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 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 possible resistance or inactivity to several other fungicides including, Manzate, Luna Experience, Phyton 27 Ag Aide and Topguard. The most effective fungicides tested were Companion, Serenade Max, Syllit and Fontelils.

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*. While *Eutypa lata* had been previously reported from pears in California, this was the first instance of any *Botryosphaeria* species on pear in California. Pathogenicity tests were conducted at UC Davis Armstrong farm using fungal mycelium from the *Botryosphaeria dothidea, Botryosphaeria iberica, Sphaeropsis sapinae, Cryptosporopsis species and Botryosphaeria obtusa* isolates previously identified. These species were successfully re-isolated from the infected wood but only caused small, slow growing lesions. Spiral gradient endpoint tests indicate several fungicides are effective against the canker causing pathogens identified.

OBJECTIVE 1: Fungicide Resistance: Determine types of fungicide resistance in *Venturia pirina* population.

PROCEDURE

Isolates of *Venturia pirina* were collected by PCA's and growers in 2012. These isolates were sub-cultured onto PDA-tet and tested for resistance to different fungicides. The Automated Spiral Plater, Autoplate 400, was used to conduct the Spiral Gradient Endpoint test which measured suceptibility of spore germination to a gradient of fungicides on an agar plate. A solution of 50 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. EC50 is determined by the point on the plate where the fungal growth is inhibited by 50% by the fungicide. Twenty two isolates collected from eleven different sites were tested against the following fungicides (active ingredients are listed in parentheses): Actinivate (*Streptomyces lydicus* WYEC108), Companion (*Bacillus subtilis* BG03), Flint (trifloxystrobin), Fontellis (penthiopyrad), Luna Experience (fluopyram), Manzate (zinc, manganese and ethylenebisdithiocarbamate), Phyton 27 Ag (copper sulphate pentahydrate), Procure (triflumizole), Serenade Max (*Bacillus subtilis* QST713), Syllit (dodine), Topguard (fultriafol), Vintage (fenarimol) and Yucca Ag Aide (steroid saponins). Three replications per isolate were conducted.

RESULTS

Lower EC values relate to better fungicide efficacy. As shown in Figure 1 the most effective fungicides were Companion, Serenade Max, Syllit and Fontelils. Yucca Ag Aide was not effective at inhibiting the growth of *Venturia pirina*. The variations between treatments are statistically significant ($F<.0001$).Pear scab resistance was seen to Flint,and fungicide inactivity or possible resistance was shown to Manzate, Luna Experience, Phyton 27 Ag and Topguard. Figures 2-6 show average EC values for each site for the fungicides with resistant strains.

Figure 1: Average EC values for *V. pirina* isolates against different fungicides using an automated sprial plater.

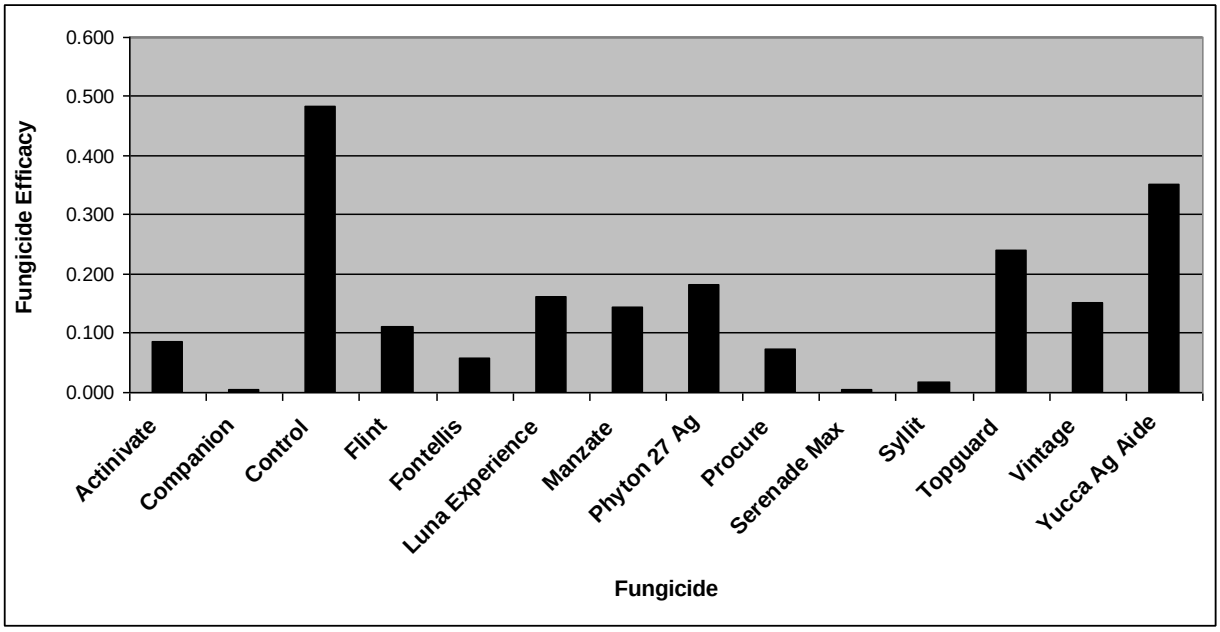


Figure 2: Average EC values for *V. pirina* isolates from different sites against Flint using an automated spiral plater.

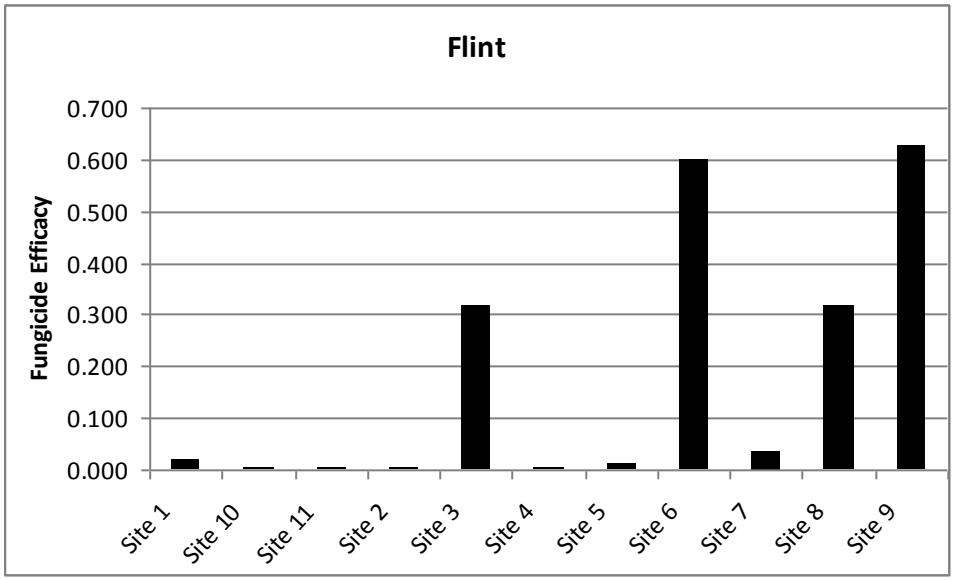


Figure 3: Average EC values for *V. pirina* isolates from different sites against Luna Experience using an automated spiral plater.

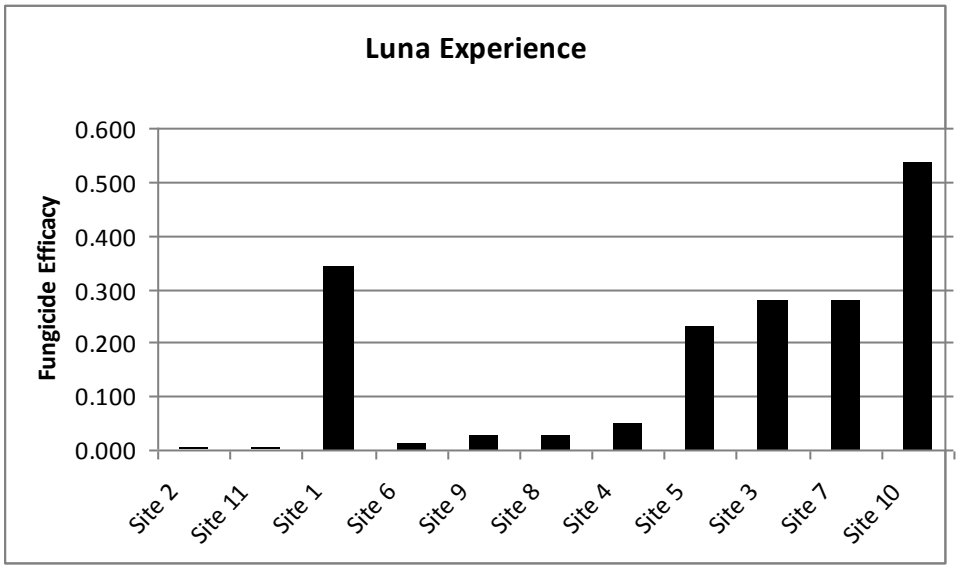


Figure 4: Average EC values for *V. pirina* isolates from different sites against Manzate using an automated spiral plater.

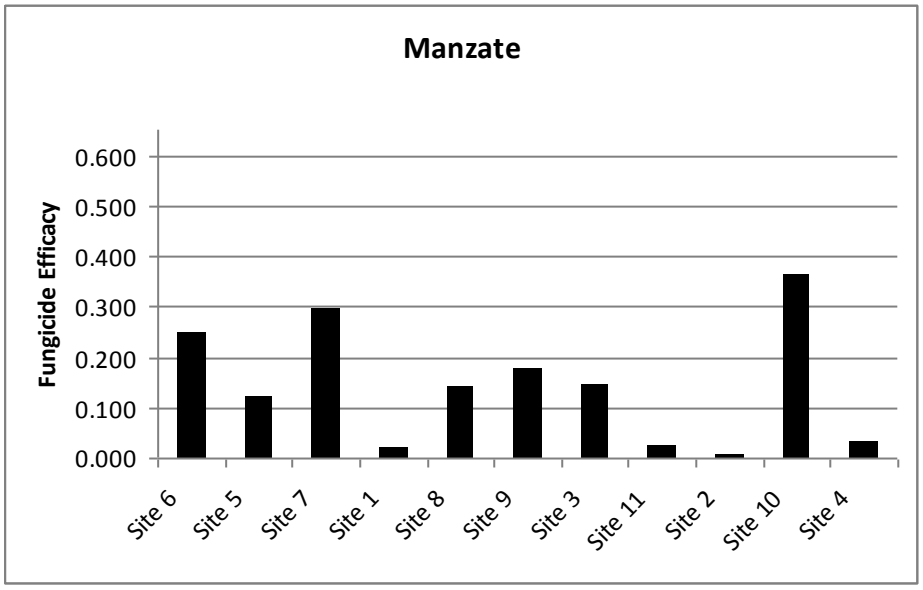


Figure 5: Average EC values for *V. pirina* isolates from different sites against Phyton 27 Ag using an automated spiral plater.

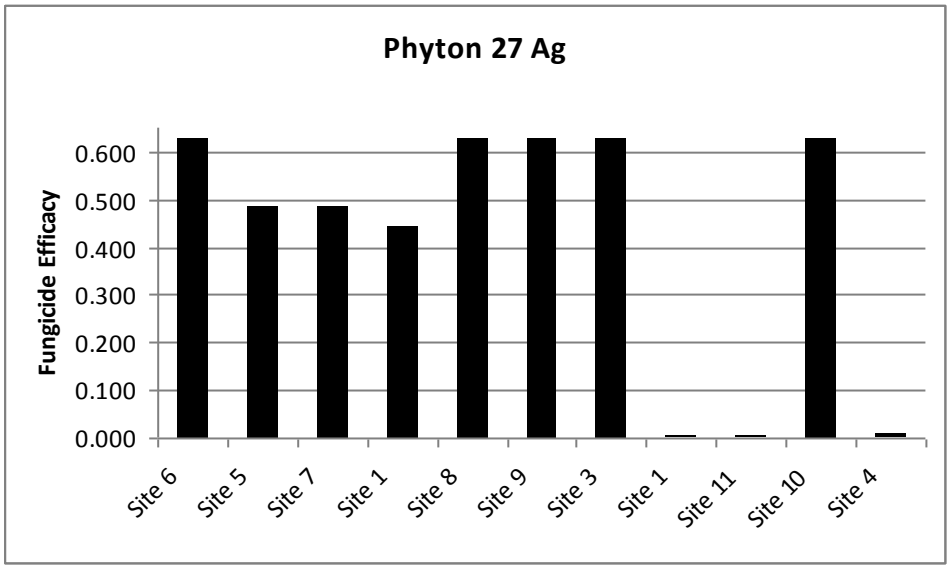
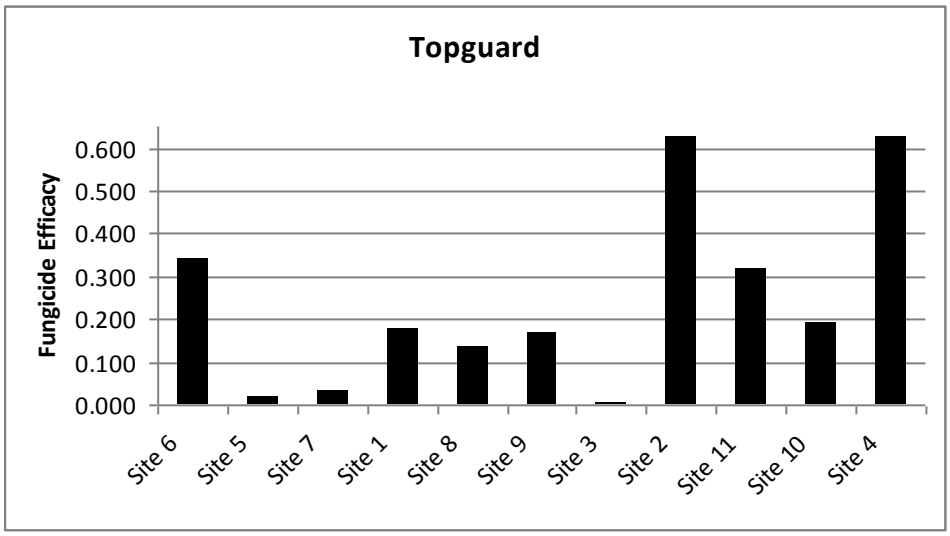


Figure 6: Average EC values for *V. pirina* isolates from different sites against Topguard using an automated spiral plater.



OBJECTIVE 1. Pear Canker: Determine the geographical distribution and incidence of *Botryosphaeria* spp. and *Eutypa lata* in pear cankers in Northern California.

PROCEDURE

Pear orchards in Mendocino county were visited and fungi were isolated from branches showing dieback and disease symptoms. Fungi were isolated from the cankers 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 also performed to confirm the morphological identification.

Spore trapping studies were conducted in different orchards in Mendocino County. Spores were trapped using microscope slides coated with Vaseline (Unilever, London, UK) placed randomly on pear branches. Spore traps were collected and changed weekly following the protocol described by Urbez Torres et al., 2010. The microscope slides were placed in 50ml screw top tubes. 10ml of sterile, distilled water was added. The tube was shaken vigorously for 60 seconds. Then a 200 microliter aliquot was spread on a PDA-tet Petri dish. A second 200 micro liter aliquot was spread on a second PDA-tet Petri dish. The different species of fungi that grew on the plates were subcultured and morphological and molecular techniques were used to identify the fungi. The different species of fungi collected were identified.

RESULTS

A survey of wood canker diseases in Lake and Mendocino County pear orchards was completed in 2010. The survey revealed the presence of several different pathogenic fungi causing cankers on stems and branches of Bartlett pears. Canker symptoms were variable based on the infectious agent. V-shaped cankers were observed on branches at several locations. Isolation and molecular identification from these cankers revealed the presence of several pathogenic fungi species, including *Botryosphaeria obtusa*, *B. dothidea*, *B. iberica*, and *Eutypa lata*. While *Eutypa lata* had been previously reported from pears in California, this was the first instance of any *Botryosphaeria* species on pear in California. *B. dothidea* was recently implicated as the causal agent of apple ring rot in China (Tang et al., 2012). Based on phylogenetic trees constructed using the ITS and beta tubulin genes, the *B. dothidea* found in Californian pear orchards was not closely related to the isolates found on apple in China. The *B. dothidea* on Californian pears seemed to be more closely related to isolates found on grapevines and coast live oaks in California.

In addition to the V-shaped cankers caused by *Botryosphaeria* and *Eutypa* spp., branches from one location had more general sunken lesions. Isolation from these lesions revealed the presence of *Leucostoma persoonii*. A limited amount of small twig

dieback was also noted in one orchard, and molecular identification suggests that *Potebniamyces pyri* was the causal agent. This fungus causes Phacidiopycnis rot, a postharvest disease on pears. It also causes cankers and twig dieback in pear trees (Xiao and Boal, 2005). A root and trunk decline was noted in one orchard; isolation and molecular identification suggest that the causal agent was a *Cryptosporiopsis* species. A *Bionectria* species was also isolated from diseased tissue. In 2012, isolations from canker lesions in Mendocino County revealed the presence of *Botryosphaeria obtusa, Cytospora austromontana, Phaeoacromonium* spp.*, Sphaeropsisi sapina,* and *Diplodia seriata.*

Spore trap studies to date have isolated the following pathogens: *Sphaeropsis sapinea, Eutypa lata, Phaeoacromonium* species, *Stereum* species, *Botryosphaeria obtusa* and *Trametes versicolor*.

OBJECTIVE 2. Identification and characterization of *Botryosphaeria* spp. associated with pear dieback in California.

PROCEDURE

Pear wood showing dieback symptoms will be collected from orchards located in Mendocino County. Pieces of wood showing necroses were isolated onto potato dextrose agar (PDA) medium amended with tetracycline. Fungal cultures were identified using both morphological characters such as colony and conidial morphology, size, shape, color, septation and wall thickness of conidial spores and using specific identifying key (Phillips 2002). Furthermore, molecular analyses were conducted to confirm the identification of the various fungal isolates. Fungal colonies were grown for 10 days at room temperature under ambient light conditions in PDA Petri plates for DNA extraction. Fungal DNA was extracted from pure cultures with the DNeasy Plant Mini kit (Quiagen Inc., Chatsworth, CA) following the manufacturer's instructions. PCR amplification was conducted using the 5.8S nuclear ribosomal DNA region (ITS1 and ITS2) using primers ITS4 and ITS5 (White et al. 1995). Partial sequence of the β-tubulin (BT) gene was amplified using primers Bt2 and Bt2B. Amplification products were purified using the QIA quick PCR purification kit. ITS and β-tubulin regions were sequenced by the University of California, Davis, Division of Biological Sciences (DBS) sequencing facility. Identification will be processed using the Blast search in GenBank.

RESULTS

Three *Botryosphaeria* species were associated with the pear decline in Northern California. Following isolation and subculturing, DNA from fungal cultures was collected, amplified and sequenced. Based on the ITS and beta tubulin genes, the causal agents of the cankers were *Botryosphaeria obtusa*, *B. dothidea*, and *B. iberica*. *B. obtusa* was the most prevalent of the three, and *B. iberica* was only isolated from one site. Fungal cultures of the three species were grown on PDA amended with sterile pine needles. After 21 days, spores from pycnidia formed on the pine needles were examined

microscopically and compared to type descriptions of the three species. The pycnidial spores from culture confirmed the identities of the fungi.

OBJECTIVE 3. Determine the pathogenicity and symptoms of *Botryosphaeria* and *Eutypa lata* associated with pear canker disease.

PROCEDURE

The pathogenicity and virulence of ten of the isolated fungi were tested in live Bartlett pear plants planted in Davis, CA. Two trials were conducted; the first trial lasted 12 months (March 2011-March 2012) and the second trial lasted five months (June 2012-November 2012). Following the protocol established for pathogenicity studies for Botryosphaeriacieae species isolated from grapevine canker (Urbez-Torres and Gubler, 2009), branches of pear trees were pruned and inoculated using mycelium plugs and then covered with petroleum jelly and parafilm. Non-colonized PDA plugs were used as negative controls. Six months after inoculation wood samples were collected near the point of inoculations, symptoms were assessed and pathogens re-isolated to PDA medium. Vascular discoloration was measured. Typical mycelial growths were transferred to new PDA-tet in Petri dishes to obtain pure cultures. Fungi were identified by their colony size, shape, color and growth rate.

RESULTS

The results from the pathogenicity and virulence trials conducted at UC Davis are shown in Figure 7. *Botryosphaeria obtusa* and *B. dothidea* were able to elicit longer lesions than *B. iberica*. This closely matches the results of Urbez-Torres and Gubler (2009), which found that *B. dothidea* isolates inoculated onto grapevine created the longest lesions of the three, and *B. iberica* isolates created the shortest. While *Phaeoacremonium angustis* and *Potebniamyces pyri* were isolated from lesions in Californian orchards, they were not able to colonize and create lesions when re-inoculated into the pear orchard in Davis, CA.

Figure 7. Pathogenicity and virulence tests of fungi isolated from cankers.

		Trial 1		Trial 2	
Species	**Isolate #**	**Re-isolated**	**Avg Lesion Length***	**Re-isolated**	**Avg Lesion Length***
Bionectria sp.	UCDX106	YES	0.2	YES	0.2
Botryosphaeria dothidea	UCDX101	YES	2.1	YES	0.9
Botryosphaeria iberica	UCDX104	YES	0.2	YES	0.8
Botryosphaeria obtusa	UCDX102	YES	1.3	YES	0.2
Botryosphaeria obtusa	UCDX103	YES	1.7	YES	0.4
Botryosphaeria obtusa	UCDX105	YES	2.3	YES	0.5
Cryptosporiopsis sp.	UCDX107	YES	1	YES	0.5
Leucostoma persoonii	UCDX109	YES	1.5	YES	0.8
Phaeoacremonium angustis	UCDX121	NO	---	nt	---
Potebniamyces pyri	UCDX122	NO	---	nt	---
* Measured in cm					
nt = not tested					

OBJECTIVE 4. Develop and implement control methods against fungi involved in pear dieback and decline.

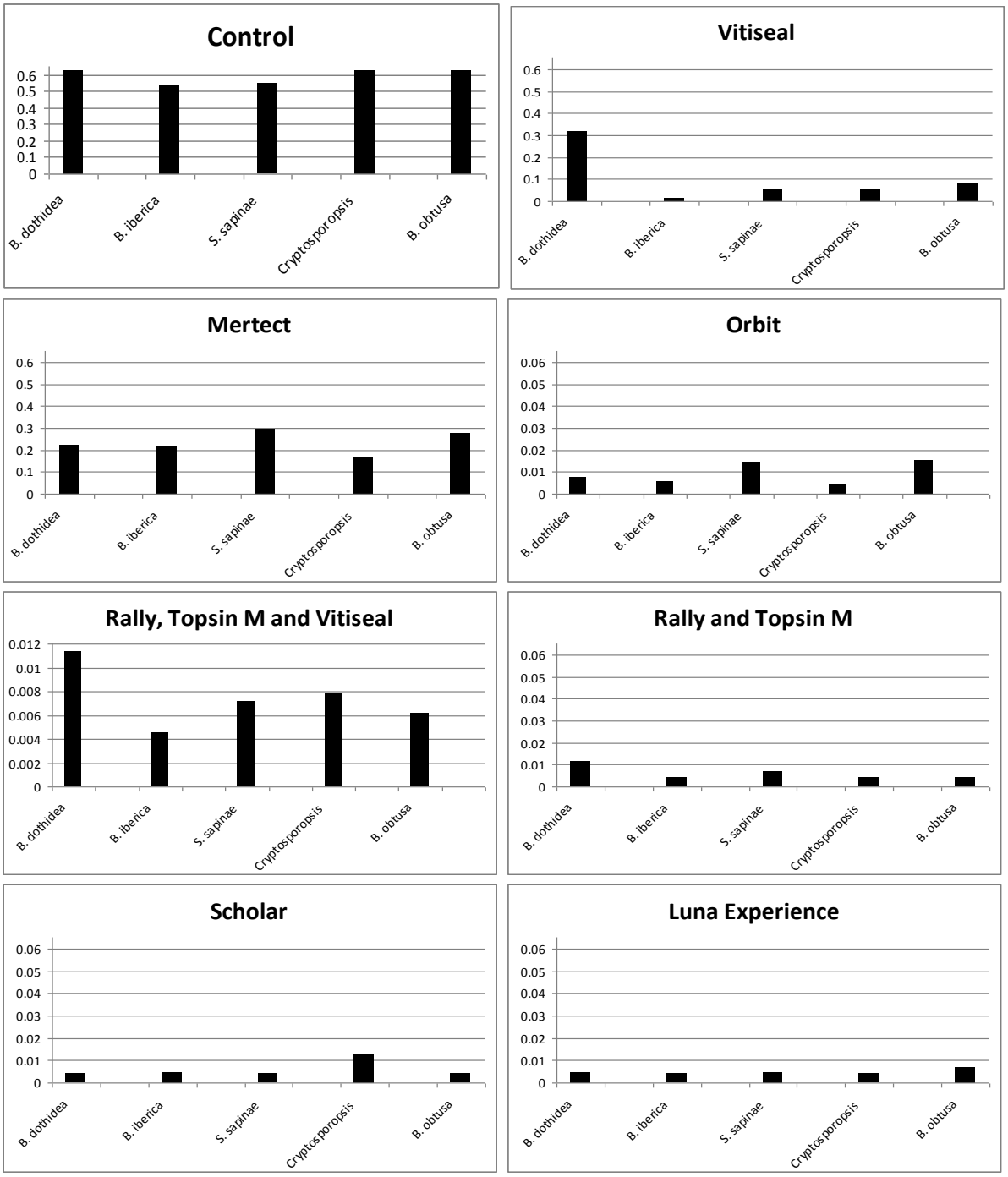
PROCEDURE

Pear canker isolates were subcultured onto PDA-tet and tested for sensitivity to different fungicides. The Automated Spiral Plater, Autoplate 400, was used to conduct the Spiral Gradient Endpoint test. A solution of 50 ppm of the fungicide was spiral plated onto a 150 mm PDA plate. The fungicides tested were Vitiseal (1:10 dilution), Mertect, Orbit, Rally+Topsin M+Vitiseal (1:10 dilution), Rally+Topsin M, Scholar, and Luna Experience. Then the plates were radially streaked with a conidial suspension of *Botryosphaeria dothidea, Botryosphaeria iberica, Sphaeropsis sapinae, Cryptosporopsis species, and Botryosphaeria obtusa* isolates previously identified. 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.

RESULTS

Spiral gradient endpoint tests show several fungicides are effective against the canker causing pathogens identified (Figure 8). The variations between treatments are statistically significant (F<.0001).

Figure 8. Average EC values for fungal pathogens against different fungicides using an automated sprial plater.



DISCUSSION

The most effective fungicides against *Venturia pirina* were Syllit, Fontellis, Companion and Serenade Max. Additional isolates will be collected and tested to obtain a larger data set to further research fungicide efficacy.

The pear canker disease complex includes several *Botryosphaeria* species as well as other pathogens. This is the first observation of *L*. *persoonii*, *P*. *pyri, Bionectria* and *Cryptosporiopsis* species on pear in California. The limited occurrence of these species could indicate they play a more limited role in recent decline compared to the more prevalent *Botryosphaeria* and *Eutypa* species. While this is the first observation of these pathogens on pears in California, they may not be recent introductions to the state. *B. obtusa*, *B. dothidea*, *E. lata*, *L. persoonii*, *P. pyri*, and a *Cryptosporiopsis* species have all been noted from pear in the Pacific Northwest. It is possible that some of these pathogens have been present in Californian pear orchards for some time but have not caused major disease or crop loss. However, the prevalence of *Botryosphaeria* and *Eutypa* species in pear orchards and the consistent association with cankers suggest these species are playing a role in the recent pear decline. Using a spiral plater, initial trials indicate several fungicides are effective against these pathogens. Further research is being conducted to determine fungicide efficacy. A field trial and in vitro bottle trial are currently being conducted.

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