Detection of resistance in populations of pear scab (*Venturia pirina)* and fireblight *(Erwinia amylovora)* in California pear orchards.

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Duration of Project: 2 years

Objectives:

1. Determine the presence and level of resistance to DMI and strobilurin fungicides in the *V. pirina* population in California.
2. Determine presence and level of resistance to copper and streptomycin in the *E. amylovora* population in California.

Pear scab and fireblight are two of the most common diseases of pear in California. Both diseases are commonly treated with fungicides and bactericides for control, respectively. In recent years the most common products used against pear scab have been products that attack the pathogen at a metabolic pathway that is genetically coded for at only one locus. As these materials are used over time, resistance to the products may develop more quickly than fungicides that act against pathways that are controlled by many loci. Growers and pest control advisers (PCAs) have stated that they have observed a reduced level of control for the strobilurin and DMI fungicides for scab control. At the same time PCAs and growers have also observed a decrease in sensitivity to the products used for fireblight control.

Trifloxystrobin (Flint) is active against a wide array of pathogens including the pear scab pathogen *V. pirina*. This product inhibits mitochondrial respiration by blocking electron transfer at the ubiquinol-oxidation center (Qo-site) of the bc1-enzyme complex (complex III). Based on evidence from a number of pathogens, trifloxystrobin, kresoxim-methyl, azoxystrobin and famoxadone belong to the same chemical group, the Qo-site inhibitors (QoIs).

During flowering and early fruit development in 2006 there was substantial precipitation, which is conducive for disease development. During the same period in 2007 there was low rainfall; disease incidence was very low. Few samples of *V. pirina* were found when the orchards from the previous year were sampled. No disease was found in some of the previously sampled orchards. Fewer samples were obtained for resistance testing in 2007 than were available in 2006. In 2006 502 isolates were available for testing; in 2007, 160 isolates were available for testing.

The baseline sensitivity of *V. pirina* towards trifloxystrobin has not been previously established. However, the mean baseline sensitivity towards *V. inaequalis,*a closely related species causing apple scab, was found to be 0.035 ppm with a factor of up to 16 between the most and the least sensitive isolates. In 2006 isolates were obtained from an orchard that had no history of strobilurin fungicide use. When these isolates were tested for trifloxystrobin sensitivity, the mean was 0.032 mg AI/mL, with a factor of 16 between the most and least sensitive isolates.

Methods

Isolates of *V. pirina* were collected from California pear orchards in 2007 and were taken to UC Davis to be mass cultured and maintained on appropriate media prior to being tested on fungicide amended agar plates for their reaction to various fungicides. In the case of screening for resistance to Flint (trifloxystrobin), the fungicide was incorporated into artificial media and each isolate was streaked onto an amended plate. Each isolate was replicated at least three times. Data analysis was conducted for determination the EC50 in mg AI/mL for trifloxystrobin and for determination of significant differences among isolates for individual products.

Pear dextrose agar amended with tetracycline (PrDA-tet) was developed to facilitate colony growth. Two hundred grams of fresh pear diced into 1 cm cubes was boiled in 1L deionized water, strained, and 16g agar and 15g dextrose added to create the media. After autoclaving and cooling to 55°C, 0.01g tetracycline, an antibiotic, was added. The media was then poured into petri plates.

In order to assess the presence of resistance in the *V. pirina* population in California pear orchards we used the spiral plating technique as described by Adaskaveg et al. Fresh leaf and fruit samples were collected and brought to the lab. The pathogen was isolated from lesions onto PrDA-tet on 60mm petri plates. Cultures were then stored at 4 C until processed for assessment of resistance. Petri plates (150mm) containing PrDA-tet were placed on the spiral plating instrument and trifloxystrobin was incorporated into the media in a spiral pattern whereby the highest concentration of fungicide was placed in the middle of the plate and as the needle which incorporated the fungicide spiraled toward the outside of the plate the fungicide became less concentrated. Spore suspensions from isolate colonies were prepared and tangentially streaked across the fungicide gradient onto the agar using sterile micropipettes, with the experimental design having three replications per plate. Spore germination was assessed at each gradient thus allowing the development of EC50 values.

Isolates were identified as to the location from which they were collected, including pear variety, orchard, and location of orchard in the county. A history of use for the product class is being put together but has not been completed yet.

Results

Statistical analyses were completed on JMP IN v.5.1 (SAS Institute Inc.) using the Tukey test to compare populations (α=0.05). Population averages for each fungicide by orchard showed some significant differences (fig.1-4). In figure 1, average EC50 for Flint® sensitivity by orchard was compared by the Tukey test. Four orchards had significantly higher average values, and 8 of the 14 orchards tested had average EC50 values greater than the baseline sensitivity. Baseline sensitivity for the strobilurin fungicides Flint® and Sovran® isolates was 0.032 mg AI/mL, and isolates below that were considered to be highly sensitive. Of the isolates tested from Mendocino County, 30.0% were considered resistant to trifloxystrobin. In Lake County, 53.3% of the isolates tested were considered resistant to trifloxystrobin. Figure 5 shows the EC50 frequency distribution for all isolates combined, showing a trend towards decreased sensitivity.

Figure 2 illustrates sensitivity to Sovran® by orchard. Five orchards were significantly less sensitive than the most sensitive population based on the Tukey test, and seven of the 14 orchards had average EC50 values higher than the baseline sensitivity. In Mendocino County 32% of the isolates tested were above the baseline 0.032 mg AI/mL. In Lake County, 60% of isolates tested were considered resistant to Sovran®. Figure 6 shows the EC50 frequency distribution for all isolates tested showing, as for Flint®, a trend towards decreased sensitivity.

Sensitivity curves by county sampled were similarly structured despite numerical differences in the number of isolates collected for each, indicating resistance is developing similarly in each location. Figures 7 and 8 are EC50 frequency distributions based on all isolates per county for Flint® and Sovran®, respectively. The shift in resistance in Lake County isolates indicates an accelerated rate of selection towards resistance to the strobilurin fungicides. This is consistent with Lake County having higher portions of the population resistant to the strobilurins, as noted above. These observations may be due to differential fungicide use between the counties, and

possibly increased disease pressure in Lake County. The observation will be further examined as fungicide use histories are collected for all locations.

Further testing is needed for the DMI fungicides. Using the current method, EC50 value averages by orchard are given. Figure 3 illustrates that for Rubigan®, seven orchards had average EC50 values significantly higher than the most sensitive orchard. Figure 4 shows average EC50 values by orchard for Procure®. Four of the orchards had significantly higher values as compared to the most sensitive orchard. These results for the DMI fungicides indicate differential sensitivity occurs in the *V. pirina* populations. These are preliminary results; due to the mode of action for DMI's, a different test is currently being used to confirm the findings. Further data will be released when that testing is complete.

Conclusions

Resistance to both strobilurin and DMI fungicides has developed in *V. pirina* populations in Mendocino and Lake Counties. In pathogen populations of agriculture crops, resistance develops due to fungicide use in response to pathogen pressure. As selection pressure increases, genetic shifts towards tolerance and resistance occur. In cases where sensitivity is controlled by one locus, resistance has been shown to develop quickly, as has happened with the DMI and strobilurin fungicides. When the trait is controlled by several loci resistance development can take much longer or does not develop at all. Thus products such as Captan® and Maneb® should be used in resistance management strategies. To minimize fungicide resistance in pathogens, FRAC guidelines should be followed. Label application rates should be strictly followed. Decreasing application rates allows greater survivability for pathogen phenotypes with some level of tolerance. Minimal applications per year also decrease selective pressure, thereby increasing the useful life of these products. Fungicide rotation of chemistry groups is very important; pathogen genotypes may have resistance to one or two fungicide chemistry groups, but not resistance to all chemistry groups. When possible, integrated pest management protocols and disease models should be followed. It is much more difficult and costly to develop fungicides with new modes of action than to avoid resistance development in the pathogen.

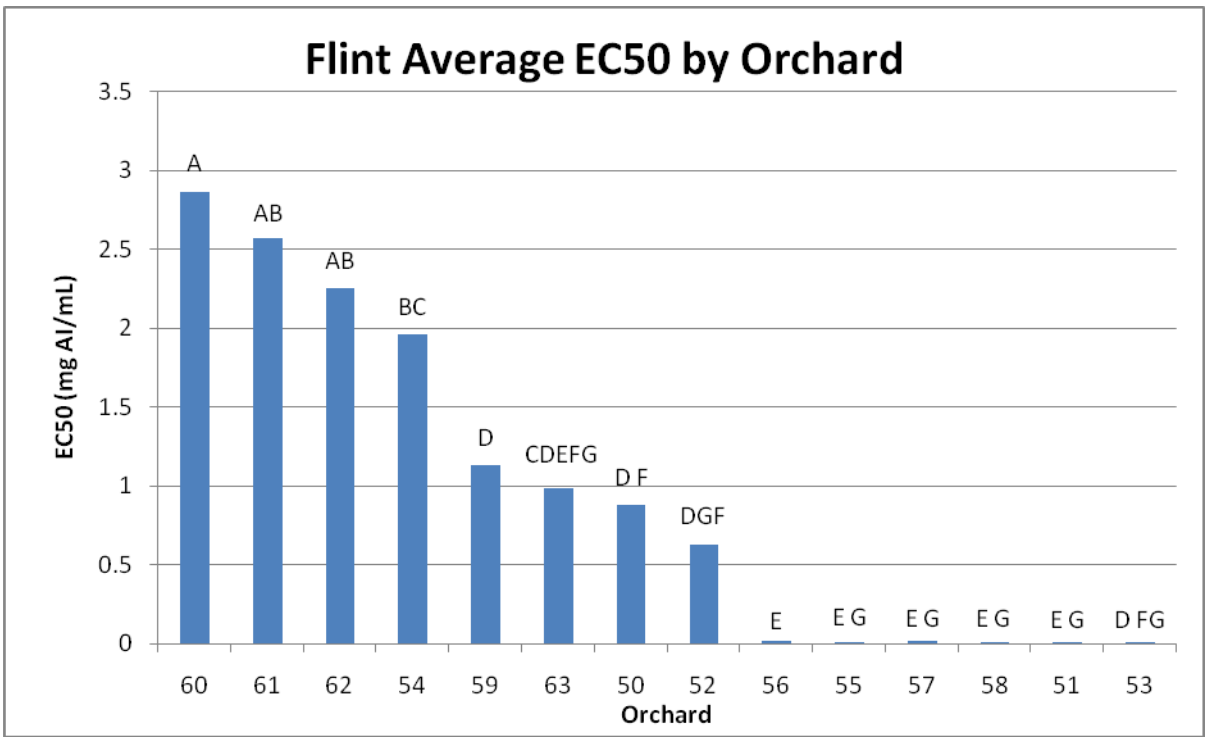

Fig.1 Average EC50 values for trifloxystrobin sensitivity (Tukey test, α=0.05). Isolates below the threshold value 0.032mg AI/mL are considered to be highly sensitive whereas those populations above 0.032 ppm are considered to be resistant to trifloxystrobin. Bar values followed by the same letter are not significantly different from one another.

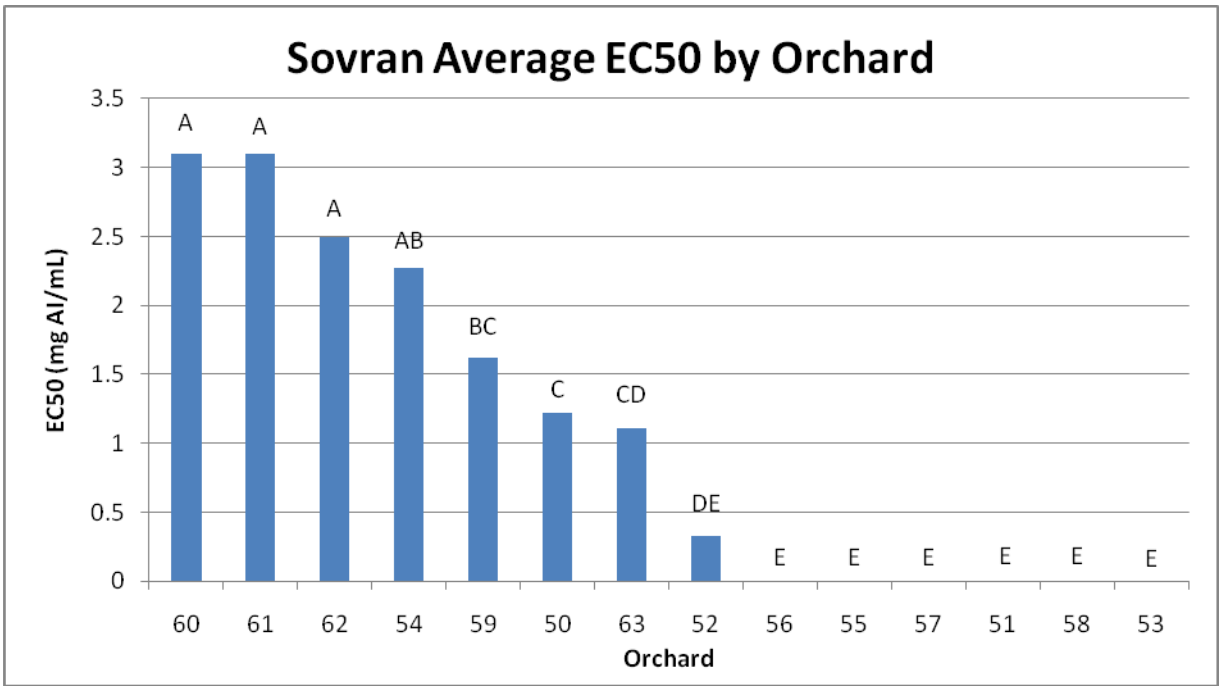

Fig.2 Average EC50 values for kresoxim-methyl for 2007 sampling. Bar values followed connected by the same letter are not significantly different based on the Tukey test (α=0.05). The baseline for sensitivity is 0.032 mg AI/mL.

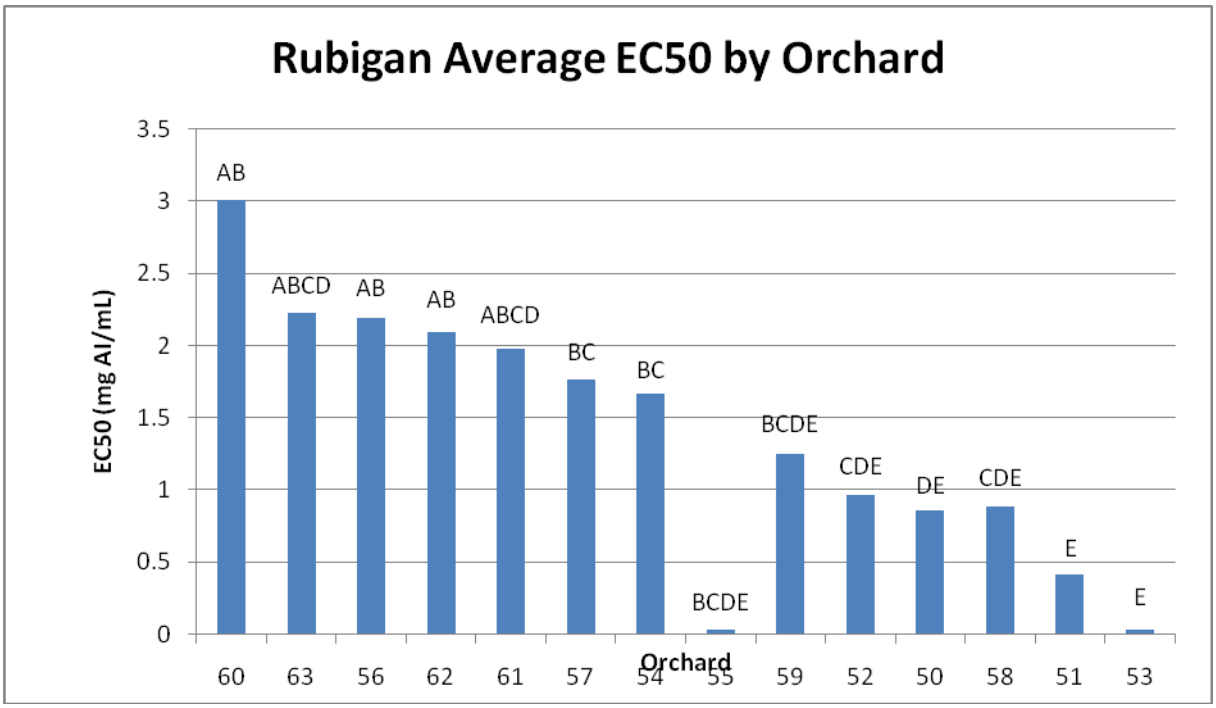

Fig.3 Average EC50 values for triflumizol for 2007 sampling. Bar values connected by the same letter are not significantly different based on the Tukey test (α=0.05).

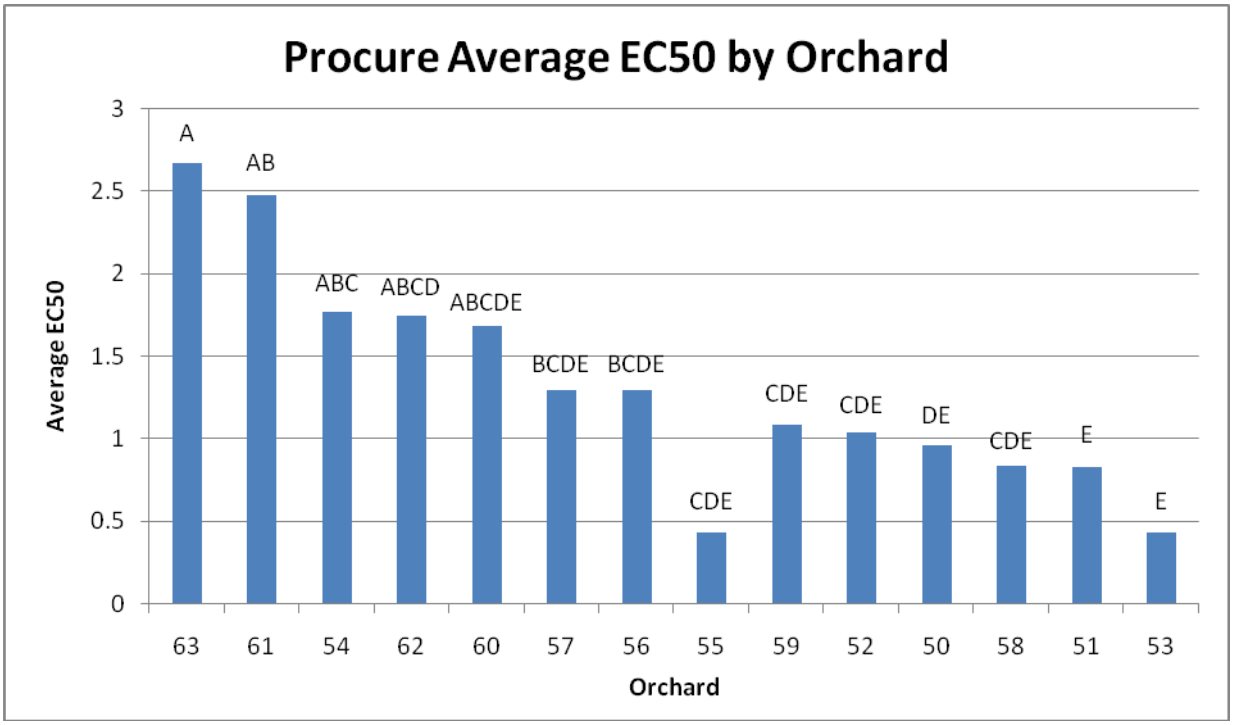

Fig.4 Average EC50 values for fenarimol. Bar values connected by the same letter are not significantly different based on the Tukey test (α=0.05).

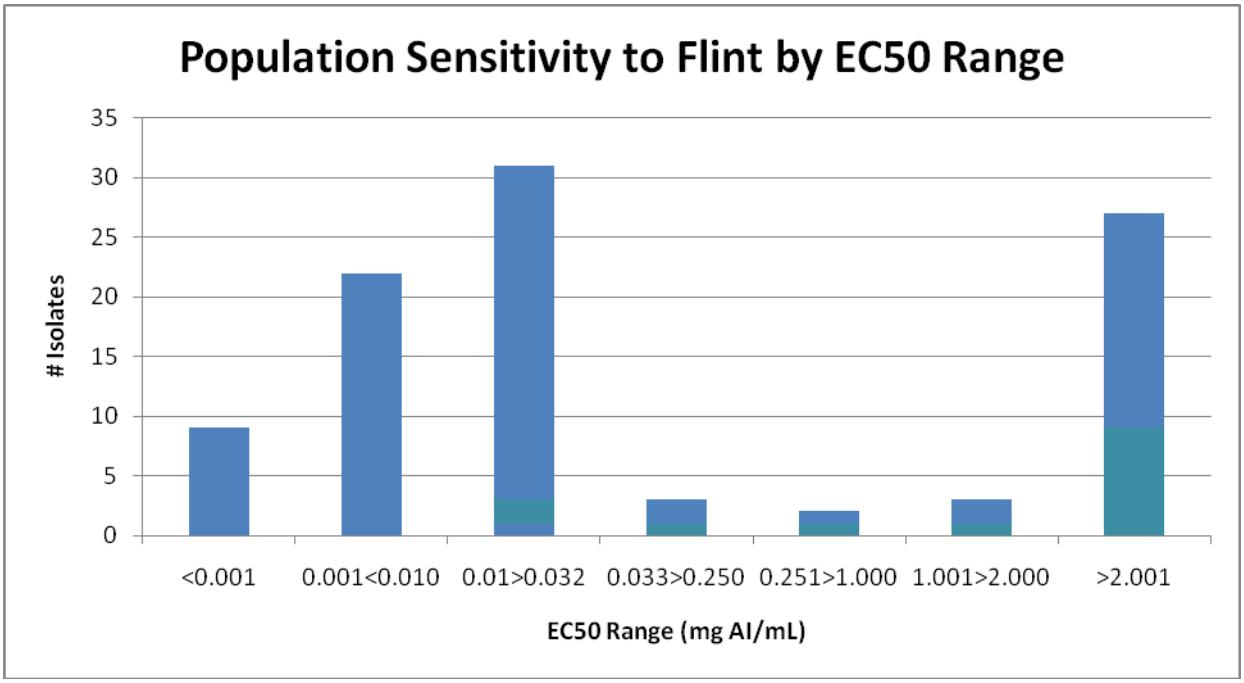

Fig. 5 The number of isolates in each EC50 category for all orchards sampled in 2007. All isolates above 0.032mg AI/mL are considered to be resistant to Flint®.

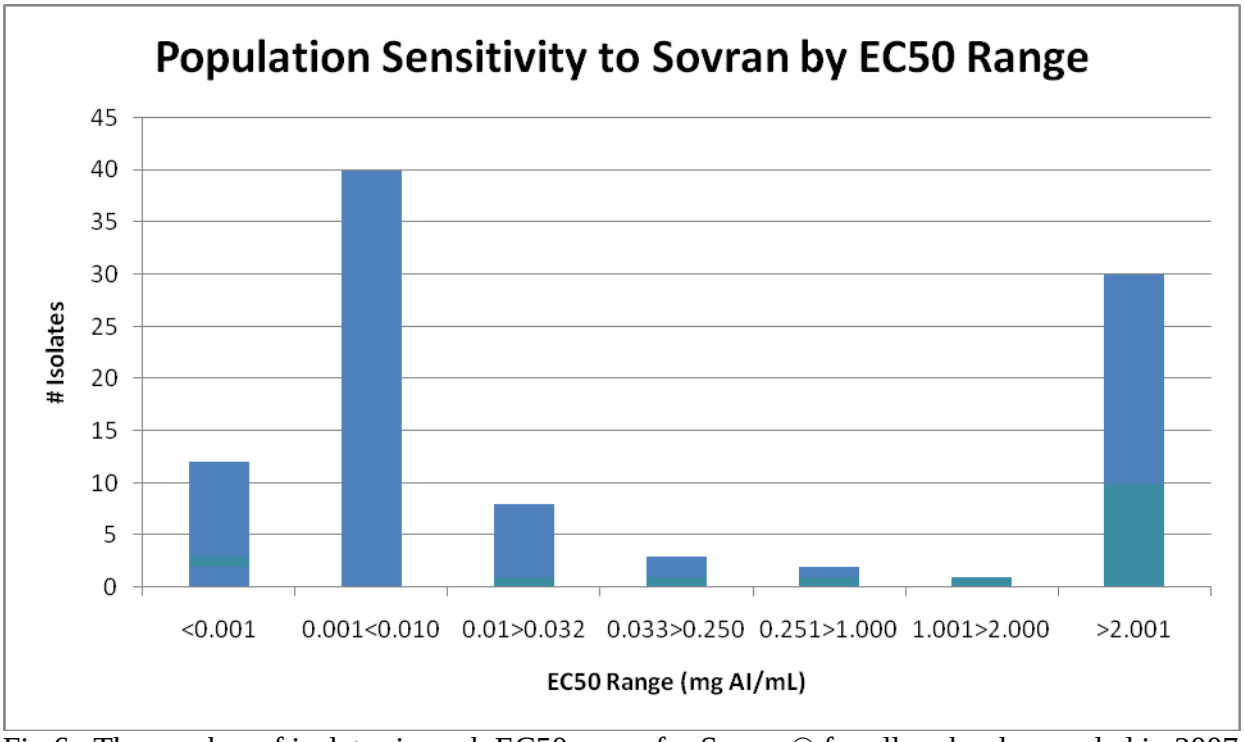

Fig.6 The number of isolates in each EC50 range for Sovran® for all orchards sampled in 2007.

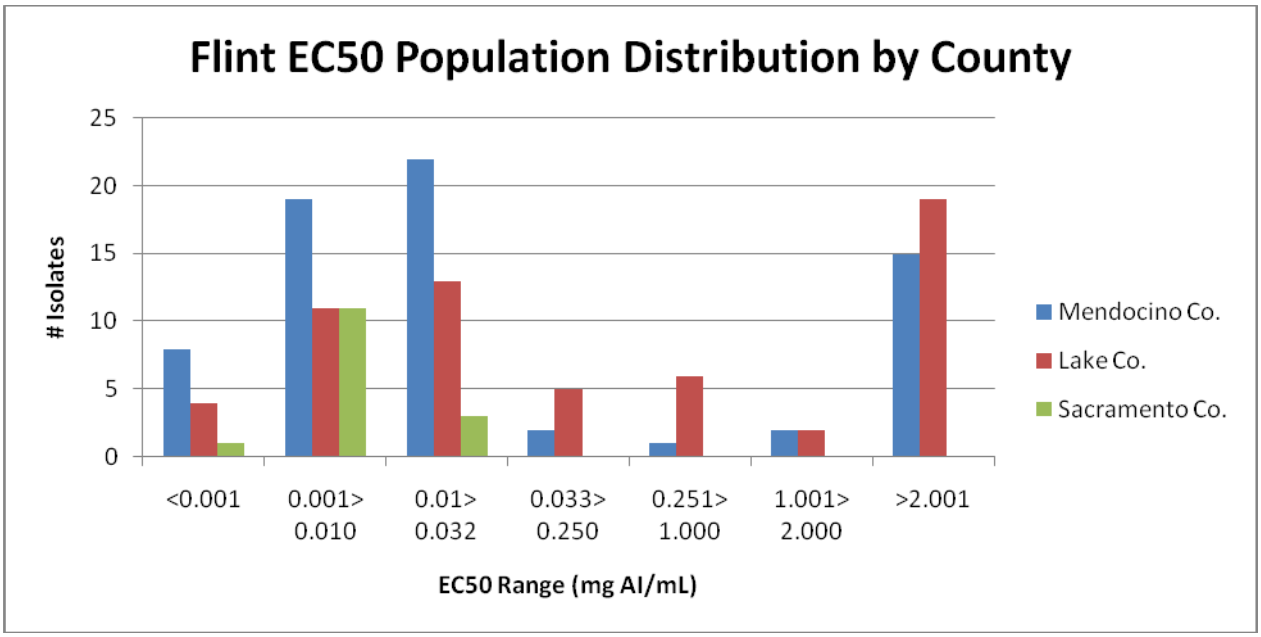

Fig. 7 Population distribution for Flint®. Bars represent EC50 values for isolates by county. The structure of the population curves is similar for Lake and Mendocino Counties whereas isolates collected from Sacramento Co exhibit no resistance.

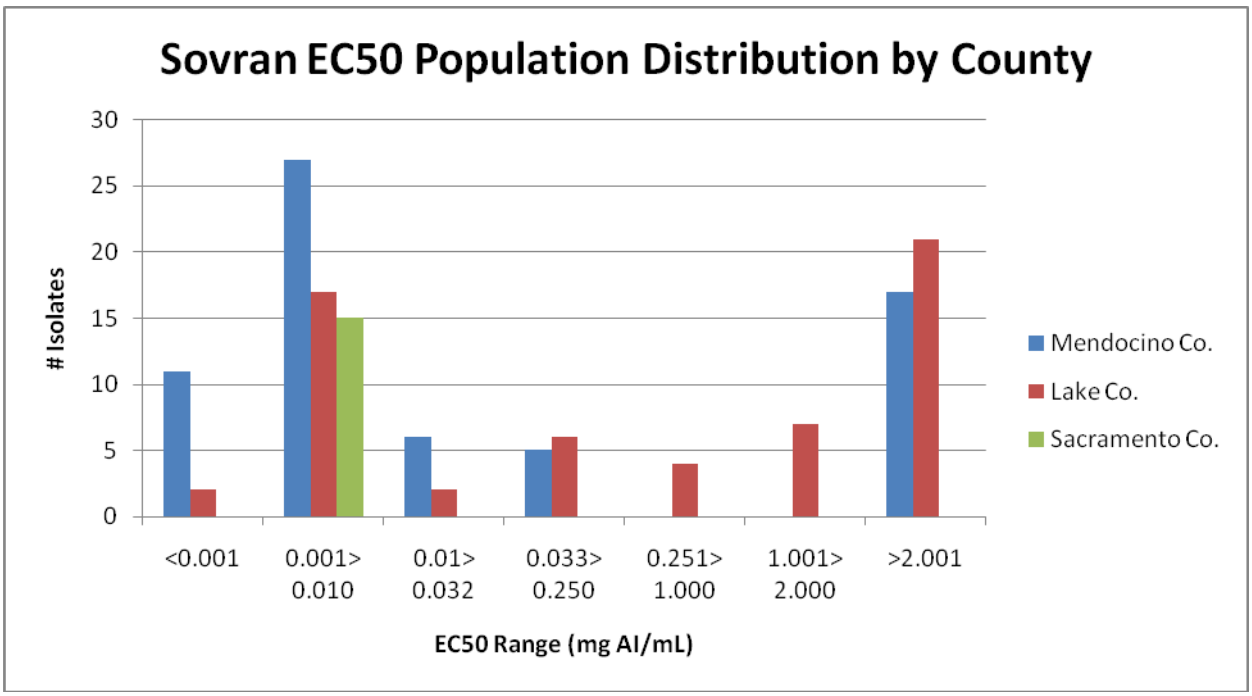

Fig. 8. Population distribution of Sovran®. Bars represent EC50 values for isolates by county. The structure of the population curves is similar between Lake and Mendocino Counties and no resistance was observed in Sacramento Co.