

DETECTION OF RESISTANCE IN POPULATIONS OF *VENTURIA PIRINA* IN CALIFORNIA PEAR ORCHARDS AND IDENTIFICATION AND CONTROL OF PEAR CANKER DISEASES IN CALIFORNIA

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ABSTRACT

Pear scab is the most common disease of pear in the north coast area of California. Scab prevention requires use of fungicides in most years for control. In recent years the most common products used against pear scab have been products that attack the pathogen in only one locus. As these materials are used over time, resistance and reduced susceptibility to the products has occurred. Spiral gradient endpoint tests were conducted to measure fungicide resistance and efficacy. Fungicide resistance in *Venturia pirina* population was shown to Flint and decreased susceptibility to several other fungicides including Elite, Inspire, Manzate, Procure, Scala, Sovran, Syllit, Topsin-M, Vanguard and Vintage.

In 2010, a decline and branch dieback of pear trees (*Pyrus communis* L.) was observed in orchards in Mendocino County. Symptoms included branch dieback, cankers and vascular necroses in tree branches and limbs. Isolation and molecular identification from pear cankers in Mendocino County revealed the presence of several pathogenic fungi species, including *Botryosphaeria obtusa*, *B. dothidea*, *B. iberica*, and *Eutypa lata*. Spore traps were placed in two orchards with pear canker diseases and spores were collected. Spore release closely followed rainfall events. Most pathogenic spores were released in late spring.

OBJECTIVE Pear Scab: Determine types of fungicide resistance in *V. pirina* population and determine distribution of fungicide resistance in *V. pirina* population.

PROCEDURE

V. pirina isolates were collected by PCA's and growers in 2013. Isolates were mass cultured and maintained on appropriate media prior to being tested using the automated spiral plater for their reaction to various fungicides. The fungicides tested included the active ingredient in Elite, Inspire, Manzate, Procure, Scala, Sovran, Syllit, Topsin-M, Vanguard and Vintage. The Automated Spiral Plater, Autoplate 400, was used to conduct the Spiral Gradient Endpoint test which measured susceptibility of spore germination to a gradient of fungicides on an agar plate. A solution of 25 ppm of the fungicide was spiral plated onto a 150 mm PDA plate. Then the plates were radially streaked with a conidial suspension of the fungal isolates. After incubation for one week, the fungi grew on parts of the plate where fungicide did not inhibit their growth. EC 50's (Effective Concentrations) were measured. EC is determined by the point on the plate where the fungal growth is inhibited by the fungicide. Each isolate was replicated at least three times and the test repeated at least one time. Appropriate data analysis was conducted for determination the EC50 against the products and for determination of significant differences among isolates for individual products. Isolates were identified as to the location they were collected from, including pear variety, orchard, location in the orchard, location of orchard in the county, and a history of use for the product class being tested.

Product	Active ingredient(s)	Class	Manufacturer
Elite (Tebuconazole 45 DF)	Tebuconazole	DMI-triazole (3)	United Phosphorous
Scala	Pyrimethanil	AP (9)	Bayer
Inspire	Difenoconazole	DMI-triazole (3)	Syngenta
Manzate	Mancozeb	Carbamate (EBDC) (M3)	Dupont
Sovran	Kresoxim-methyl	QoI (11)	Chemnova
Syllit	Dodine	Guanidine (7)	Agriphar
Vanguard	Cyprodinil	AP (9)	Syngenta
Topsin-M	Thiophanate-methyl	MBC (1)	UPI
Procure	Triflumizole	DMI-triazole (3)	Chemtura
Vintage	Fenarimol	DMI-pyrimidine (3)	Gowan

RESULTS

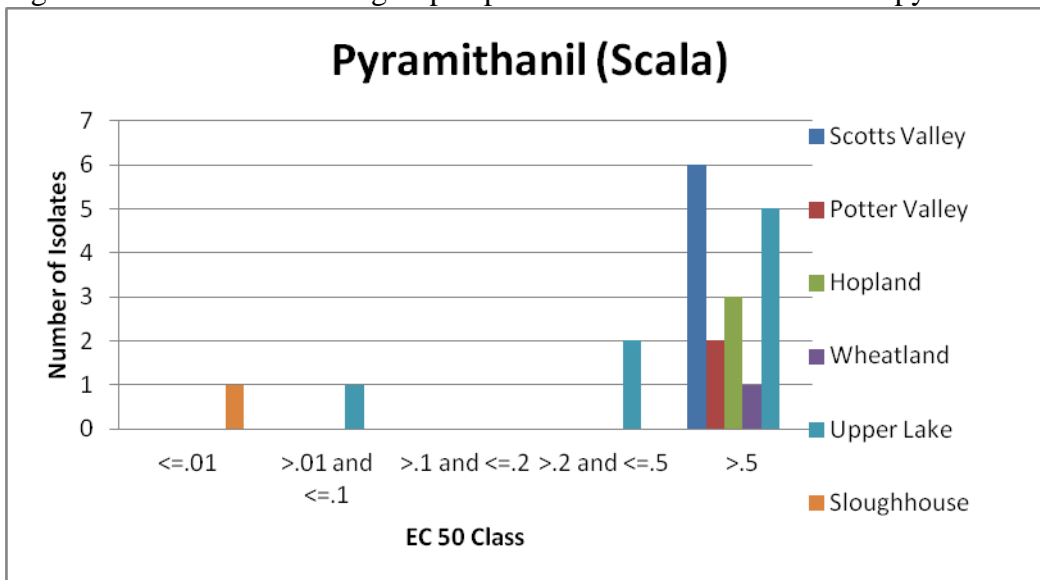
Susceptibility to fungicides varied greatly among the isolates and areas tested. Table 1 shows the number of isolates tested from each area.

Table 1. List of the numbers of isolates tested from each area.

Area	County	Number of Isolates
Hopland	Ukiah	3
Wheatland	Yuba	1
Scotts Valley	Ukiah	6
Potter Valley	Mendocino	3
Upper Lake	Ukiah	9
Sloughouse	Sacramento	1
Total		25

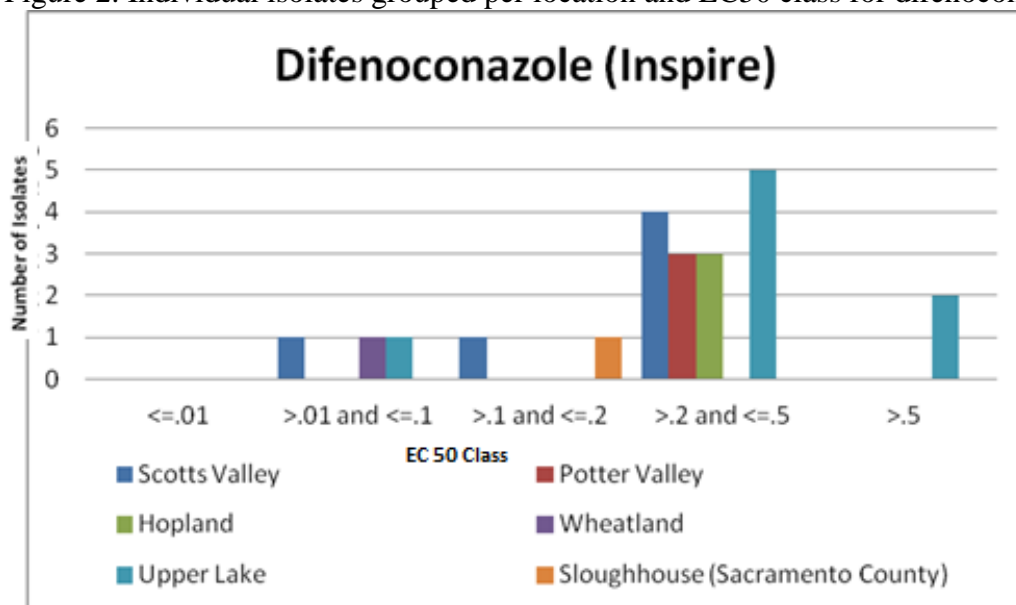
The average EC50 value for pyramethanil, the active ingredient in Scala, was high in many isolates. Only the isolate from Sloughouse in Sacramento County and one isolate from Upper Lake were highly sensitive (Figure 1). .

Figure 1. Individual isolates grouped per location and EC50 class for pyramithanil.



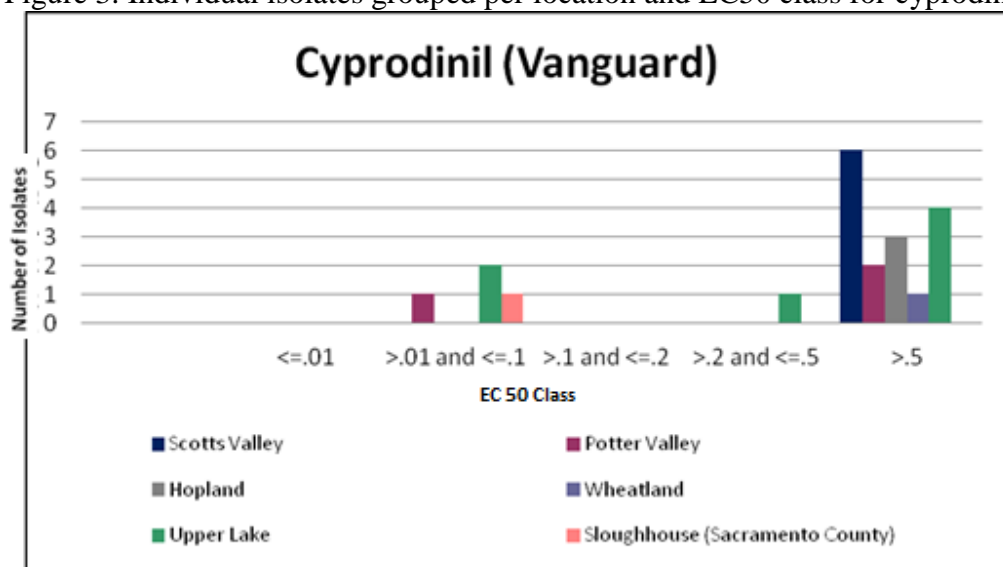
Difenoconazole, the active ingredient in Inspire, had a range of EC 50 values. Wheatland, Scotts Valley and Upper Lake had the most sensitive isolates.

Figure 2. Individual isolates grouped per location and EC50 class for difenoconazole.



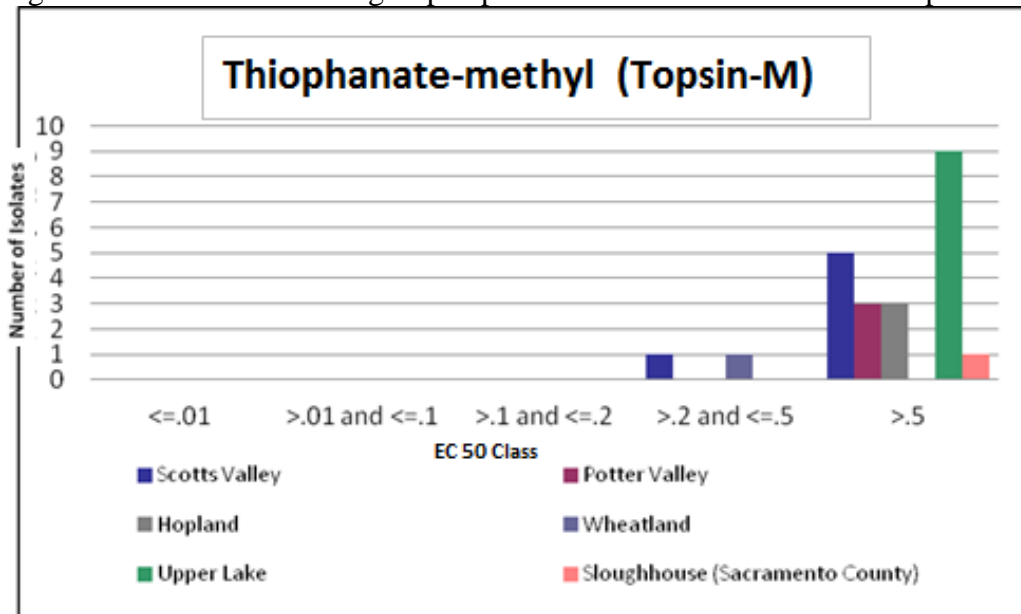
Many isolates show reduced susceptibility to cyprodinil, the active ingredient in Vanguard. The Sloughhouse isolate was highly sensitive. Isolates collected either had reduced susceptibility or were susceptible. There were no isolates with moderate sensitivity (Figure 3).

Figure 3. Individual isolates grouped per location and EC50 class for cyprodinil.



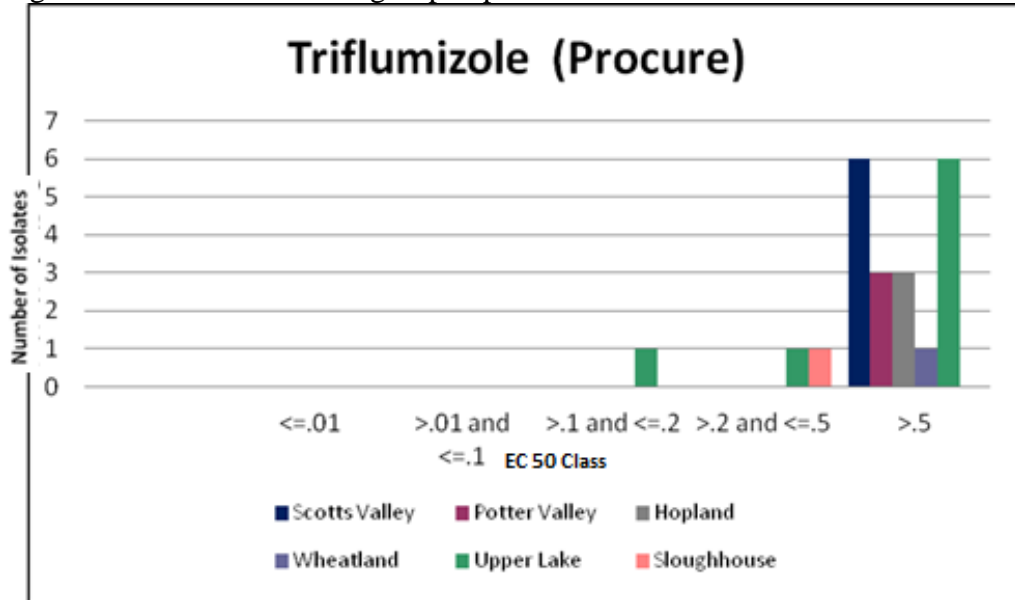
Thiophanate-methyl is the active ingredient in Topsin-M. All areas had moderate to high EC 50 values. All areas except Wheatland had isolates with reduced sensitivity to Topsin-M (Figure 4).

Figure 4. Individual isolates grouped per location and EC50 class for thiophanate-methyl.



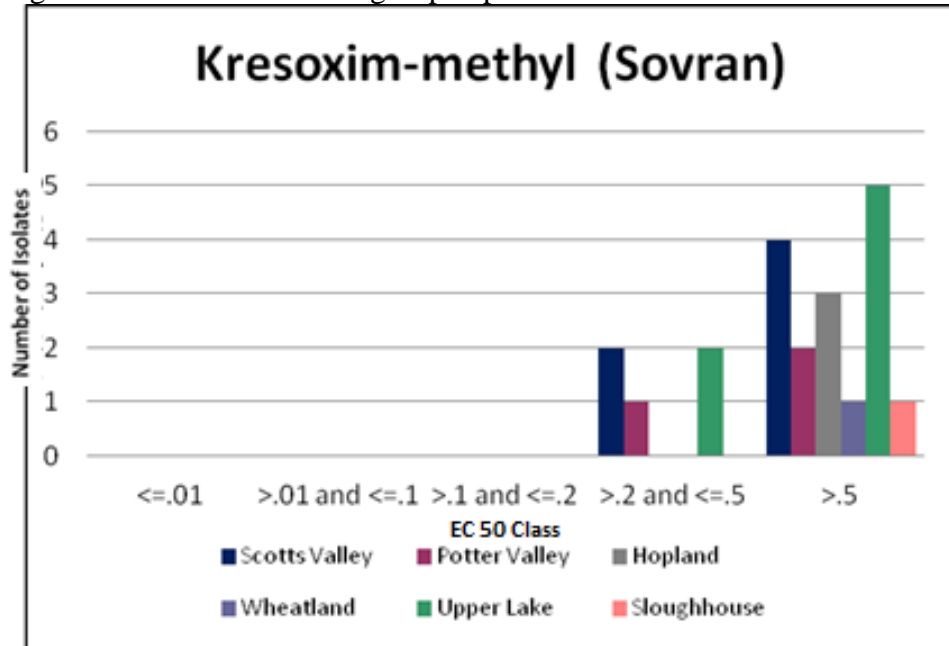
Triflumizole, the active ingredient in Procure, was as effective against pear scab as in the past. Nearly all isolates showed reduced sensitivity but the product is still usable one time in a season (Figure 5).

Figure 5. Individual isolates grouped per location and EC50 class for triflumizole.



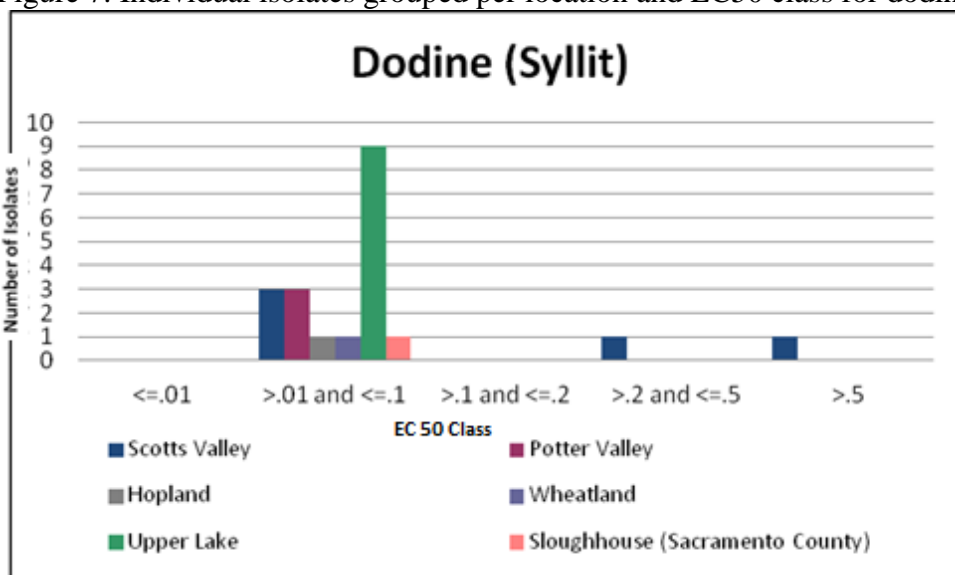
Overall, isolates were less sensitive to kresoxim-methyl, the active ingredient in Sovran. Figure 6 shows most isolates were in the least sensitive class (>0.2 and >0.5).

Figure 6. Individual isolates grouped per location and EC50 class for Kresoxim-methyl



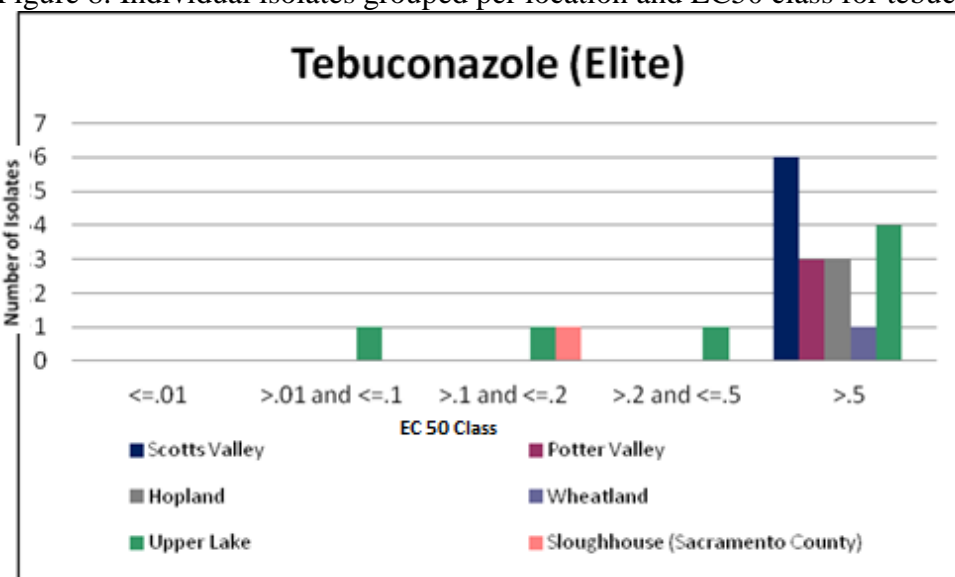
Average EC 50 values for dodine were low for all areas. Most isolates were sensitive to dodine with figures in the range of .01 to 0.1 (Figure 7).

Figure 7. Individual isolates grouped per location and EC50 class for dodine.



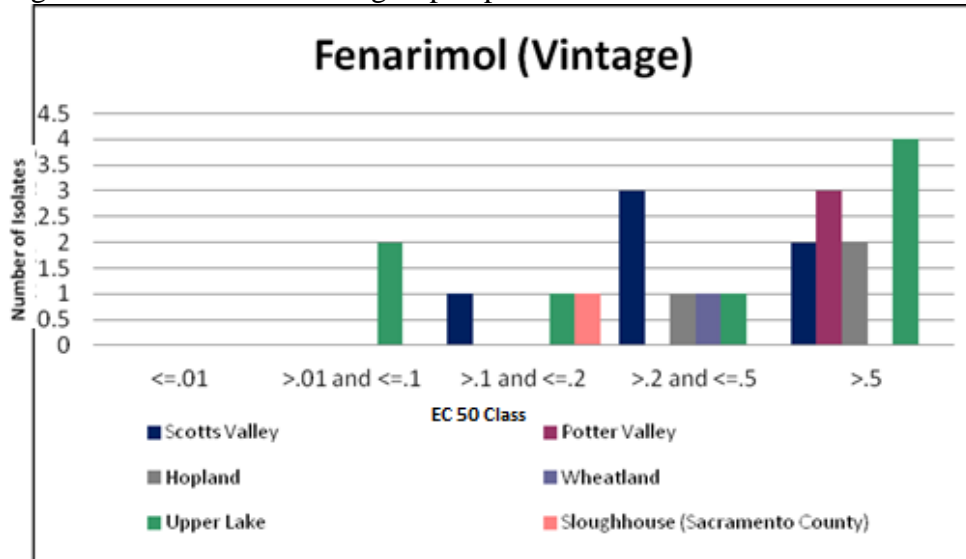
Elite (active ingredient tebuconazole) had high EC 50 values. Only some Upper Lake isolates and the Sloughhouse isolate showed sensitivity (Figure 8).

Figure 8. Individual isolates grouped per location and EC50 class for tebuconazole.

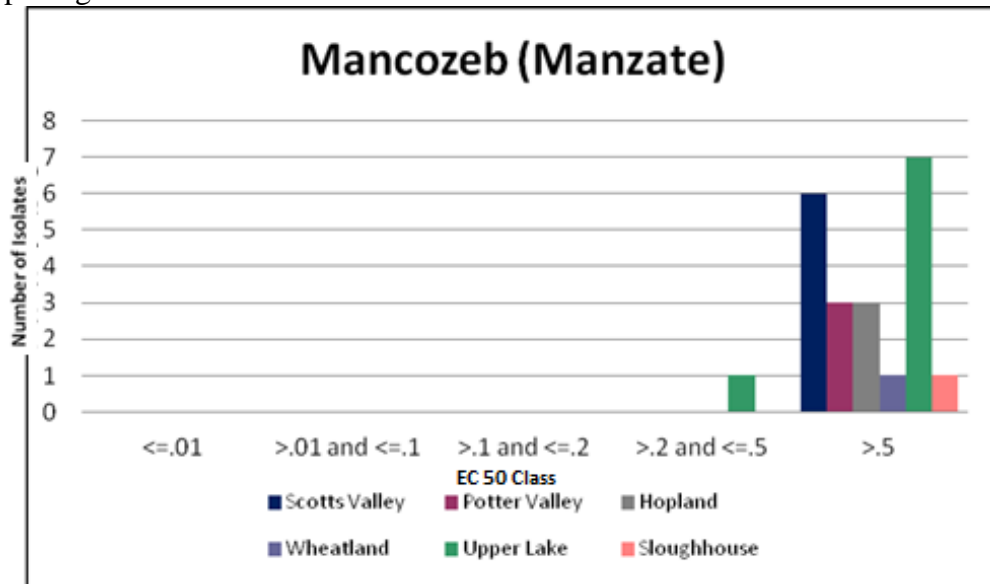


Fenarimol (active ingredient in Vintage), had a wide range of EC 50 values from the different areas. Some isolates showed reduced sensitivity to fenarimol especially in Upper Lake, Potter Valley, Scotts Valley and Hopland.

Figure 9. Individual isolates grouped per location and EC50 class for Fenarimol.



Mancozeb (active ingredient in Manzate), had reduced susceptibility in all areas (Figure 10.) but we .dont know what this means as far as efficaceousness against the pathogen.



Discussion

We did not get too many isolates in 2014 due to low disease pressure. However, there was evidence of resistance in many isolates that presumably had had significant selection pressure over the past few years. Resistance to the strobilurins and DMI's was still fairly prevalent. Even though we have resistance it does not mean we cannot use the products. We just have to never apply two consecutive applications of one class of chemistry. Managing resistance means we use multiple chemistries in a given year and from year to year.

OBJECTIVE Spore Trap Study: Conduct spore traps in two pear orchards to study the release of fungal spores.

PROCEDURE

To evaluate the distribution, incidence and severity of pear canker diseases, field surveys were conducted in the main pear growing areas in Northern California, including Lake and Mendocino Counties, as well as the Sacramento Delta regions. Pear orchards were visited and branches showing disease symptoms or large knots of wood were collected and processed for fungal isolation and pathogen identification. Isolation was conducted by placing small pieces of necrotic tissue obtained from the margin of cankers onto petri dishes containing 4% potato dextrose agar amended with tetracycline hydrochloride (0.01%) (Sigma-Aldrich, St. Louis) (PDA-Tet). Cultures were incubated at room temperature (24-25 °C) for ten days. Isolates recovered from cankers were identified based on morphological characters. DNA isolation and amplification was performed to confirm the morphological identification.

Spore trapping studies were also conducted in different orchards in Mendocino County. Spores were trapped using microscope slides coated with Vaseline (Unilever, London, UK) placed randomly on pear branches. Eighteen spore traps were distributed between two orchards. Spore traps were collected and changed weekly following the protocol described by Urbez Torres *et al.*, 2010. The number of spores were quantified weekly.

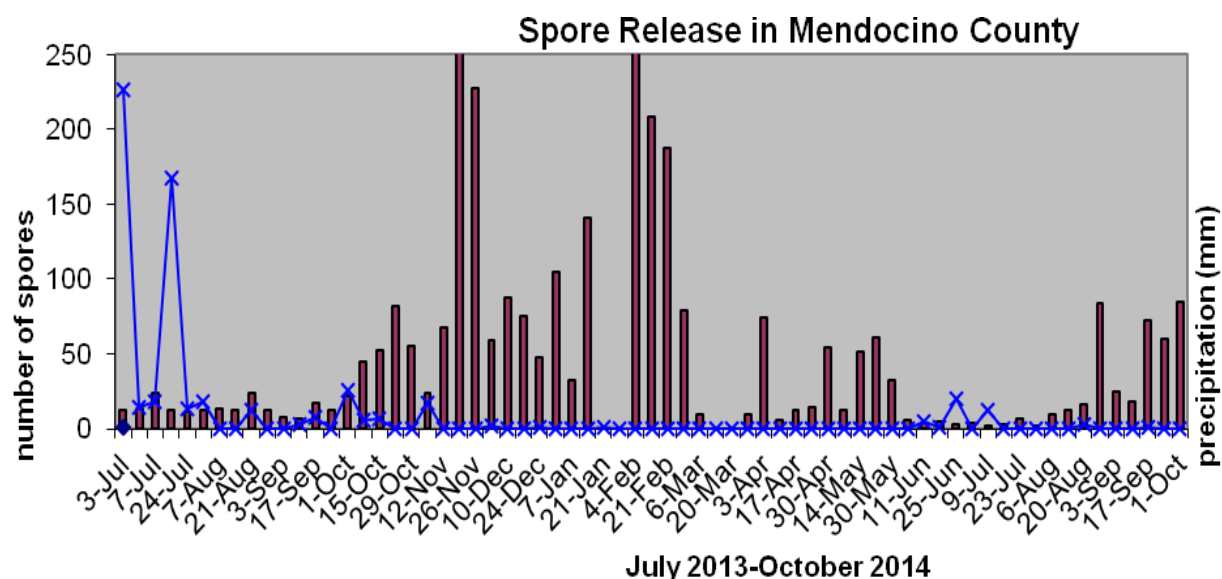
RESULTS

Pathogens isolated from diseased wood in Lake and Mendocino County included *Eutypa lata*, *Botryosphaeria iberica*, *Microdiplodia sp.*, *Cryptosporiopsis californiae*, *Cryptosporiopsis ericae*, and *Leucostoma personii*.

Spore trap studies to date have isolated the following pathogens: *Sphaeropsis sapinea*, *Eutypa lata*, *Phaeoacromonium* species, *Phaeoacromonium rubrigenum*, *Stereum* species, *Botryosphaeria obtusa*, *Botryosphaeria stevensii*, *Botryosphaeria dothidea*, *Botryosphaeria iberica*, *Botryosphaeria rodina*, *Lasodiplodia theobromae*, *Ganoderma lobatum*, *Cryptovalsa ampelina* *Fusarium venenatum*, and *Trametes versicolor*.

Spore trap studies showed most spores were released during February and March (Figure 21). Some pathogenic species captured were trapped in May, when nearly all spores trapped were *Botryosphaeria* species. Here we show the total spore catch of all species. We will break out individual species.

Figure 11. Average number of spores collected per week in Mendocino County.



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No disease was observed in the pear scab trial for 2014. The trial as set up with products is shown below. Trial will be repeated in 2015.

Fungicide control of Pear Scab: 2014 field trial

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Materials and Methods

A. Trial layout

Experimental unit	1 tree = 1 plot		
Row and tree	ft (row) and ft (tree)	Plot unit area	200 ft ²
Area/treatment	800 ft ² or 0.0184 acre/treatment (4 replicate trees = 1 treatment)		
Fungicide applications	<p>A. Green tip, 13 March, 100 gallons/acre, 1.8 gallons/4 replicates</p> <p>B. Early bloom, 24 March, 100 gallons/acre, 1.8 gallons/4 replicates</p> <p>C. Full bloom, 4 April, 100 gallons/acre, 1.8 gallons/4 replicates</p> <p>D. Petal fall, 10 April, 125 gallons/acre, 2.3 gallons/4 replicates</p> <p>E. Cover spray, 21 April, 25 gallons/acre, 2.3 gallons/4 replicates</p>		

B. Trial Map

Pear Scab 2014 Map														
● = skipped tree														
									OS	BS	●			
									LP/HP/K	●	●			
								PKS	KS	Y	●			
							B	●	HP	PKS	●			
							B	YKS	BS	RS	PKD	●		
							Y	RB	R	YS	●	YG		
							KS	●	LP/HP/K	W	Y	B	●	
							O	●	●	OS	O	RS	OS	R
							YS	●	●	YG	●	PKD	O	PKS
			HP	R	BS	●	YS	●	RD	●	KS	YKS		
			RD	Y	PKD	●	LP	O	●	YG	LP/HP/K	●		
		LP	●	RS	LP/HP/K	RD	●	●	●	R	●	RD		
		B	KS	PKS	YG	HP	RS	BS	YS	HP	●	●		
●		W	OS	YKS	●	YKS	PKD	W	LP	W	LP	●		
1	2	3	4	5	6	7	8	9	10	11	12			
ROAD		ROAD		ROAD		ROAD		ROAD		ROAD				

Pear Scab – 2014 Experimental treatments

No.	Flag	Product(s)	FP/Acre	FP/Treatment
1	W	Unsprayed control	non	non
2	B	Vanguard 75WG then Manzate Pro-stick then A15457 then Inspire super 2.82EW	5 oz then 96 oz then 6.84 fl oz then 13.7 fl oz then	2.6 g then 50.1 g then 3.7 ml then 7.5 ml then 3.7 ml
3	LP	Vanguard 75WG then Manzate Pro-stick then A15457 then Inspire Super 2.82EW then A15457 (2 weeks interval after green tip) (3/13 1.8 gal, 3/27 1.8 gal, 4/10 2.3 gal, 4/24 2.3 gal)	5 oz then 96 oz then 6.84 fl oz then 12 fl oz then 6.84 fl oz	2.6 g then 50.1 g then 3.7 ml then 6.5 ml then 3.7 ml
4	HP	Vanguard 75WG then Manzate Pro-stick then A19334 then Inspire Super 2.82EW	5 oz then 96 oz then 7.04 fl oz then 13.7 fl oz then	2.6 g then 50.1 g then 3.8 ml then 7.5 ml then 3.8 ml
5	RD	ISOFETAMID (4x) then Manzate Pro-stick	12.5 fl oz (4x) then 3 lb	6.8 ml (4x) then 25.0 g
6	KS	ISOFETAMID + IB18111 (4x) then Manzate Pro-stick	6.85 fl oz + 5.57 fl oz (4x)	3.7 ml + 3.0 ml (4x) then
7	OS	ISOFETAMID + IB18111 (4x) then Manzate Pro-stick	6.85 fl oz + 2.97 fl oz (4x) then 3 lb	3.72 ml + 1.61 ml (4x) then 25.0
8	YKS	ISOFETAMID + IB18111 (4x) then Manzate Pro-stick	3.43 fl oz + 5.57 fl oz (4x) then 3 lb	1.86 ml + 3.02 ml (4x) then 25.0
9	PKS	Sovran	4 oz	2.1 g
10	RS	Syllit (3 apps) then Manzate Pro-stick	3 pt (3x) then 3 lb	26.1 ml (3x) then 25.0 g
11	Y	Syllit (36 hrs eradicant after rain event) (3/27 1.8 gal, 4/3 1.8 gal)	3 pt	26.1 ml
12	R	Tebuconazole	2 oz/100 gal	1.0 g
13	O	Manzate Pro-stick	3 lb	25.0 g
14	YS	Ziram (4x) then Manzate Pro-stick	6 lb then 3 lb	50.0 g then 25.0 g
15	BS	Microthiol (sulfur)	30 lb	250.0 g

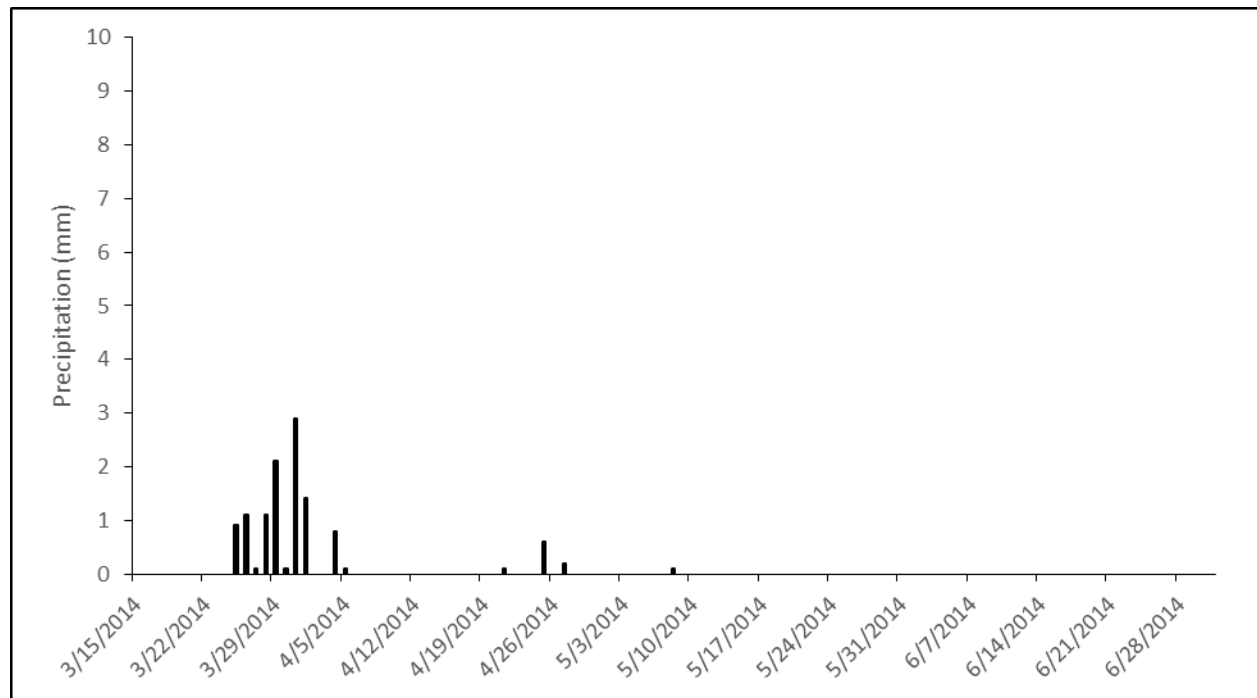
16	PKD	Merivon (4x) then Manzate Pro-stick	5 fl oz (4x) then 3 lb	2.7 ml (4x) then 25.0 g
17	LP/HP/K	Topsin-M	16 oz	8.4 g
18	YG	Serenade Optimum	24 oz	12.5 g

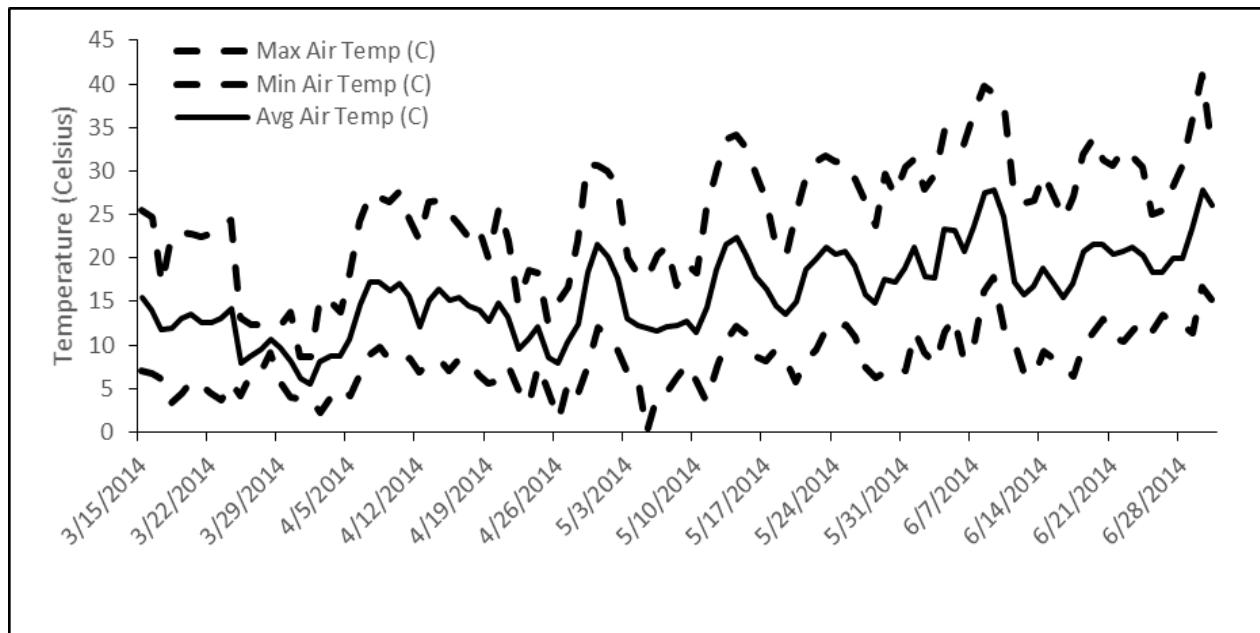
. Disease and Statistical Analysis

Disease was assessed on June 18 2014 when fruits were large enough to observe scab lesions. No disease was present due to dry conditions.

D. Weather and Disease

Weather for the spray season was dry with 8 rain events (Mar 15 – July 1) of 0.6 – 2.9 mm of rain.





Acknowledgements

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Appendix: Products tested

Product	Active ingredient(s) and concentration	Class	Manufacturer
A15457	Proprietary	N/A	Proprietary
A19334	Proprietary	N/A	Proprietary
IB18111	Proprietary	N/A	Proprietary
Inspire Super 2.82EW	Cyprodinil (24.1%)/ Diflufenican (1.2%)	DMI-triazole (3)/ AP (9)	Syngenta
Isofetamid	Proprietary	N/A	Proprietary
Manzate Pro-stick	Mancozeb (75%)	Carbamate	United Phosphorous
Merivon	Fluxopyroxad (21%)	SDHI (7)/QoI (11)	BASF
Microthiol Disperss	Sulfur (80%)	Inorganic (M2)	United Phosphorous
Serenade Optimum	Bacillus subtilis (26%)	Microbial	Bayer
Sovran	Kresoxim-methyl (50%)	QoI (11)	Cheminova
Syllit	Dodine (40%)	Guanidine (M7)	Agriphar
Tebuzol 45 DF (Elite)	Tebuconazole (45%)	DMI-triazole (3)	United Phosphorous
Topsin-M	Thiophanate-methyl (70%)	MBC (1)	UPI
Vanguard 75WG	Cyprodinil (75%)	AP7 (9)	Syngenta
Ziram 76DF	Ziram (76%) Zinc (16.25%)	Carbamate (DMDC)3 (M3)	UPI

Appendix references: (1) Adaskaveg, et al. 2012. Efficacy and timing of fungicides, bactericides and biologicals for deciduous tree fruit, nut, strawberry, and vine crops 2012, available at <http://www.ipm.ucdavis.edu/PDF/PMG/fungicideefficacytiming.pdf>. (2) Gubler Lab fungicide efficacy field trials, available at http://plantpathology.ucdavis.edu/Cooperative_Extension/. (3) Various sources including product labels and/or MSDS, product websites, and personal communications.

