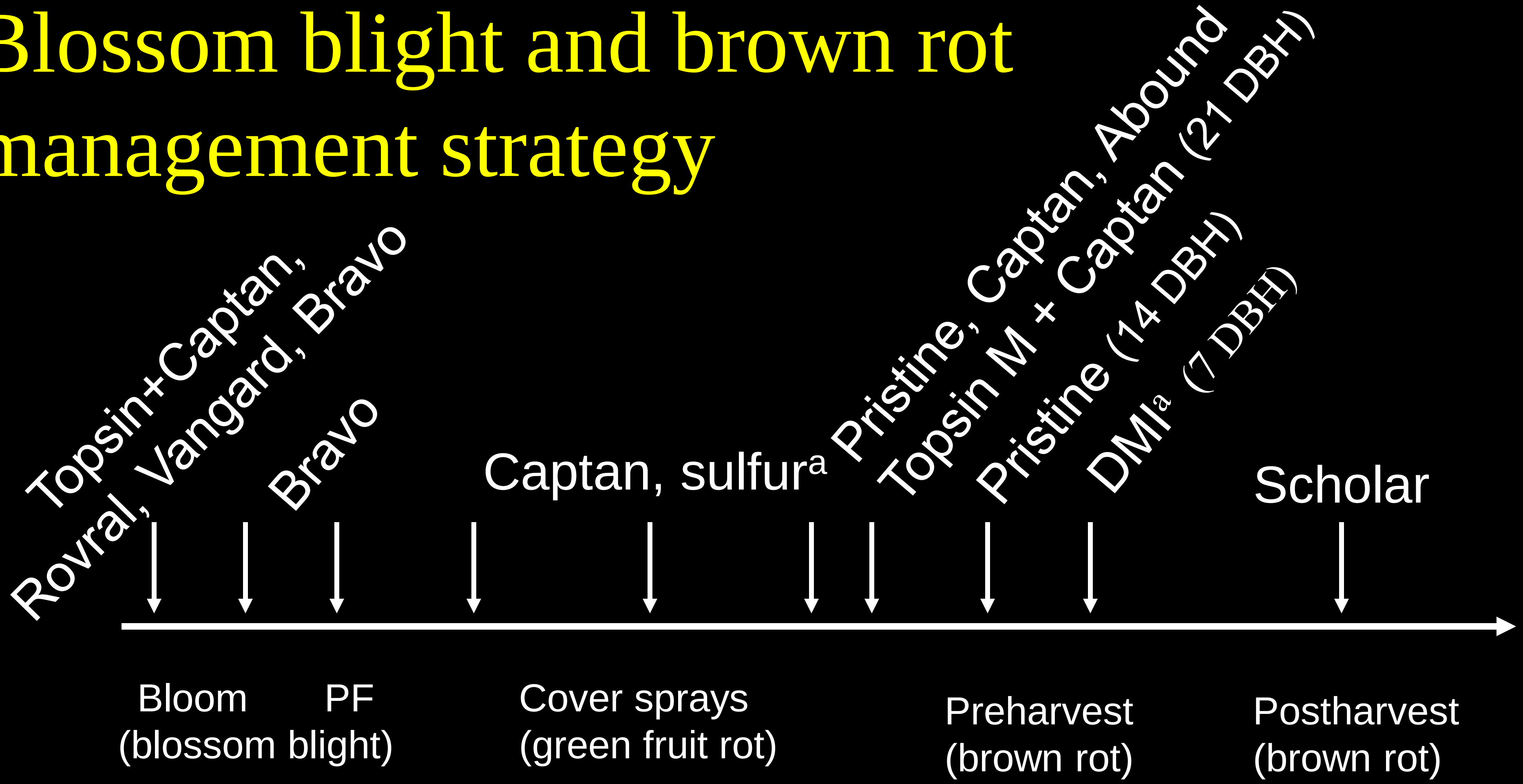


(1) Benzimidazole resistance is minimal (possibly background).

(2) The lack of use in recent years may have helped to reduce resistance in *M. fructicola* populations.

(3) Topsin should be used with Captan (once per year max). Resistance develops readily.

Blossom blight and brown rot management strategy



[a] If DMI resistance is suspected or documented, use captan instead of sulfur in cover sprays and use high rate of Elite or Indar preharvest

Estimated Application Costs

- Indar (high rate); $26.50 per acre
- Elite (high rate); $25.00 per acre
- Pristine (high rate); $30.45 per acre
- Propimax (high rate); $7.50 per acre

DMI resistance in *Monilinia fructicola* is not stable in vivo.

Nuninger-Ney et al. Neth. J. Pl. Path. (1989) 95:137-150

Cox et al. Phytopathology (2007) 97:448-453

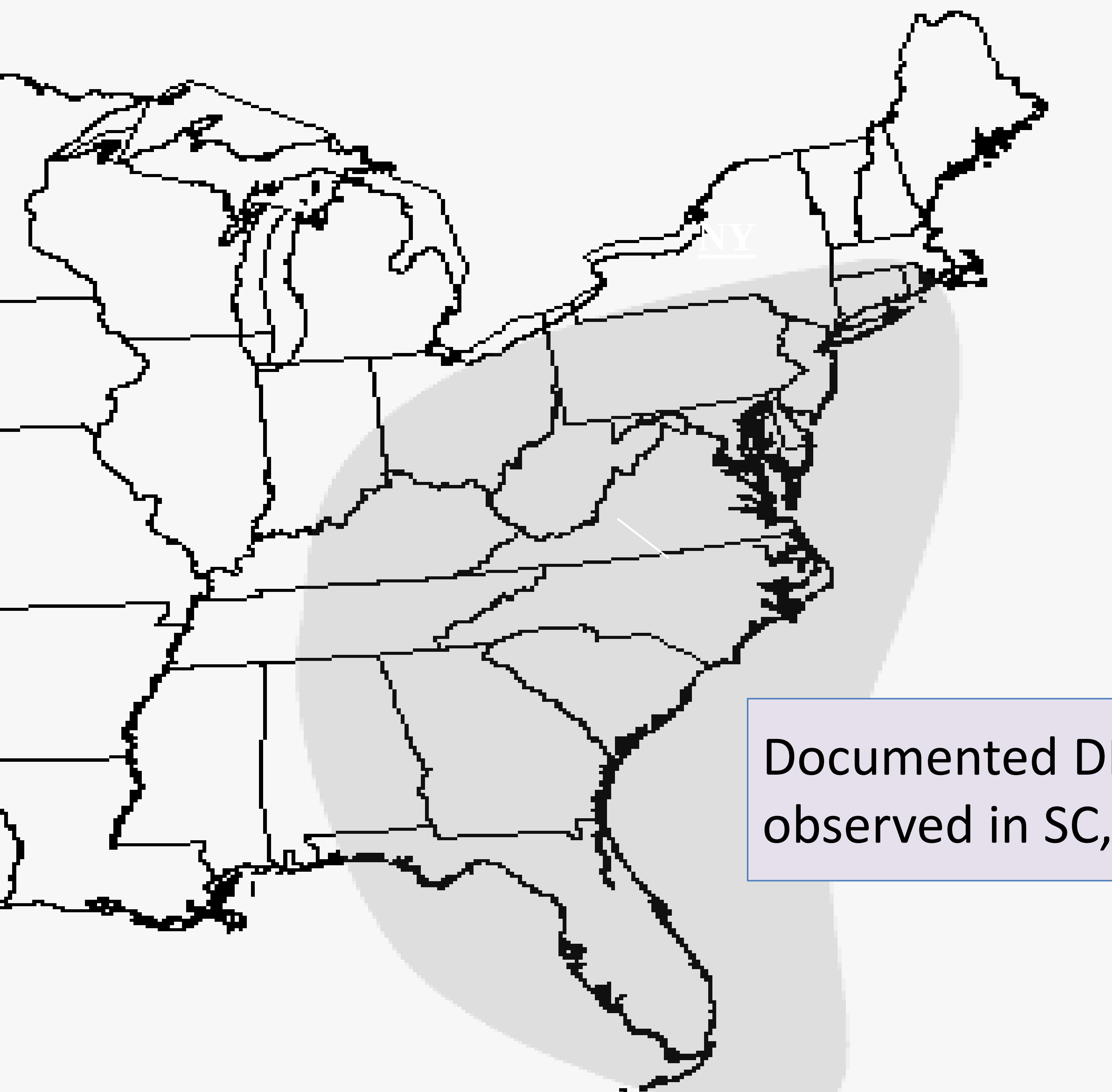
Zhu et al. Pest Manag Sci (2012) 68(7):1003-1009

Table 4. Occurrence of resistance in samples from Georgia and South Carolina collected between 2008 and 2010

	10-fruit samples (%) with at least 1 resistant isolate		
Resistance to	2008	2009	2010
DMIs	35.7a*	31.4a	3.7b
QoIs	35.7a	13.7b	3.7b
MBCs	10.7a	19.6a	14.8a
	n=28	n=51	n=54

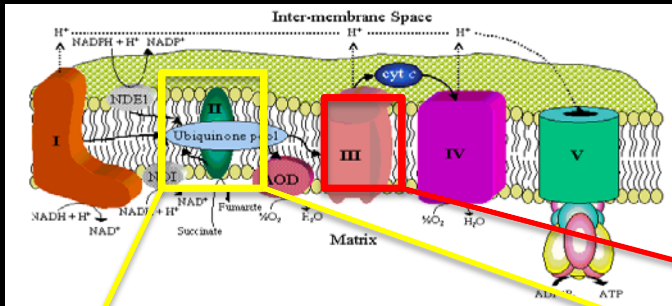
*Values with different letters within rows are significantly different according to ANOVA Pairwise Multiple Comparison Procedure; mean separation by Holm-Sidak method.

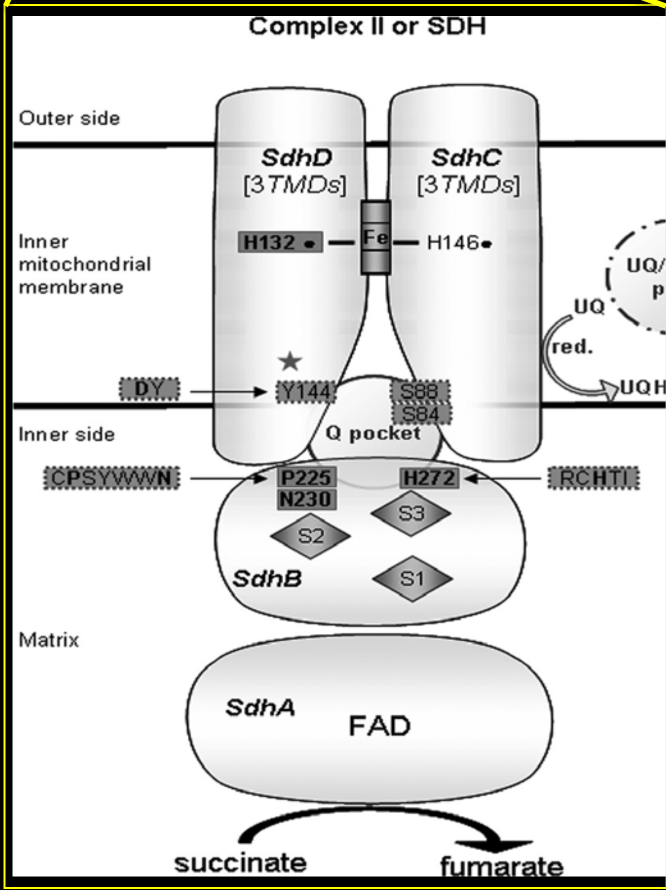
Schnabel et al. (2012) Fungicide Resistance in Crop Protection: Risk and Management



Documented DMI resistance now observed in SC, GA, OH, NJ, and NY

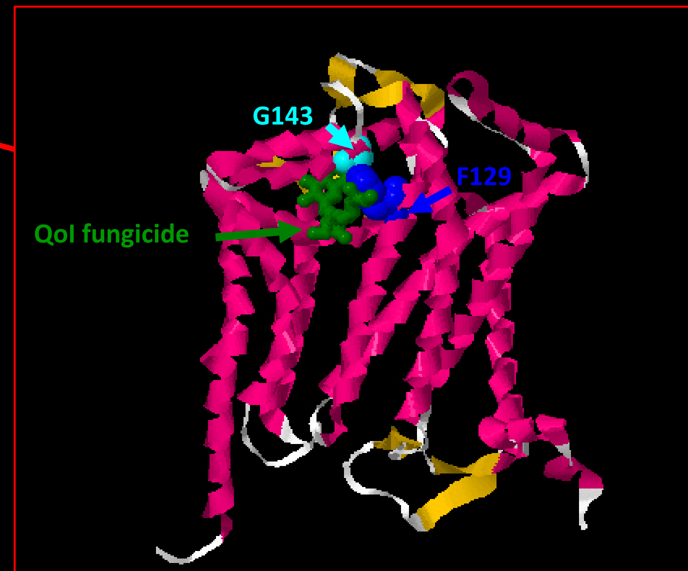
Assumed Molecular mechanism of SdhIs and QoIs Resistance





SdhIs

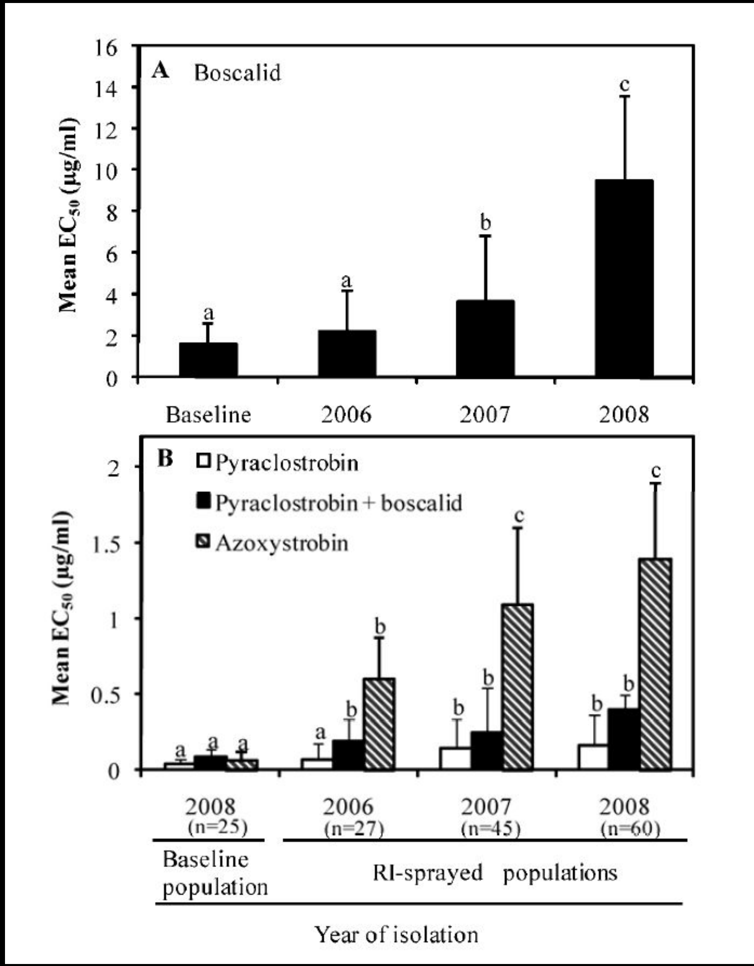
Substitutions in the SdhB subunit

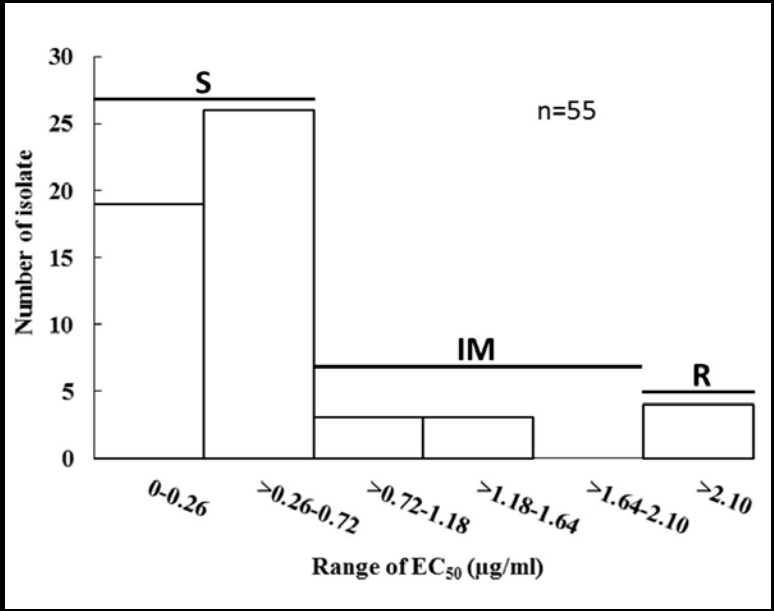


QoIs

G143A substitution in cytochrome *b* gene

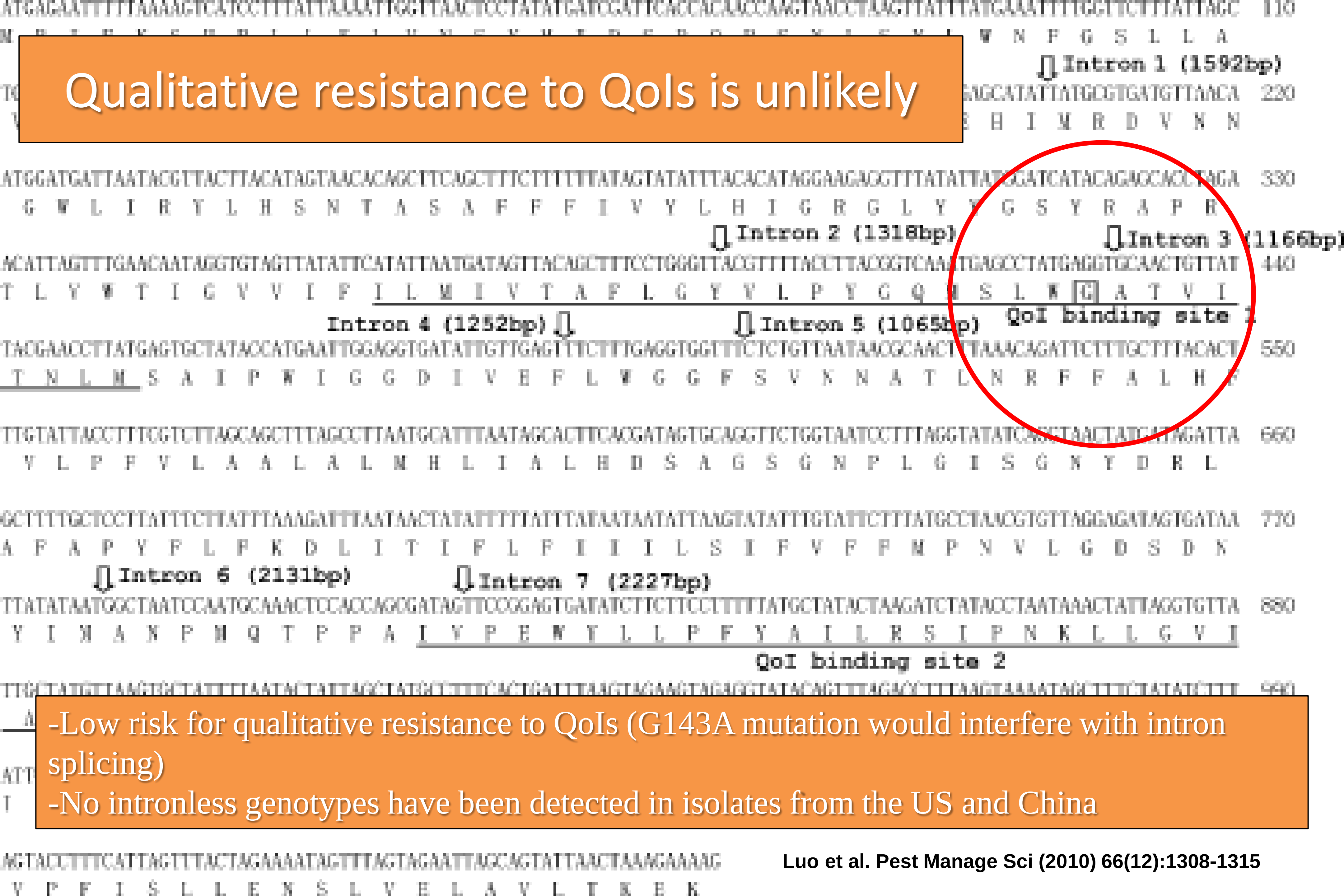
Quantitative resistance observed for SDHI and QoI fungicides





Resistance to SDHIs occurs even in baseline populations.

Qualitative resistance to QoIs is unlikely

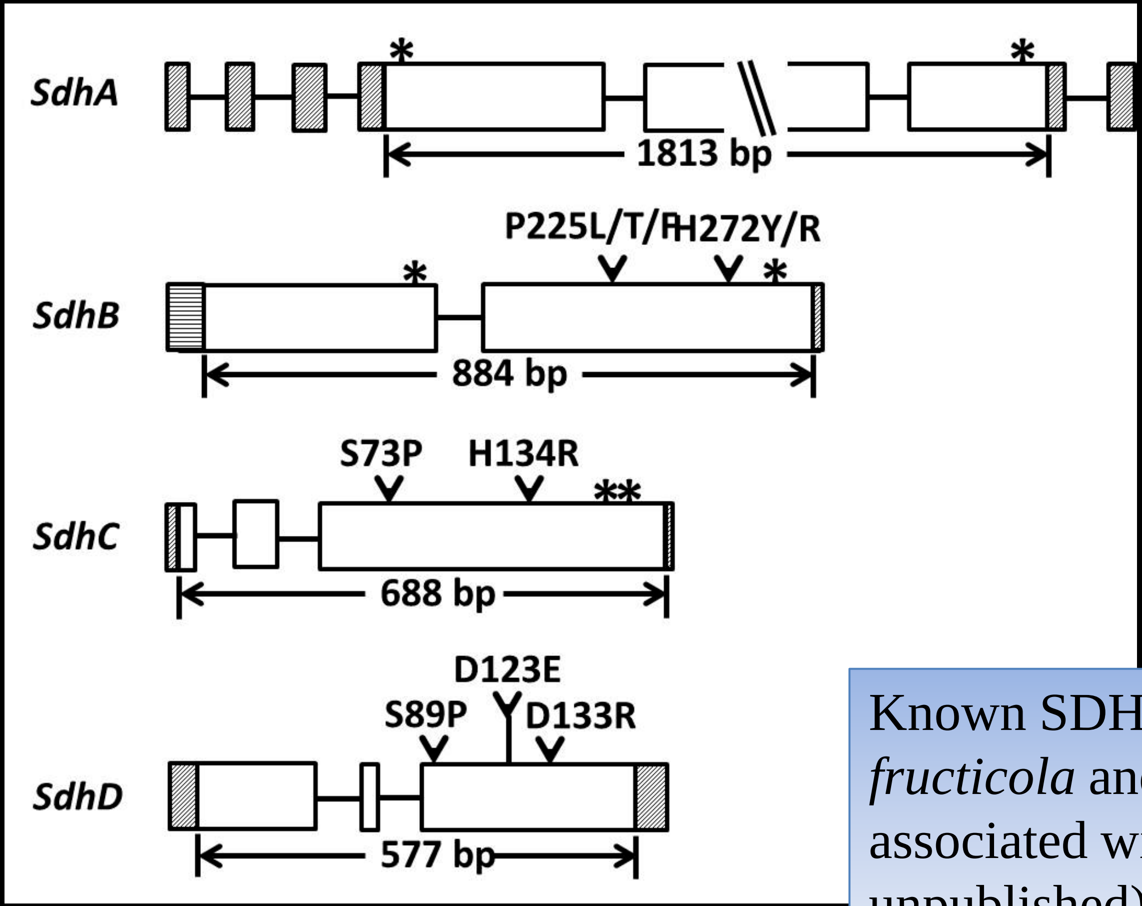


-Low risk for qualitative resistance to QoIs (G143A mutation would interfere with intron splicing)

-No intronless genotypes have been detected in isolates from the US and China

Luo et al. Pest Manage Sci (2010) 66(12):1308-1315

Qualitative resistance to SDHIs has not been reported



Known SDH subunit sequences from *M. fructicola* and commonly found mutations associated with SDHI resistance (Schnabel unpublished)

Path Forward

(1) Continue and expand monitoring for resistance.

(2) Incorporate new fungicides with different modes of action, and determine efficacy of other DMIs and combinations.

(3) Cultural and novel approaches to management may be incorporated into management schemes.