SCREENING FUNGICIDES TO CONTROL PEAR SCAB (*Venturia pirina*) AND DETECTION OF FUNGICIDE RESISTANCE IN SCAB POPULATIONS IN CALIFORNIA PEAR ORCHARDS

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ABSTRACT

The fungus *Venturia pirina* causes pear scab. It is a serious disease of pear in California, resulting in loss due to severe surface blemishing of fruit. It is most severe in North Coast production areas where spring and early summer weather is cool and moist. However, it can be a problem wherever pear grow when conditions are favorable for pathogen development. 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. This report details the findings of our annual pear scab fungicide trials on pear (*Pyrus* spp., Cultivar Bartlett – <40- yrs-old). The trials consisted of soft chemistry products and synthetic fungicides. Spray frequencies varied from 7 day to 21-day intervals. All treatments applied preventively significantly reduced pear scab on fruits of Bartlett pears. However, the best result was obtained with applications of the fungicides Syllit, Ziram, Inspire Super, Pristine, Merivon and Rango, applied, seven times, during the critical period of leaves and fruits for development pear scab. Spiral gradient tests were conducted to measure fungicide resistance and efficacy. Fungicide resistance in *Venturia pirina* population was shown to Flint, Sovran and Topsin-M, and a decreased susceptibility to Procure and Scala.

INTRODUCTION

The European pear (*Pyrus communis*) is commercially important crop with 11,200 acres planted in California (CDFA 2018). California is the nation's leading producer of Bartlett pears for both fresh and processed markets, followed by Washington and Oregon. Furthermore, California produces 57% of the canned pear and canned fruit cocktail product sold in the United States (Mitcham and Elkins, 2007).

Pear Scab (*Venturia pirina*) is the most common disease of pear in the north coast production area of California. Pear scab is an economically important disease throughout the world and can cause severe crop loss in susceptible cultivars. Pear scab prevention requires use of fungicides in most years for control. As older broad-spectrum fungicides with post-infection activity were taken off from the market due to health concerns. The introduction of new single-site fungicide chemistries in the United States occurred in a slow successive manner and led to the unfortunate practice of overreliance on the newest fungicide class and the development of fungicide resistance to each class in succession (Cox 2015).

In recent years, growers have observed a reduced level of control when using demethylation inhibitors (DMIs), benzimidazoles and the quinone outside inhibitors (QoIs) in California's North Coast pear production areas. Other practices that often lead to resistance are the practices of using reduced concentrations of product and poor coverage (Elkins et al. 2012). New fungicides are being developed with new modes of action against fungal disease. As we tend to lose single site of action fungicides due to resistance it becomes necessary to evaluate new fungicides to establish efficacy against pear scab.

OBJECTIVES

Objective 1. Establish field fungicide screening trial for scab control in Lake County.

Objective 2. Monitor potential resistant *V. pirina* isolates against register commonly used fungicide.

PROCEDURES

Field fungicides trial. This trial was conducted at Lubich pear farm (*Pyrus communis*, Cultivar Bartlett – <40- yrs-old), Ukiah (38°19'14.2"N, -121°30'25.7"W) (Table 1) from March to June 2020. Treatments (Table 2) were placed in a complete randomized block design (Figure 1). The treatments were evaluated for disease incidence and severity on June 3rd, 2020. The trials consisted of soft chemistry products and synthetic fungicides. Spray frequencies varied from 7 day to 21-day intervals

Disease was assessed on June 3rd. Fifty fruits were randomly selected from each tree. The number of lesions were scored for each fruit. Disease incidence per replicate was determined as the proportion of fruits that were infected by at least one lesion. Data was analyzed using ANOVA Fit Model test for data. Comparison of the means was made using Fisher’s LSD test with α=0.05.

Daily temperature and relative humidity were obtained from the trial site (Figure 2).

Table 1. Location experimental design and application timing

Location	Lakeport, California, 39°05'08.9"N 122°56'35.5"W		
Experimental design	Randomized complete block design with 4 replicates		
Experimental unit	1 tree = 1 plot		
Row and tree spacing	19 ft (row) and 15 ft (tree)	Plot unit area	285 ft2
Area/treatment	1140 ft2 or 0.02617acre/treatment (4 replicate trees = 1 treatment)		
Fungicide applications	A green tip, Mar 12th, 100 gallons/acre 2.6 gallons/4 replicates B green cluster, Mar 20th, 100 gallons/acre 2.6 gallons/4 replicates C early bloom, Mar 31st, 100 gallons/acre 2.6 gallons/4 replicates D full bloom, Apr 14th, 100 gallons/acre 2.6 gallons/4 replicates E petal fall, Apr 24th, 100 gallons/acre 2.6 gallons/4 replicates F 1st cover spray, May 08th, 100 gallons/acre 2.6 gallons/4 replicates G 2nd cover spray, May 22nd, 100 gallons/acre 2.6 gallons/4 replicates		
Equipment	Stihl SR 450 Backpack Sprayers		

Table 2. Treatment programs. “FP” = formulated product

No.	Flag	Product(s)	Application	FP/Acre	FP/Treatment
1	Y	Unsprayed control		none	none
2	YS	Ziram 76 DF	ABCDEFG	6 pounds	70.7 gr
3	PKC	Syllit	ABCDEFG	3 pints	36.9 ml
4	KC	Inspire Super 2.82 EW	ABCDEFG	12 fl oz	9.2 ml
5	OS	Cueva	ABCDEFG	1 gal	98.4 ml
6	P	Pristine	ABCDEFG	16.5 Oz	12.1 gr
7	BC	Merivon	ABCDEFG	5 fl oz	3.8 ml
8	GS	Rango	A	230 fl oz	176.8ml
		Rango	BCDEFG	160 fl oz	123.1 ml
9	YKC	Aprovia	ABCDEFG	6.25 fl oz	4.8 ml
10	B	Rango	ACEG	160 fl oz	123.1 ml
		TER 1291 + Nu Film P	BDF	0.8% (v/v) + 16 fl oz	78.7 ml + 12.1 ml
11	BD	Mastercop	BDF	0.5 pt	49.2 ml
12	RKD	Sonata	ABCDEFG	4 qt	98.5 ml

N

Row 1	2	3	4	5	6	7
					BC-7	B-10
			OS-5	RKD-12	OS-5	x
		YS-2	YS-2	YKC-9	B-10	Y-1
	PKC-3	x	BD-11	BC-7	GS-8	YKC-9
x	B-10	P-6	x	PKC-3	KC-4	PKC-3
X	RKD-12	Y-1	Y-1	KC-4	BC-7	BD-11
x	0S-5	GS-8	P-6	B-10	BD-11	KC-4
	x	x	BD-11	GS-8	0S-5	RKD-12
		YKC-9	KC-4	YS-2	Y-1	GS-8
			BC-7	P-6	PKC-3	YS-2
				RKD-12	YKC-9	P-6

Block 1
Block 2
Block 3
Block 4

Figure 1. Trial layout

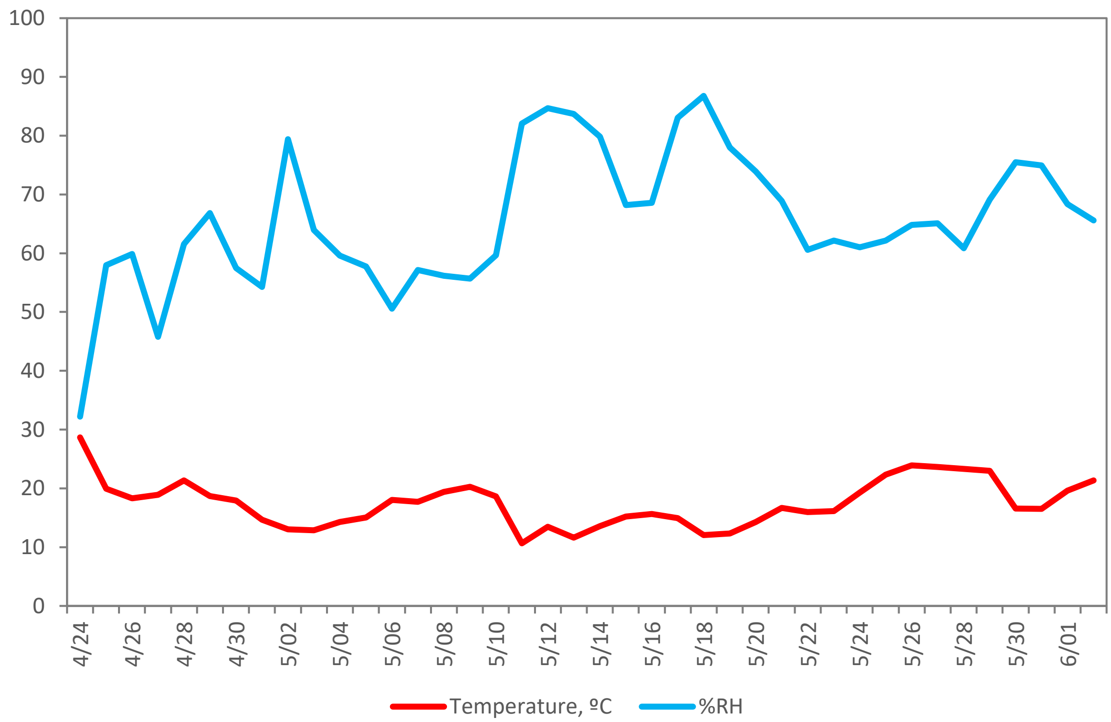

Figure 2. Average daily relative humidity (%RH) and average daily temperature (°C) were recorded from April 2020 to June 2020.

Determination of fungicide resistance *Venturia pirina* isolates were collected from an orchard in Ukiah, isolates were tooked from young, fully expanded leaves and fruits with isolated sporulating primary pear scab lesions. Single lesions were removed using a sterile cork-borer (5 mm in diameter), placed in 2.0 ml of sterile distilled water, and shaken for 60 s to dislodge conidia from the lesion. The resulting conidial suspensions (10^2 to 10^3 *V. pirina* conidia ml^{-1}) were stored at −20°C until the evaluation of fungicide sensitivity (Villani et al. 2016).

Common fungicides used by growers will be tested to identify their ability to inhibit *V. pirina* isolates via a spiral plating assay *in vitro*. Fungicides tested were: Flint, Procure, Sovran, Syllit, and Topsin M (Table 3). An Eddyjet-2 Spiral Plater was used to dispense the test fungicides onto a 150 mm x 15 mm plate containing 50 ml of potato dextrose agar. Compounds were diluted to 1000 ppm concentration in sterile distilled water and 54.3 µl were dispensed in an Archimedean spiral, beginning at 12 to 13 mm from the center of the plate, towards the edge. Then the plates were radially streaked with a conidial suspension of the fungal isolates Plates were incubated at 20^{O}C for 14 days. Control plates will consist of potato dextrose agar without fungicides. Conidial suspensions were applied in the same manner. Each isolate was replicated at least three times and the test repeated at least one time. An R package designed by Torres- Londoño et al (2016) was used to determine EC_{50} values of each treatment. The following formulation was used for the calculation of EC_{50} values:

$$\text{ECcal(rad1 = c(w), mw =x, ppm =y, AH=z)}$$

In this formula, w is the desired EC_{50} measurement, x is the molecular weight of the test compound, y is the parts per million concentration of the test compound and z is agar height.

Table 3. Fungicide tested for resistance in *Venturia pirina* isolates

Product	**Active ingredient(s) and concentration**	**Manufacturer or distributor**	**Chemical class (Frac Code)**
Flint	Trifloxystrobin (50%)	Bayer CropScience	QoI (11)
Procure	Triflumizole	Chemtura	DMI-triazole (3)
Rango	cold pressed neem oil	Terramera Inc.	N/A
Sovran	Kresoxim-methyl	Cheminova	QoI (11)
Syllit	Dodine (40%)	Agriphar	Guanidine (M7)
Topsin M	Thiophanate-methyl (70%)	UPI	MBC (1)

RESULTS

Field fungicides trial

In the field, a moderate pressure of pear scab (Figure 3A) was observed, 14.5% on average in the untreated controls (Table 3). Under these conditions, there were statistically significant differences between the respective fungicide treatments and the untreated control. All treatments exercised a significant control, reducing the prevalence pear scab (Table 4).

Evidence of phytotoxicity were observed on fruits treated with Cueva, Mastercop, Rango and Terramera Biological (Figure 3B, C)

Table 4. Pear scab fruit incidence (means). Product names are followed by rate (per acre). Treatment means followed by the same letter are not significantly different according to Fisher's LSD test at α=0.05.

Treatment	Flag	Mean Incidence (%)	
Syllit 3 pt	PKC	2.5	a
Ziram 76 DF 6 lb	YS	3.5	a
Inspire Super 2.82 EW 12 fl oz	KC	3.5	a
Pristine 16.5 Oz	P	4.0	a
Merivon 5 fl oz	BC	4.0	a
Rango 230 fl oz (1st app) / Rango 160 fl oz	GS	4.0	a
Aprovia 6.25 fl oz	YKC	5.5	ab
Rango 160 fl oz / TER 1291 + 0.8% (v/v) + Nu Film P 16 fl oz	B	6.0	ab
Cueva 1 gal	OS	6.5	ab
Mastercop 0.5 pt	BD	7.5	ab
Sonata 4 qt	RKD	10.5	bc
Unsprayed control	Y	14.5	c

Figure 3. A) Pear scab lesion on fruit from untreated control. **B)** Symptoms of phytotoxicity on fruit treated with Cueva and Mastercop. **C)** Symptoms of phytotoxicity on fruit treated with Rango and Terramera Biological.

Determination of fungicide resistance

Thiophanate-methyl is the active ingredient in Topsin-M. Resistance to Thiophanate-methyl was present in one of the isolates evaluated, and approximately 45% of isolates had a moderate resistance to this fungicide, a 45% of isolates were sensitive to Thiophanate-methyl (EC_{50} < 0.6 µg/ml, Chapman et al. 2011, Figure 4).

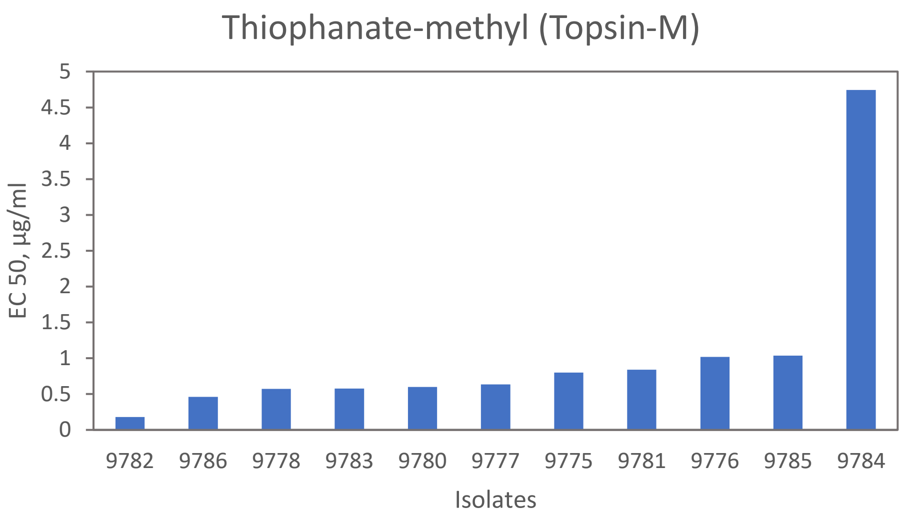

Figure 4. *Venturia pirina* isolates and EC50 for Thiophanate-methyl

The average EC50 value for Pyrimethanil, the active ingredient in Scala, was high in many isolates (EC_{50} > 0.5µg/ml, Figure 5). The isolates 9785 and 9786 showed a reduced sensitivity (EC_{50} > 2 µg/ml, Figure 5).

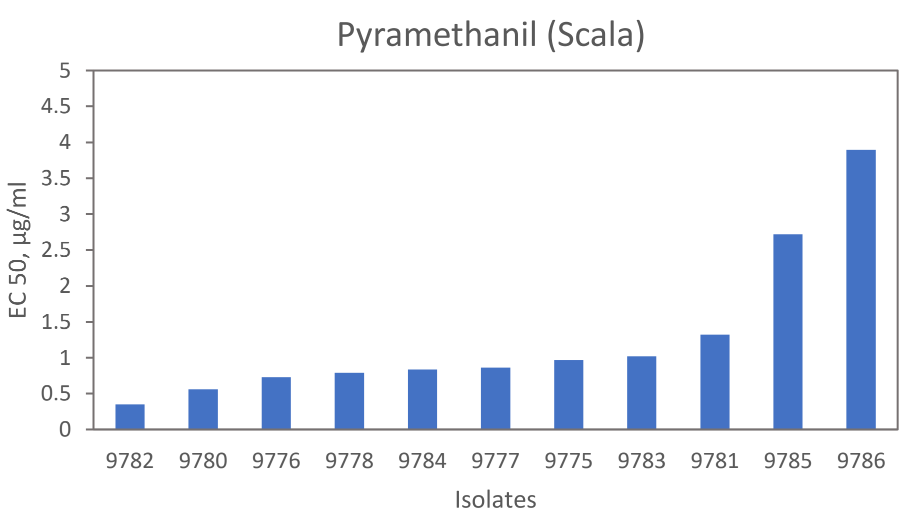

Figure 5. *Venturia pirina* isolates and EC50 for Pyrimethanil

Nearly all isolates showed sensitivity to Triflumizole, the active ingredient in Procure. Just the isolates 9785 and 9786 showed a reduced sensitivity (EC_{50} > 0.4 µg/ml) (Figure 6).

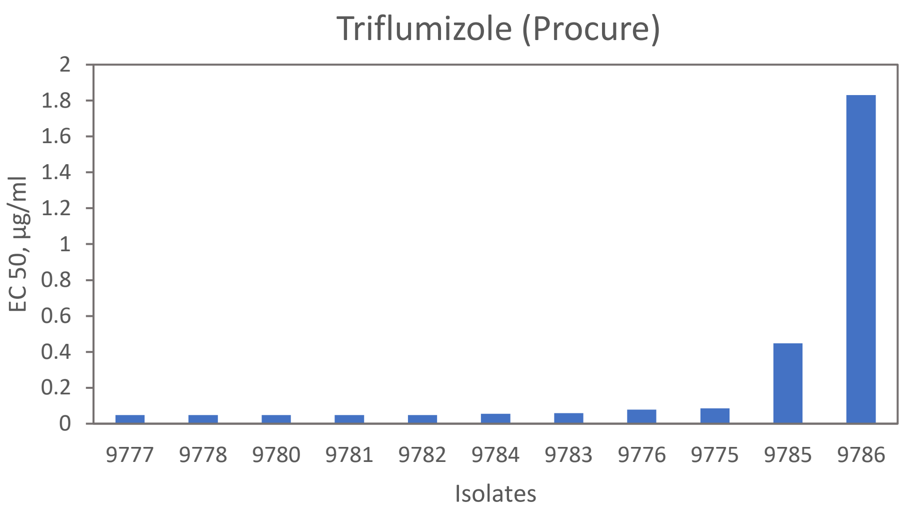

Figure 6. *Venturia pirina* isolates and EC50 for Triflumizole

Average EC_{50} values for dodine were low. All isolates were sensitive to dodine with EC_{50} values in the range of 0.058 to 0.28 µg/ml (Figure 7)

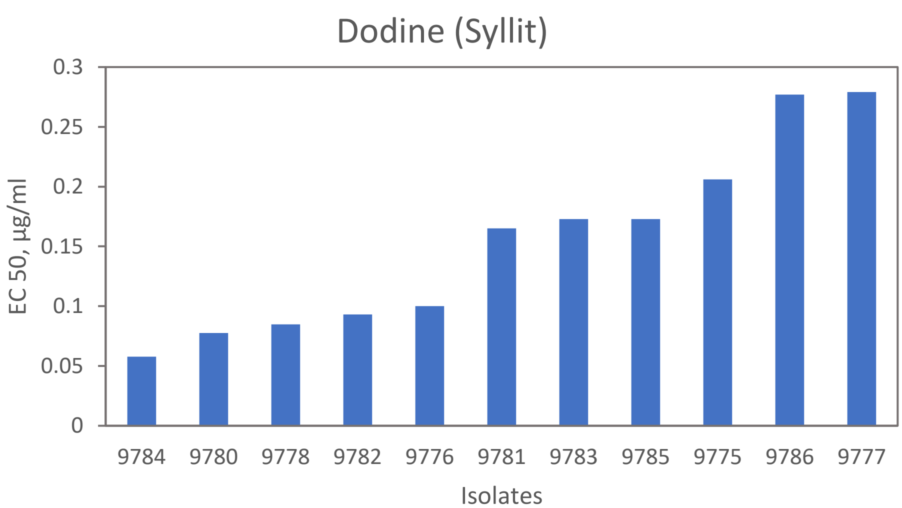

Figure 7. *Venturia pirina* isolates and EC50 for Dodine

Average EC_{50} values for Kresoxim-methyl and Trifloxystrobin were low except for isolate 9776 which showed to be resistant with EC50 > 5 µg/ml. (Figure 8, 9)

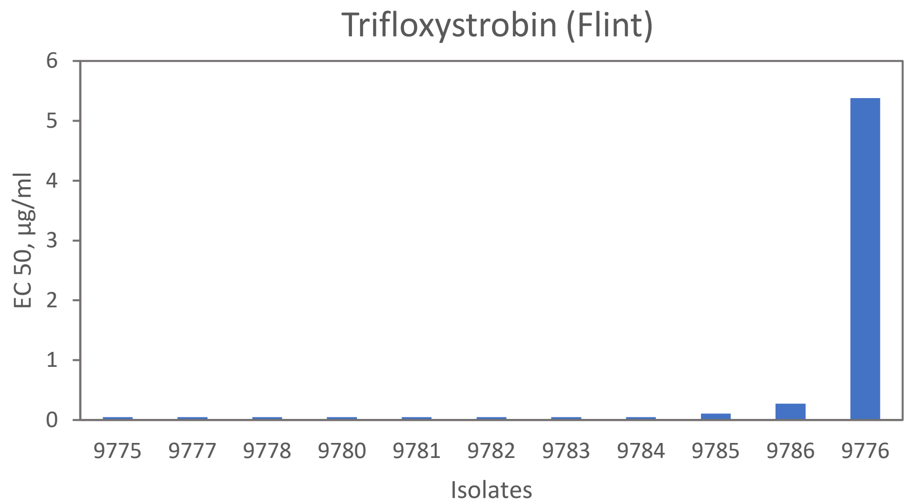

Figure 8. *Venturia pirina* isolates and EC50 for Trifloxystrobin

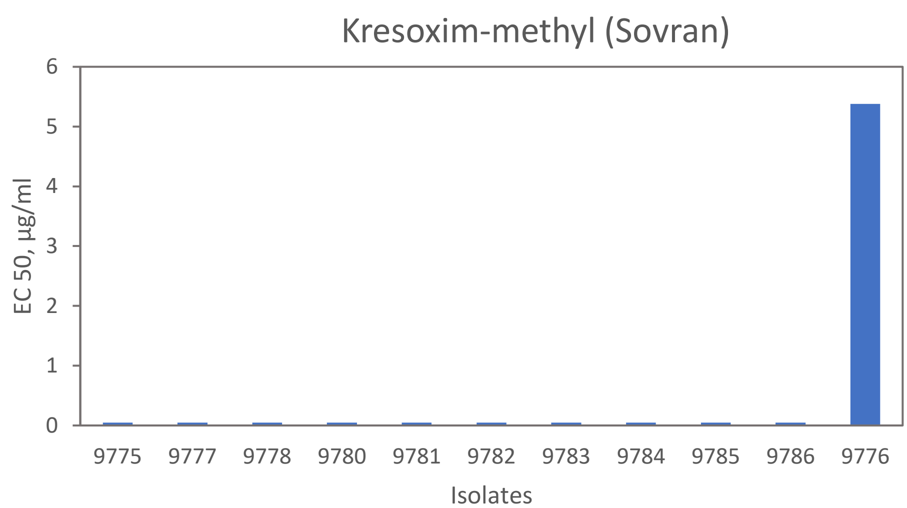

Figure 9. *Venturia pirina* isolates and EC50 for Kresoxim-methyl

DISCUSSION

A moderate pressure of pear scab was observed in this year's trial site. Under these environmental conditions, all of treatments applied preventively showed significant reduction of pear scab on Bartlett pears. Among them, the following fungicides Syllit, Ziram, Inspire Super, Pristine, Merivon and Rango performed the best as preventative of the disease. However, Rango and Terramera showed minor phytotoxicity on fruits.

Due to COVID-19 restriction, we received a limited number of isolates for fungicide resistance screening this year. However, several isolates showed evidence of resistance against common fungicide in our spiral gradient screening process. These results suggest that the fungicide applications should be alternated with different FRAC group.

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Appendix: Materials

Product	Active ingredient(s) and concentration	Manufacturer or distributor	Chemical class (Frac Code)
Aprovia	Benzovindiflupy	Syngenta	SDHI (7)
Cueva	Copper octanoate (10%)	Certis USA	inorganic (M01)
Flint	Trifloxystrobin (50%)	Bayer CropScience	QoI (11)
Inspire Super	difenoconazole (8.4%), cyprodinil (24.1%)	Syngenta Crop Protection, Inc.	DMI-triazole (3)/AP(9)
Mastercop	copper sulfate pentahydrate (21.46%)	ADAMA	inorganic (M01)
Merivon	pyraclostrobin (21.26%), fluxapyroxad (21.26%)	BASF	QoI(11)/SDHI (7)
Pristine	pyraclostrobin (12.8%), boscalid (25.2%)	BASF	QoI(11)/SDHI (7)
Procure	Triflumizole	Chemtura	DMI-triazole (3)
Rango	cold pressed neem oil	Terramera Inc.	N/A
Sonata	Bacillus pumilus qst 2808 (1.38%)	Bayer CropScience	biological
Sovran	Kresoxim-methyl	Cheminova	QoI (11)
Syllit	Dodine (40%)	Agriphar	Guanidine (M7)
Terramera Biological	cold pressed neem oil (52%) octanoid acid (25%)	Terramera Inc.	N/A
Topsin M	Thiophanate-methyl (70%)	UPI	MBC (1)
Ziram 76DF	Zinc (76%)	UPI	Carbamate (DMDC)3 (M3)