Identifying Sources of Resistance in Pistachio and Almond (resistance of new and commercial cultivars)

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Introductory statement:

"We as researchers expect that there are sources of resistance among pistachio and almond cultivars that are attached by a number of canker fungi in the Botryosphaeriaceae family. We performed experiments to identify these sources of resistance"

1. Experiments in 2015-2017:

ALMOND: Cultivars in pots.

PISTACHIO:

A) Cultivars in the Kearney field: **Cvs.** Kerman, Kalehghouchi, Lost Hills, Golden Hills, Aria, Peters, Randy, & Pete-1

B) Cultivars in potted trees: **Cvs.** Kerman, Kalehghouchi, Lost Hills, Golden Hills, Aria, Peters, Randy, Red Aleppo, and Joley
Wound –canker pathogens: *Botryosphaeria dothidea, Lasiod. citricola, & Neofusicoccum mediterraneum, L. gilanensis*

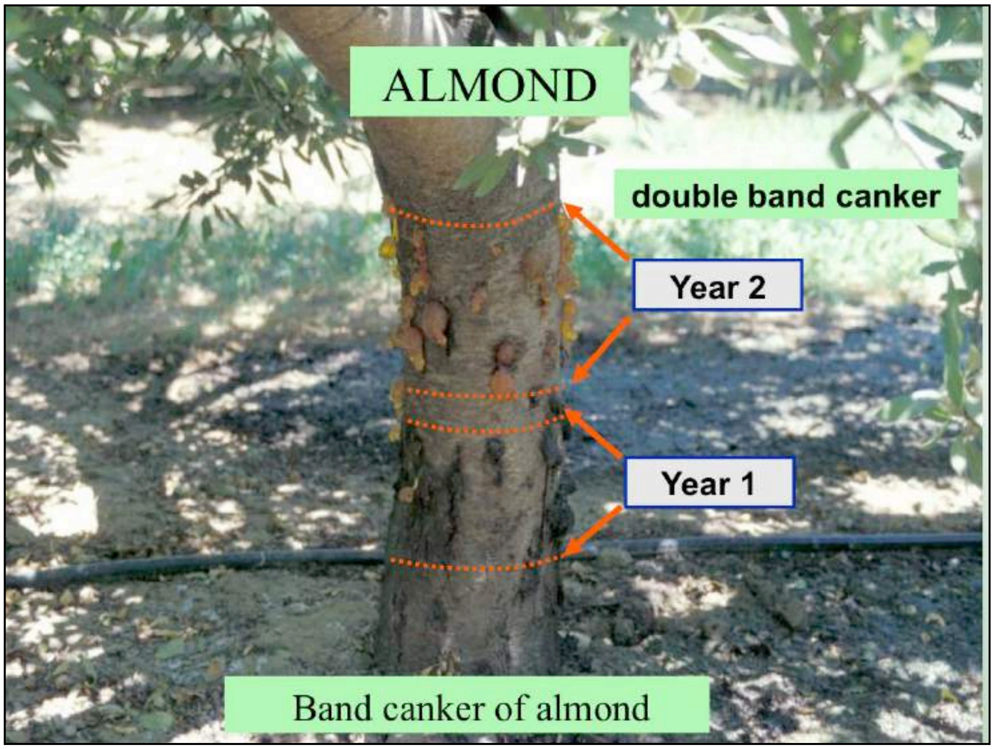
2. Determine differences in latent infection and whether they correlate with disease resistance among cultivars.

A) Use cultured-based method (ONFIT)

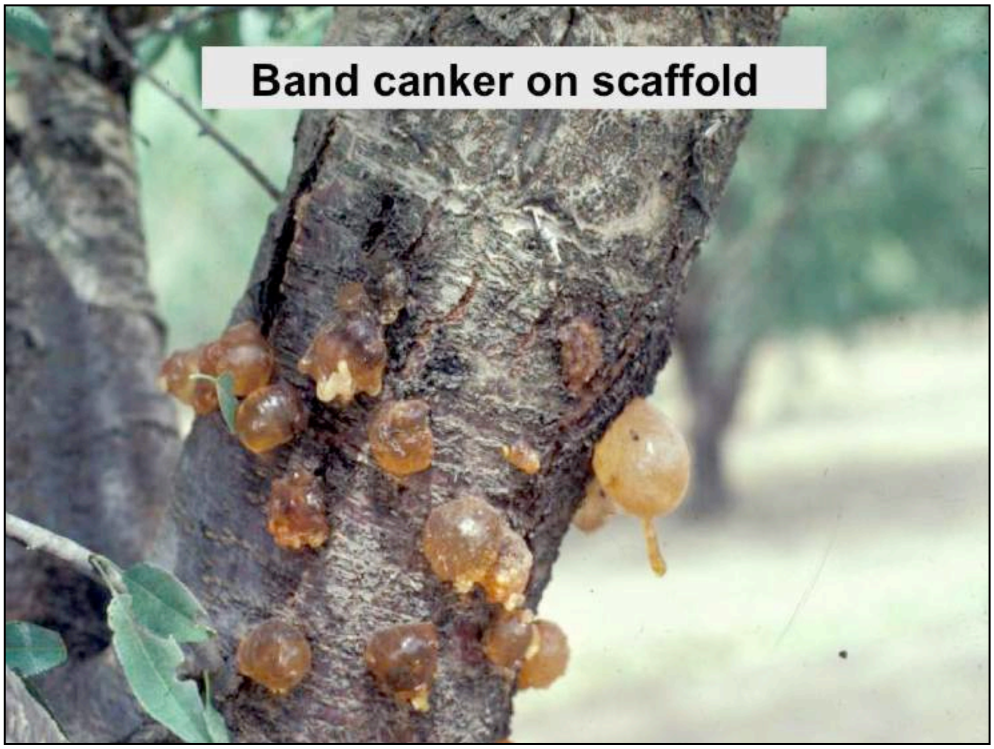
B) Use pathogen –specific primers for *in planta* (fruit) detection of latent infections by the pathogens (EARLY DETECTION OF INFECTION).

3. Build on the germplasm surveys (evaluate the Wolfskill *Pistacia* germplasm).

This is a presentation abut some of the progress we've made on the disease-resistance cultivars and germplasm accessions. For this purpose, we used various pistachio cultivars in pots and in the cultivar research orchard located at the Kearney Agric. Research and Extension Center. For checking resistance in almond cultivars, we used 10 different cultivars only in pots. The cankers pathogens used in these studies were *Botryosphaeria dothidea, Lasiodiplodia citricola,* and *Neofusicoccum mediterraneum.* In addition to inoculations after wounding of tissues, we also performed inoculations without wounding and for these we wanted to determine whether there were differences in latent infection and whether the latent infections correlate with disease resistance among the various cultivars. We used a cultured-based (conventional) method, which involves the freezing of the inoculated tissues and incubation to allow the fungal infection to develop and determine any differences in disease expression. The second method involves the detection of these latent infections using specific primers. Furthermore, although we planned to evaluate the *Pistacia* germplasm available at the Wolf skill Experimental Field in Winters, California.



We are presenting a short introduction of each disease as it occurs in almond and pistachio because they are very different in symptomatology, although the causes can be the same fungi attacking both these hosts. For instance, in almonds the disease is called “band canker” since the infections occur in the trunk or the basal part of scaffolds or around major pruning wounds of young trees. Cankers on the trunk and/or the scaffolds can form a kind of a band and they can kill either the entire tree or portions of the trees. The canker are characterized by profuse amber colored sap. Cankers develop yearly and sometimes a double bank can be found in trees as shown in this photo. Reduction in yields is due mainly to the fact that these cankers kill entire trees or major scaffolds of trees. In addition, additional expenses are associated with the removal of killed trees and parts of trees, replanting costs, and a period of at least 4 years until the newly planted trees will be back into production.

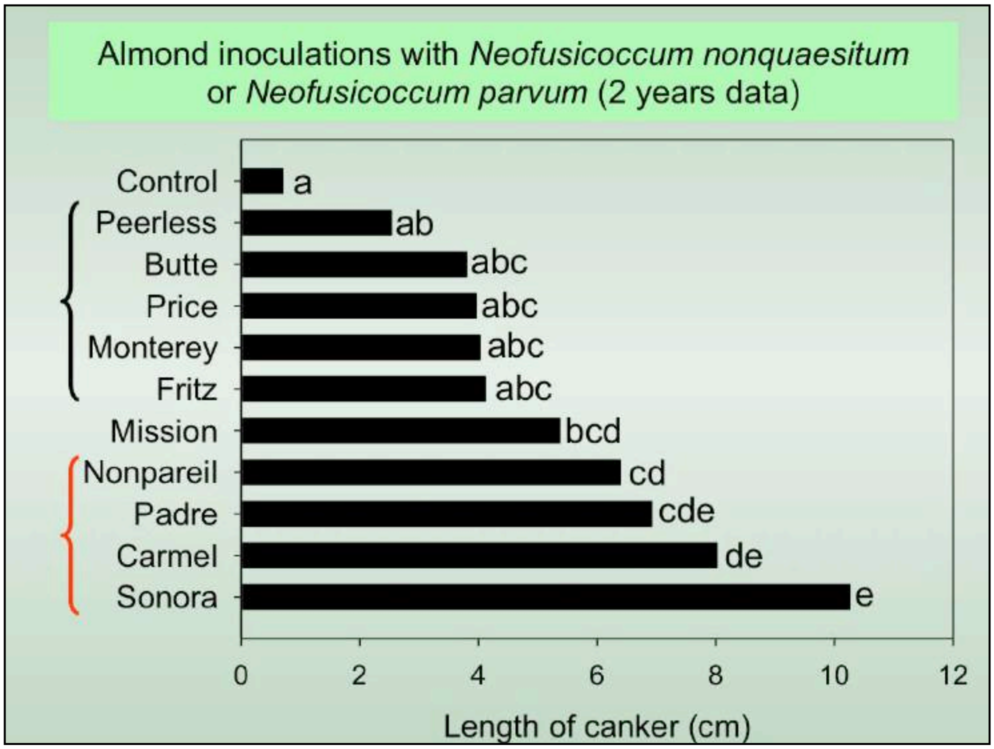


A close up of band canker on the scaffold of a Nonpareil tree showing the typical production of sap.

Another example of a young tree showing infection of a pruning wound made on the trunk by the grower to develop the primary scaffolds of the tree.

These are examples of the band canker disease with typical cankers surrounding pruning wounds of a 7 –year Padre almond tree in Butte County, California. Again, notice the profuse production of sap, an indication that the hosts “fights” the fungal infection.

Two pathogens were used in inoculations of potted almond trees to compare susceptibility of 10 different almond cultivars. As you can see infections are very distinct and evaluations are done by measuring the vertical length of the cankers.



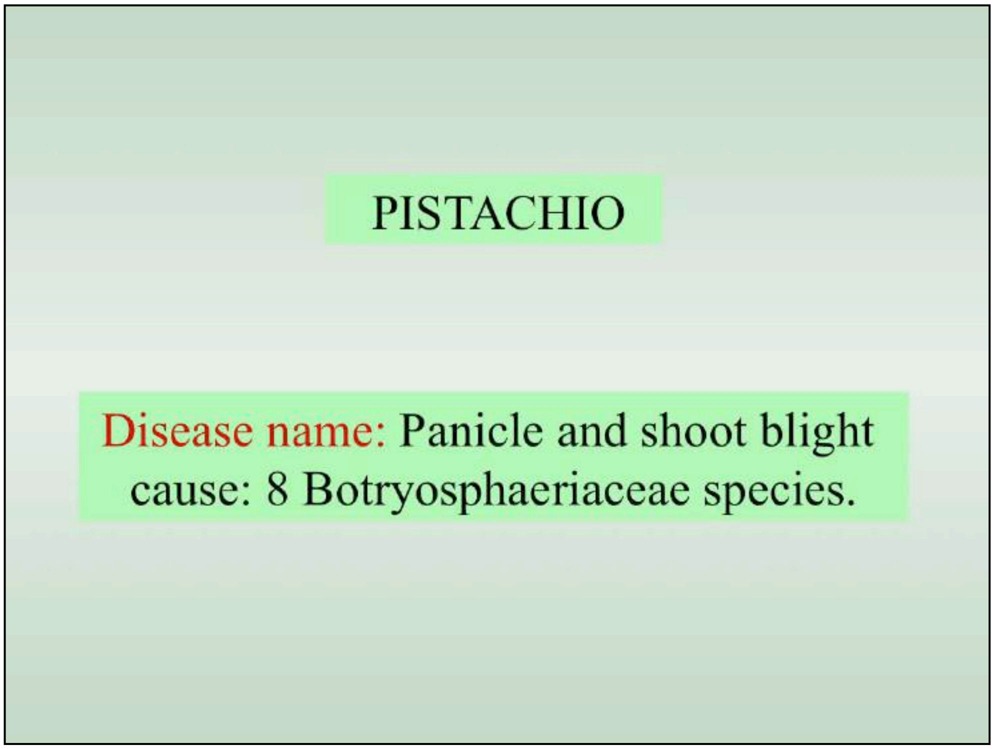
These are the results of 2012 and 2013 inoculations (mycelial plug inoculation method) experiments. Because similar trends were found for each of the two fungal pathogens used in these inoculations, the data were combined for *Neofusicoccum nonquaesitum* and *N. parvum* used in these experiments. These two fungal pathogens represent two aggressive species of Botryosphaeriaceae isolated from band cankers of almond. Although there is some variation among these inoculations, it is apparent that five cultivars (Mission, Nonpareil, Padre, Carmel, and Sonora) seem to be more susceptible to these pathogens than the other group of cultivars (Peerless, Butte, Price, Monterey, and Fritz). Interestingly, under commercial plantings, banker disease has been problematic more in Nonpareil, Padre, and Carmel cultivars than other commercial cultivars.

Almond varieties to be inoculated in 2016

Avalon	Padre
Butte	Peerless
Carmel	Price
Fritz	Sonora
Marcona	Supareil
Mission	Winters
Monterey	Wood Colony
Nonpareil	

In 2015, we were able to secure and obtain more cultivars as shown here with red in addition to the commercially susceptible cultivars (Nonpareil, Padre, and Carmel) and some others used in previous inoculation experiments.

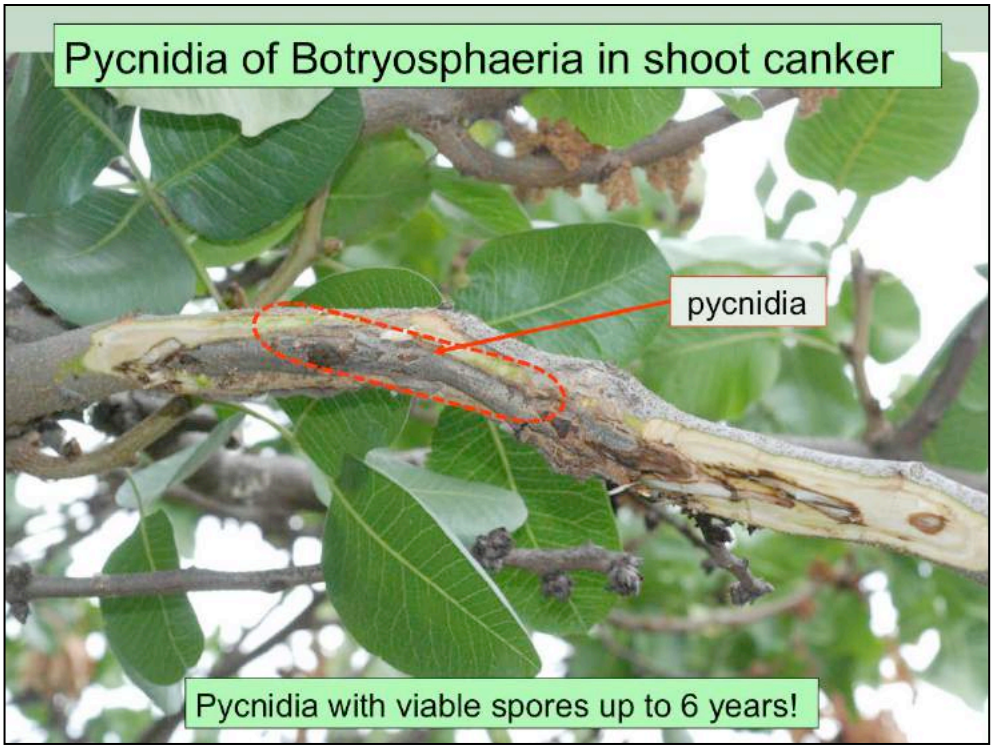
These trees were of a very small caliber in 2015. They were potted in larger pots and they should be ready to be inoculated in 2016.



Again, we present a short introduction to the Botryosphaeria disease on pistachio.

In contrast to the band canker disease of almond, in pistachio, the disease is called Botryosphaeria panicle and shoot blight because in addition to cankers also causes a very devastating phase, the blighting of the fruit clusters. Eight species of the Botryosphaeriaceae were identified as causes of this blight and canker disease. (8 species were identified) prod cause a different

The Botryosphaeria panicle and shoot blight (referred by the growers as "Bot blight" or "Bot disease") is direct yield reducing disease killing the fruit clusters. It was discovered in 1984, following an El Niño yield in 1982-83. The photo shows a total destruction of the clusters and foliage in a commercial orchard in Tehama County, California, following a second El Niño in 1997-1998. The inset shows a close-up of pistachio clusters killed by the disease.



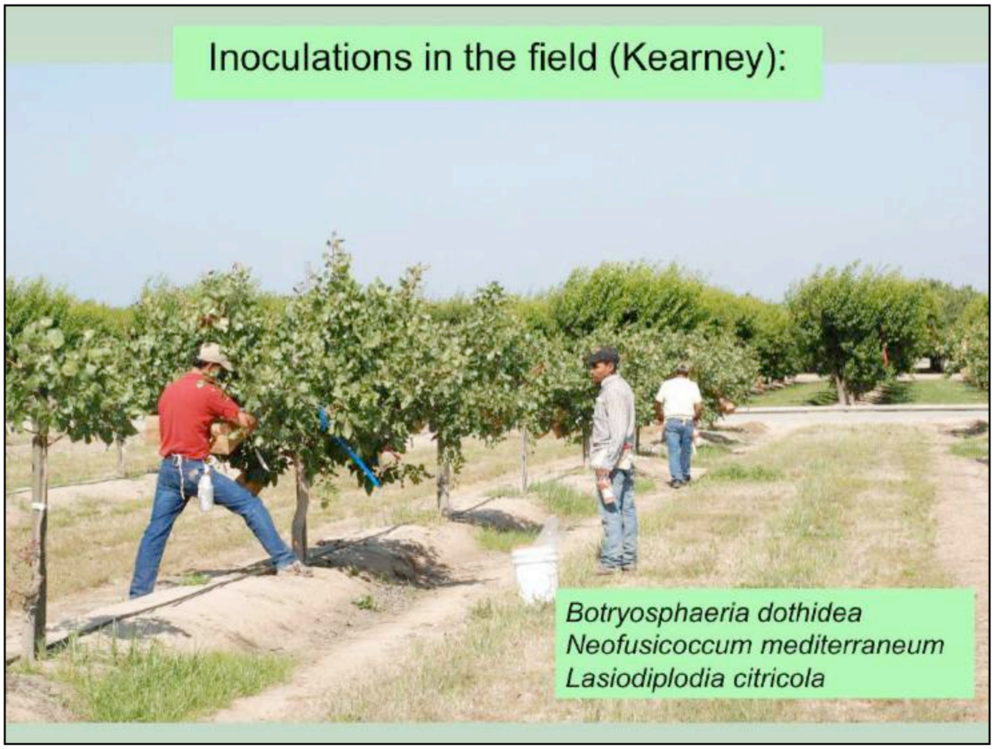
In addition to fruit clusters, flowers of male trees can be infected by Botryosphaeriaceae. Infections from the flowers invade the shoot and develop cankers as the one shown in this photo. The pathogen will produce abundant pycnidia, particularly in the central portion of the cankers. As a note, similar cankers bearing pycnidia can develop in female trees from infections of fruit clusters that invade the shoots. These cankers can provide viable spores for up to 6 years; pseudothecia have not been found in any of the pistachio tissues examined so far over the last 30 or so years.

This is an aggressive disease and can attack any part of the pistachio as listed above. Notice leaf lesions with pycnidia in the center, a blighted shoot cut superficially to show the pycnidia, a partially- and a totally-blighted female bud, a blighted fruit with hulls bearing a dense layer of pycnidia, and a blighted rachis (main axis of the fruit cluster) with a superficial cut to show the pycnidia. Cankers in shoots, blighted rachises and shoots, and blighted buds serve as sources and provide spore inoculum. Although some cluster infection can be seen even in years of drought, bud killing and leaf lesions usually develop following a wet winter and rainy spring.

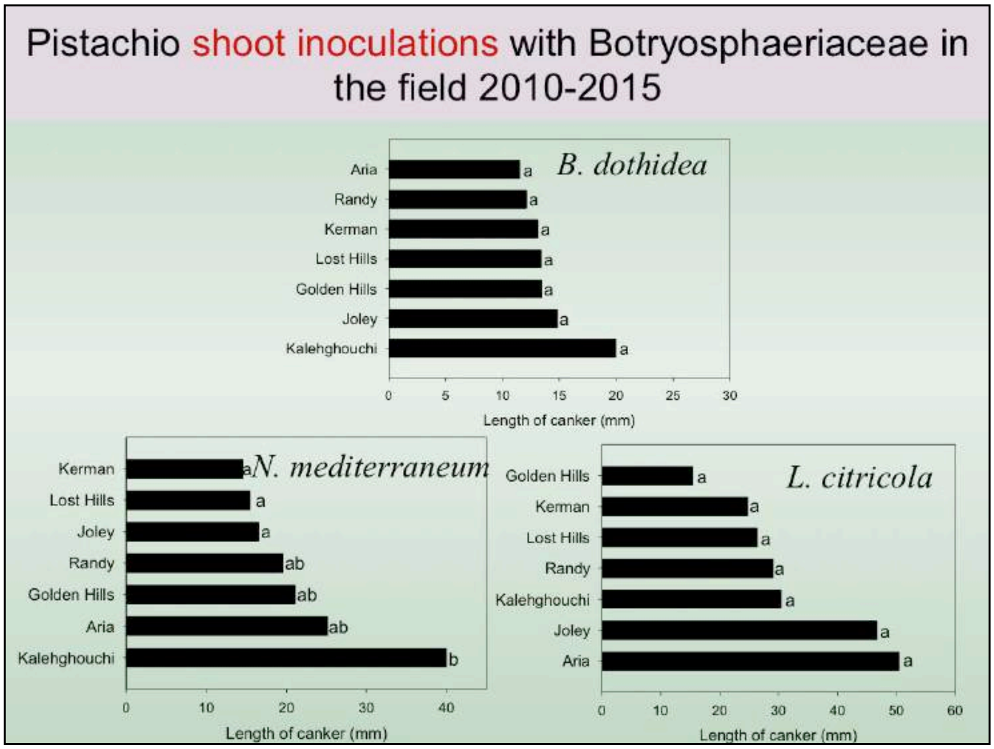
Map of pistachio cultivars at KAC

↑	Joley	Kalehghouchi	Kerman	Lost Hills
	UCB1	Kalehghouchi	Kerman	Lost Hills
Area	Joley	Kalehghouchi	Kerman	Lost Hills
to	Clonal	Kalehghouchi	Kerman	Lost Hills
plant	UCB1	Kalehghouchi	Kerman	Lost Hills
more	Clonal	Kalehghouchi	Kerman	Lost Hills
cultivars	Clonal	Kalehghouchi	Kerman	Lost Hills
↓	Joley	Kalehghouchi	Kerman	Lost Hills
Clonal	Pete 1	Aria	Randy	Golden Hills
Clonal	Pete 1	Aria	Randy	Golden Hills
Clonal	Pete 1	Aria	Randy	Golden Hills
Clonal	Pete 1	Aria	Randy	Golden Hills
Chico 1	Pete 1	Aria	Randy	Golden Hills
Clonal	Pete 1	Aria	Randy	Golden Hills
Clonal	Pete 1	Aria	Randy	Golden Hills
Clonal	Pete 1	Aria	Randy	Golden Hills

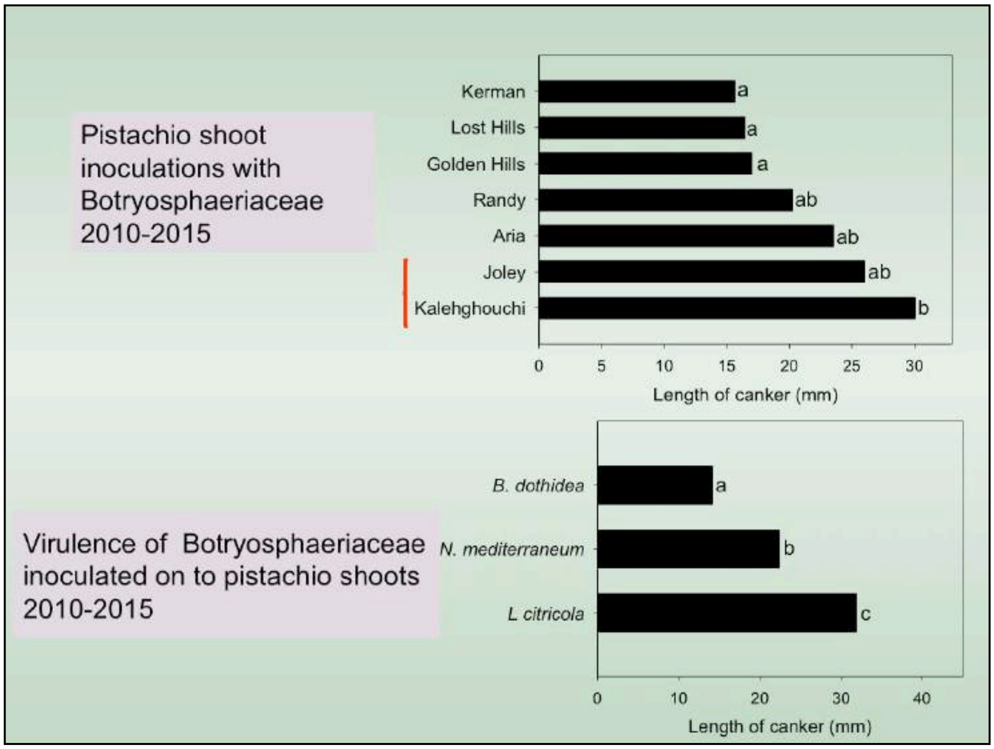
Inoculations of various cultivars were made in the Cultivar Research plot located at the Kearney Agric. Research and Extension Center (Parlier, California). A map of the cultivars is shown above. Inoculations were done by spraying a spore suspension on shoots bearing fruit clusters; evaluation of disease were made in both the fruit clusters and the leaves of shoots bearing the clusters.



A photo showing inoculations of pistachios with 3 species, *Botryosphaeria dothidea, Lasiodiplodia citricola,* and *Neofusicoccum mediterraneum*). Inoculations included a) shoots were flagged, wounded and inoculated with spore inoculum, and wounds were covered with Parafilm; b) clusters were sprayed with spore inoculum , covered with a plastic bag and the bag was removed at 10:00 am PT the day following the inoculation. Disease recording were done by cutting the inoculated shoots and the fruit clusters, bringing them to the lab, and recording blighted fruit and length of canker.

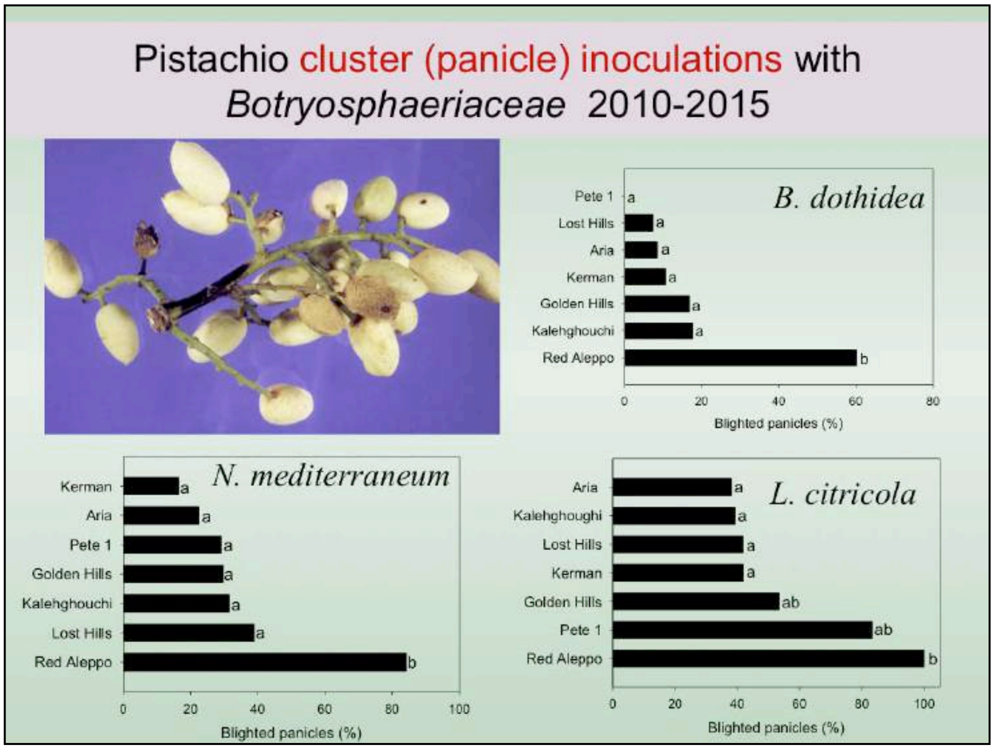


There was a lot of variation among the shoot inoculation in the field. No significant difference among the cultivars were found when they were inoculated with *B. dothidea* or *l. citricola,* however, there were some differences among the cultivars for inoculations done with *N. mediterraneum.* The Kalehghouchi cv. developed significantly longer cankers than the major commercial Kerman cultivar. These results summarize 5 years of experiments.

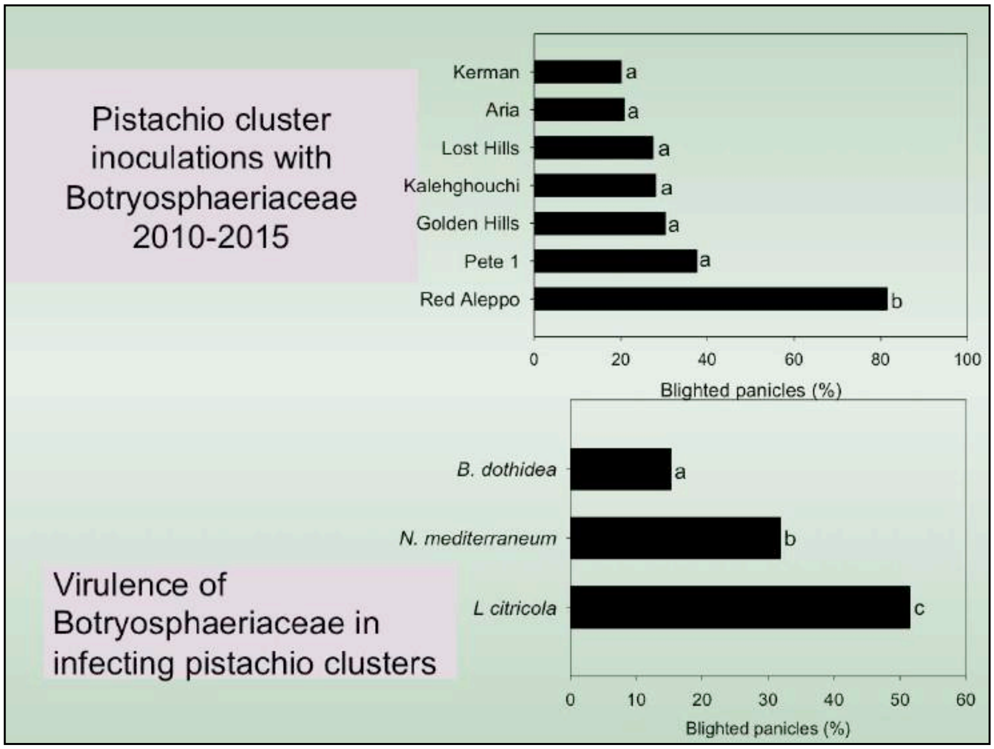


When all the data were pooled regardless of the pathogen used, again the cv. Kalehghouchi was significantly more susceptible than the commercial cv. Kerman. In the field, a grower who has orchards with Kalehghouchi suffered major losses because of Botryosphaeria panicle and shoot blight at a time when his Kerman orchards had much lower levels of disease.

Among the three species of Botryosphaeriaceae used as inoculum, the *Lasiodiplodia* was the most aggressive (resulting in the longest cankers), the *Botryosphaeria* was the least aggressive (resulting in the smallest cankers) and the *Neofusicoccum* was intermediate in aggressiveness (results from 2010-2015)

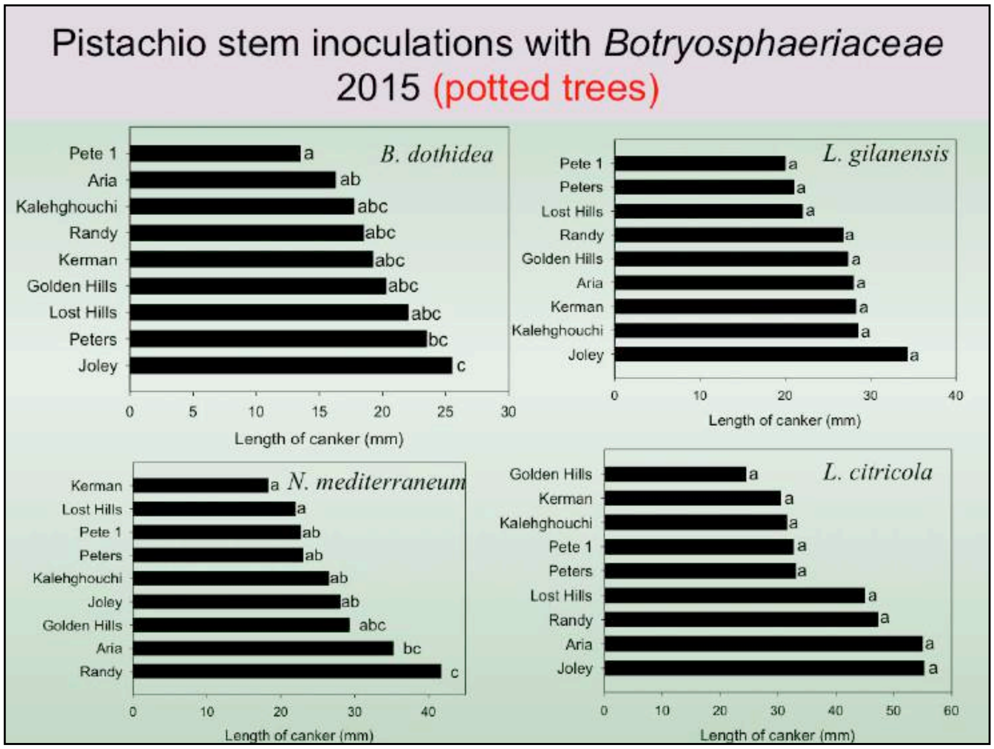


Results from the cluster inoculations in the field are shown here (photo shows infection of the cluster rachis which will eventually lead to cluster blighting): Regardless of the species used as inoculum, the cv. Red Aleppo developed the highest incidence of blighted clusters among all the other cultivars. The cultivars Golden Hills and Pete 1 showed an intermediate level of blighted clusters in comparison with the susceptible Red Aleppo and the less susceptible Kerman, Lost Hills, Kalehghouchi, and Aria when they were inoculated with the aggressive *Lasiodiplodia citricola* (results of 2010 to 2015).

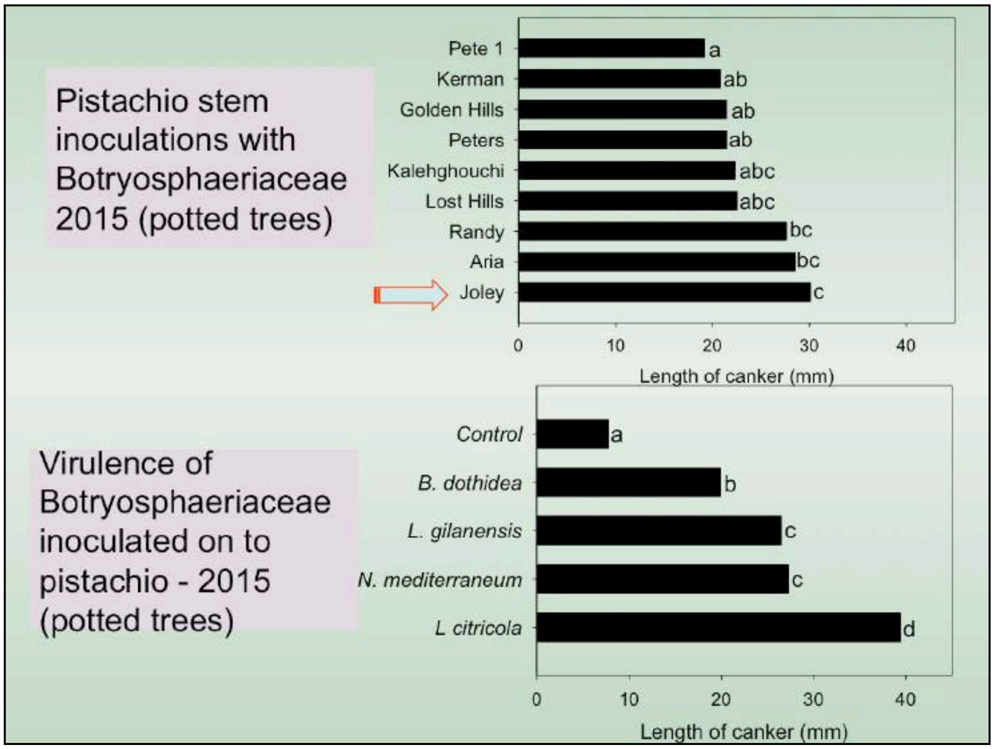


When all the data were pooled regardless of the pathogen used, again the cv. Red Aleppo was significantly more susceptible than all the other cultivars used in these experiments.

Among the three species of Botryosphaeriaceae used as inoculum, the *Lasiodiplodia* was the most aggressive [resulting in the highest incidence of blighted panicles (clusters)], the *Botryosphaeria* was the least aggressive (resulting in the lowest incidence of blighted panicles) and the *Neofusicoccum* was intermediate in aggressiveness (results from 2010-2015).

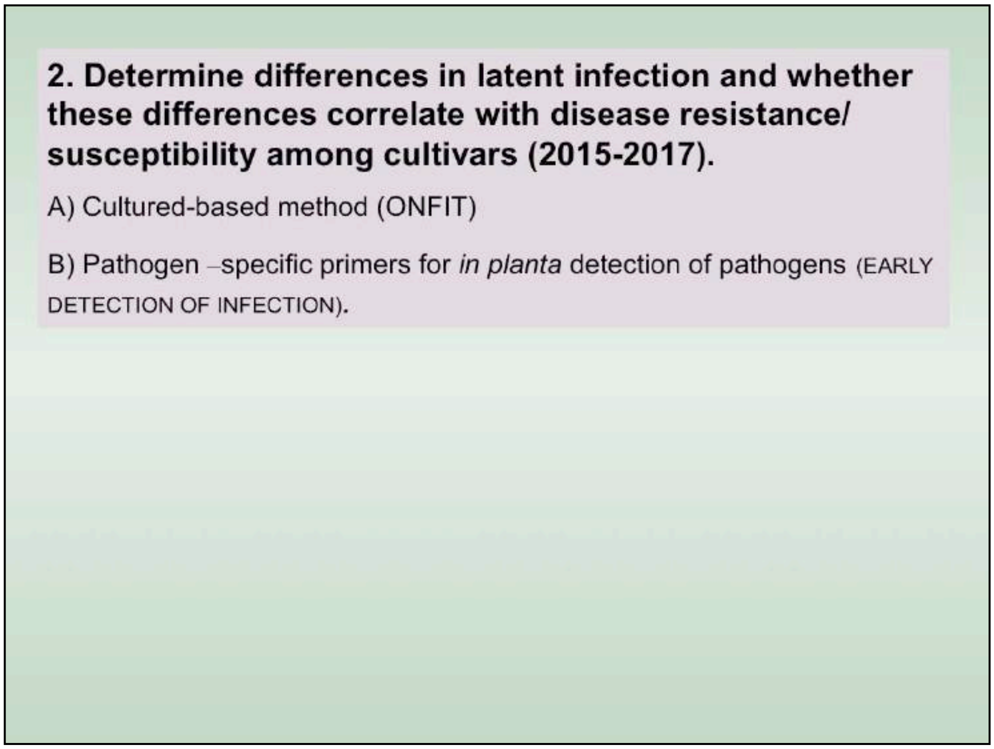


In 2015, we were able to obtain 9 cultivars of pistachio in pots and inoculated with mycelial plugs in their stems after wounding. Four species of Botryosphaeriaceae were used in these inoculations as shown above. There were significant differences among the cultivars when they were inoculated with *B. dothidea* or *N. mediterraneum.* However, there were no significant differences when the cultivars were inoculated with either of the *Lasiodiplodia* species. These are 1 year's results and these inoculations need to be repeated in order to make any conclusions.

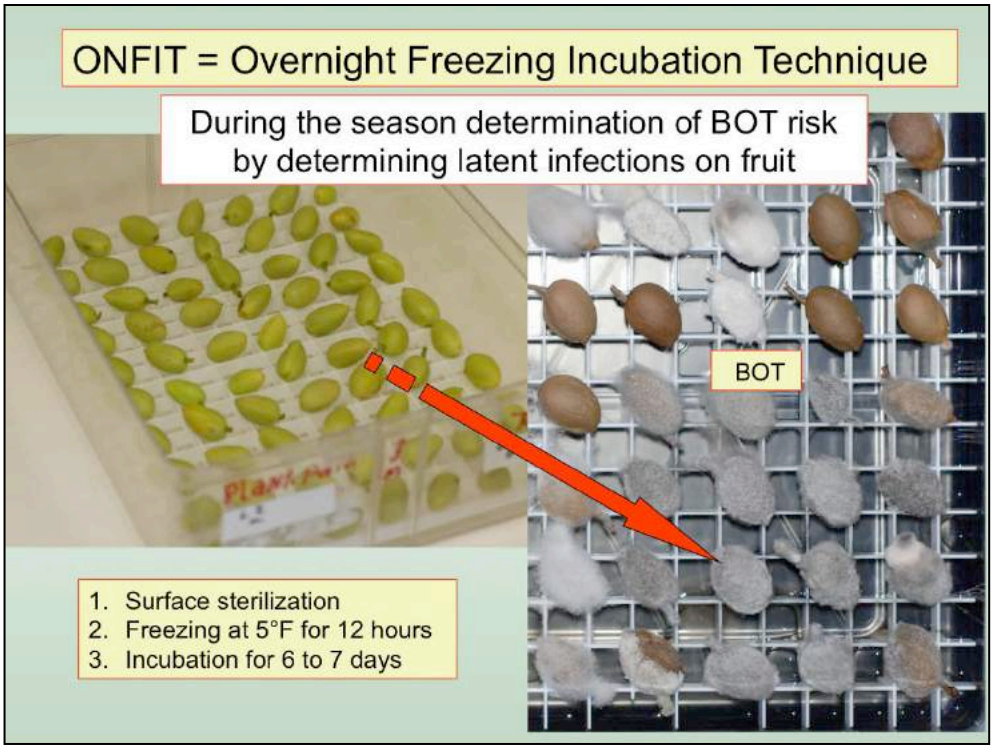


When all the data were pooled, regardless of the inoculated species, there were some differences among the cultivars, but because these are only 1 year's results with potted trees, we cannot make any conclusions yet or any correlations with the results obtained with the susceptibility of some of these cultivars in the field.

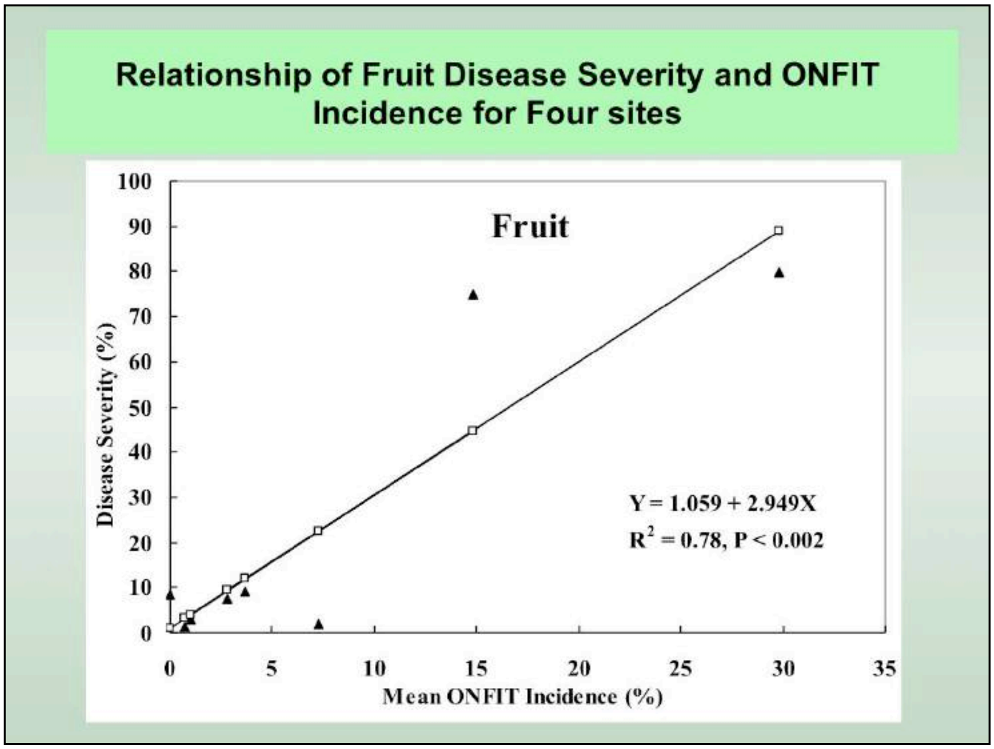
Interestingly, the pattern of aggressiveness of the four species in causing cankers matches to that determined with inoculations of shoots in the field with *L. citricola* being the most aggressive and *B. dothidea* the least aggressive.



In 2015, we initiated another approach to determine if differences in latent infection caused by Botryosphaeriaceae on pistachio fruit correlate with differences in resistance/susceptibility of the various pistachio cultivars. To do this, we used the conventional ONFIT method (see explanation in next slide) and we plan to use the species specific primers for *in lanta* detection of pathogens (which also contribute to the “Early Detection of the Disease” objective of this project).



The conventional method, ONFIT stands for the overnight freezing incubation technique. It involves a collection of about 100 fruit randomly from a 5 to 10 acre orchard, surface sterilize them at 1:10 dilution of household bleach for 4 minutes, placing the fruit in plastic containers as shown in this photo, transferring the container in a -15C freezer overnight, and then incubating the containers with the fruit on a laboratory bench at 25C for about 6-75 days. Then, record the fruit that will show Botryosphaeria development (recognized by the mouse gray color on acidified PDA). Fruit with Alternaria will be covered with black sporulation while fruit with Fusarium or Phomopsis will be white.

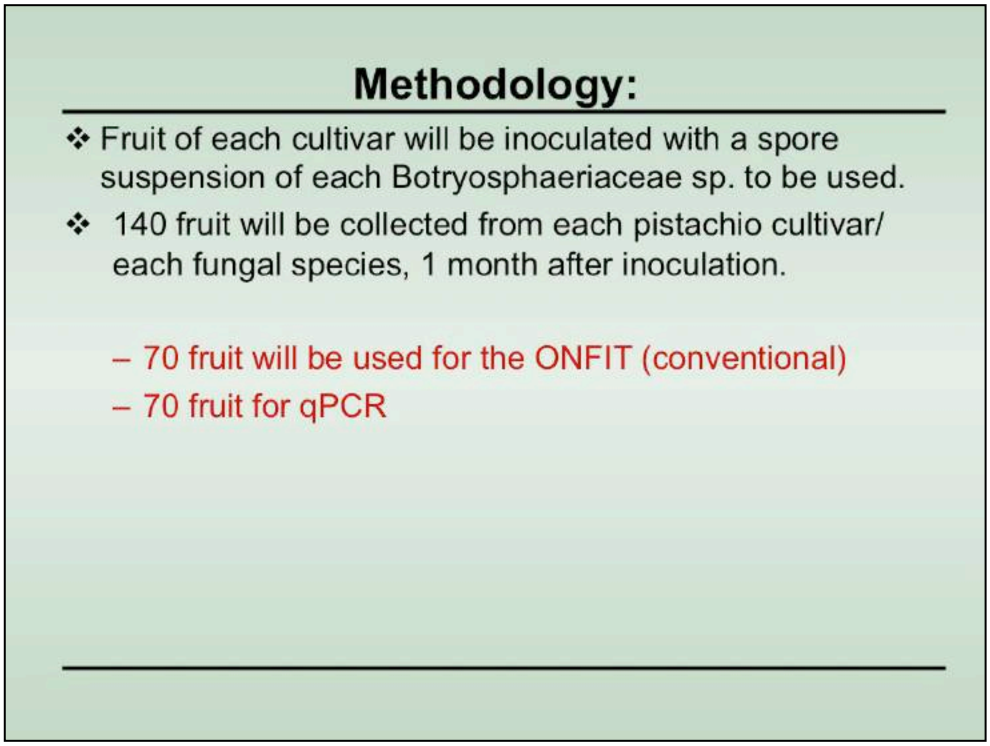


The results of the incidence of Botryosphaeria on immature fruit correlate with the disease severity in the fields where the fruits samples were collected. The ONFIT, the freezing of the tissues, triggers the latent infection to develop and colonize the entire fruit.

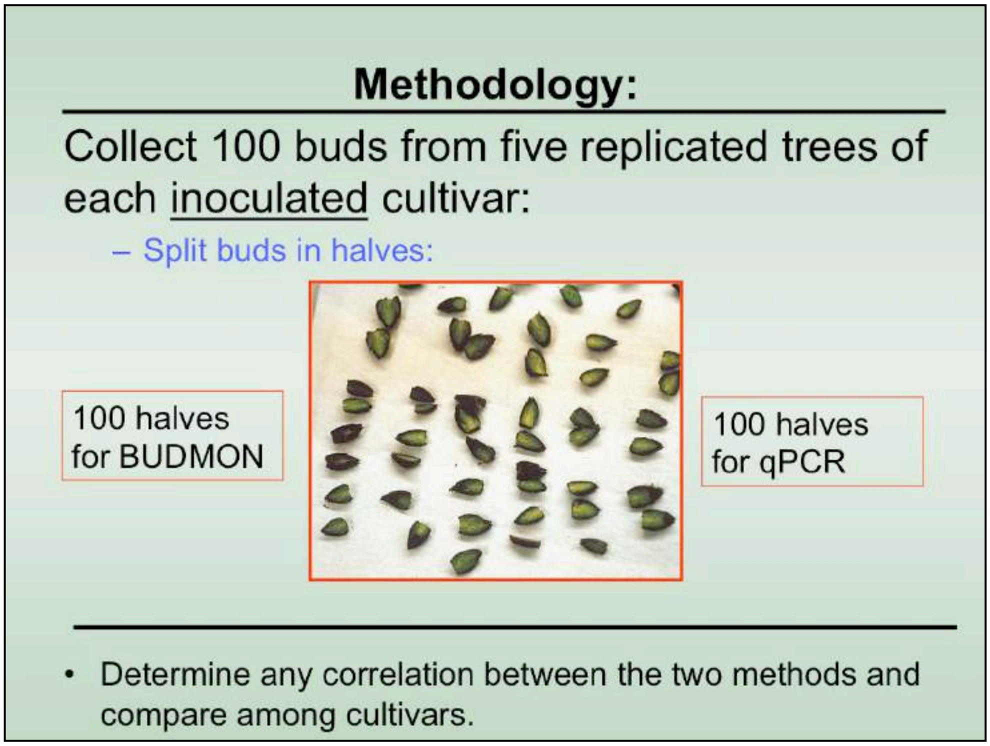
Primer pairs designed to specifically target canker-causing fungal pathogens that were used in the qPCR systems (c/o Postdoc Yong Luo)

Primer name	Sequence (5` - 3`)(forward/reverse)	Target species	PCR product size (bp)	peak of PCR product temperature (°C)
PhBT-F1	CATCGTTACTGACCTCGACTTT /	Phomopsis spp.	102	82.5
PhBT-R1	ACGAGATTTGAAGACAGGGAATAG			
BdF	CAGCGTGGGAGAACATCAA /	Botryosphaeria dothidea	103	81.5
BdR	GTGAGAGAGTACCTCGTTGAAATAG			
LcBT-F2	CTGCTTTCTGGTTTGTTGCC /	Lasiodiplodia spp.	128	86
LcBT-R2	GAGAAGGCGCACACTTACA			
CtBTFF1	GAGCGCATGAACGTCTACTT /	Cytospora spp.	106	82.6
CtBtFR1	GGAAGAAAGCGCGTCAGTAA			
NpBT-F2	ACCACAGGCAGACCATTTC/	Neofusicoccum spp.	118	86.4
NpBT-R2	GTCGGAGGTGCCATTGTAG			
DpF	GTGTAAGTTTGCGCTGTCTTTG /	Diplodia spp.	118	84.8
DpR	GTAGAGAGTACCTCGTTGAAGTAGA			

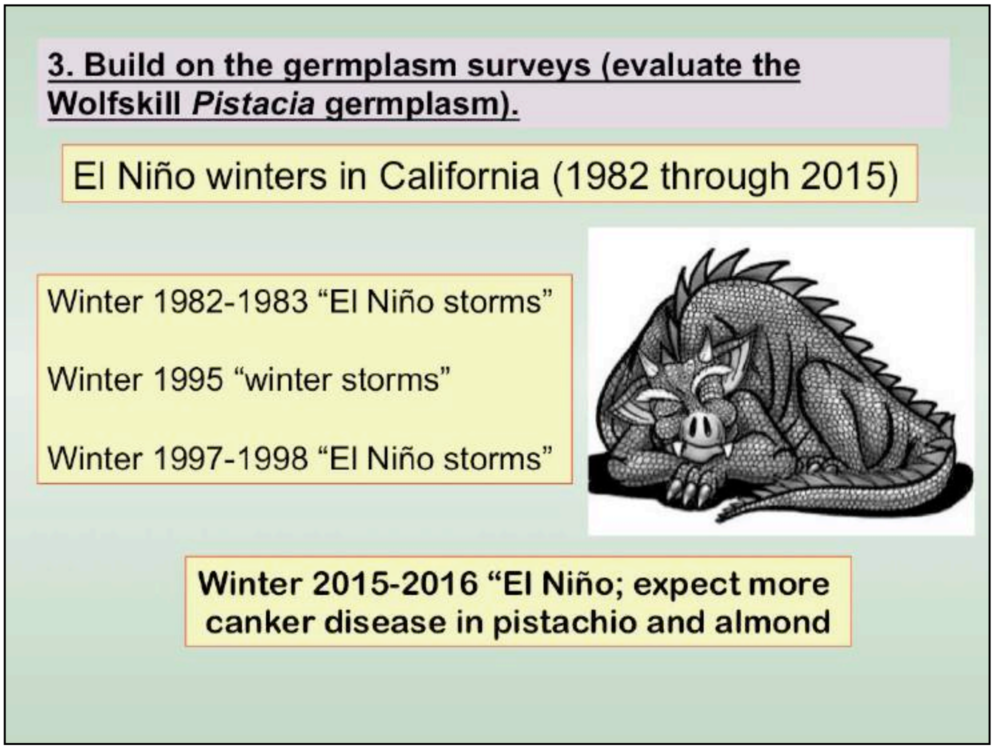
This are the genus specific primers that are used now to detect the major genera (with the exception of *Cytospora*) attacking pistachio. This method is in progress and no results are presented.



Another method that we plan to use is the BUDMON which stands for bud-monitoring of Botryosphaeria. What is described in this slide is to compare the ONFIT with the qPCR method (future work) and eventually to compare the BUDMON with the qPCR method (future work) as shown diagrammatically in the next slide..



BUDMON method vs. qPCR 9future work).



Because of the drought there was not any disease in the germplasm accession block in Winters, California. However, there is inoculum of Botryosphaeriaceae that is waiting (like the sleeping giant) for the right conditions to cause disease. With the El Nino winter expected in 20156, we expect to have more disease in the germplasm collection and be able to evaluate the various accessions.

Thank you