MANAGING BOTRYOSPHAERIA/PHOMOPSIS CANKER AND BLIGHT AND ANTHRACNOSE BLIGHT OF WALNUT IN CALIFORNIA

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ABSTRACT

The Botryosphaeriaceae fungi and *Phomopsis* species are now widely distributed in walnut orchards in all the counties where walnuts are grown. In fact, almost 50% of the 275 samples submitted to our laboratory for diagnosis were walnut samples and the majority of them had pycnidia of *Botryosphaeria* only, *Botryosphaeria* and *Phomopsis, or Phomopsis only.* Only several of the samples were diagnosed as branch wilt caused by *Neoscytalidium dimitiatum.* Five samples had both pycnidia and pseudothecia of *Botryosphaeria*, an indication that these fungi can spread by two kinds of spores: a) water splashed spores, and b) spores spread by wind (ascospores). To determine the mode of infection periodic inoculations with two aggressive species showed that, under favorable environmental conditions, immature walnut fruit can be infected, infections can remain latent (without noticeable symptoms), and lead to fruit blight later in the season as the fruit mature. Latent infections from May to early August remained low but increased three-fold in August and September. Infected and blighted fruit lead to invasion of the pathogens into the spur and caused cankers. Inoculations in August and September resulted in higher incidence of cankers caused by *N. parvum* than by *L. citricola.* Both these pathogens are very aggressive on walnut and both were shown to infect fruit and shoots after wounding and in 2014 without wounding. Interestingly, although *L. citricola* was an excellent kernel colonizer, *N. parvum,* did not colonize the kernels. Inoculations of walnut husks indicated that they can serve as a good nutrient source for growth and reproduction of Botryosphaeriaceae in walnuts. To determine the time period pruning wounds remain susceptible to infection by Botryosphaeriaceae, inoculations of wounds immediately after pruning and 3, 7, 4, 21, and 28 days were done in Vina, Chandler, and Tulare cultivars with *L. citricola* and *N. parvum.* The majority (43 to 100%) of the wounds were infected. No significant differences were found among the cultivars or between the two pathogen species. However, what was surprising was 4 weeks after pruning, the wounds were still susceptible to infection and the length of cankers developed was not much different from that resulting from earlier inoculations or inoculations done immediately after pruning. A fungicide trial was established in San Benito Co. but due to lack of rain no anthracnose disease developed in 2014. This trial will be evaluated for Botryosphaeria in early 2015. There were 5 more fungicide trials to control Botryosphaeria canker and blight, one each in Butte (cv. Chandler) and Colusa (cv. Chandler) counties, and 3 in Sutter County (two Chandler and one Howard). In all trials where sprays were applied in mid-May, mid-June, and mid-July, the majority of fungicide treatments were effective in reducing infections and cankered spurs. In at least one trial where nuts were evaluated, the fungicide treatments reduced infection of kernels by Botryosphaeriaceae species in nuts that had black or brown kernels. In this trial, it seems that at least one fungicide treatment also reduced the incidence of blighted nuts in comparison with the unsprayed control. In a trial where bloom and postharvest sprays were compared, only the trees sprayed both at bloom and postharvest showed a trend in reducing the disease while a spray at bloom only or at postharvest only was not sufficient to reduce the disease.

OBJECTIVES

1) Continue surveying orchards and diagnosing walnut samples brought to the laboratory by growers, farm advisors, and pest control advisers.

2) Determine the time and mode of infection of stems, branches, and fruit by Botryosphaeriaceae fungi.

3) Determine infection of fruit/husks in the field at harvest and postharvest.

4) Determine whether and how long major pruning wounds are susceptible to infection by *Botryosphaeriaceae* fungi.

5) Determine efficacy of fungicides to control Botryosphaeria and anthracnose blights in walnut.

PROCEDURES

1) Continue surveying orchards and diagnosing walnut samples brought to the laboratory by growers, farm advisors, and pest control advisers.

We continued providing support to walnut growers, farm advisors, and pest control advisers throughout California, in diagnosing putative diseased walnut samples brought or mailed to our laboratory at Kearney Agric Research and Extension Center. In many cases, we visited problematic orchards and consulted the grower and his/her PCA on fungal disease and collected samples ourselves for further diagnosis of the pathogens. We believe that until growers, farm advisors, and PCAs become very familiar with the various symptoms and signs of the Botryosphaeria/Phomopsis and Anthracnose diseases, this activity is essential to help diagnose these diseases correctly at an early stage, thus giving the growers the opportunity to make timely disease management decisions. For instance, we strongly believe that by helping a grower, a farm advisor, a pest control adviser, or a foreman make an early and correct disease diagnosis in an orchard, there is plenty of time to act on disease management, especially since successful control of these diseases depends on protective early fungicide treatments and cultural controls, such as timely pruning and removal of prunings to reduce inoculum.

To diagnose samples brought or collected we follow a series of steps which depend on the type of samples For cankered and/or blighted shoots, the bark of the cankered areas or blighted spurs and shoots was first examined for the presence of fruiting structures. These observations were made using a dissecting microscope to help us determine whether we are dealing with a Botryopshaeriaceae species (10 were idenitfied from walnuts [1]), or a species of *Phomopsis* and/or species of *Diaporthe* (which is the sexual stage of *Phomopsis*). Then, a micrsocopic slide is prepared and examined under the compound microscope to determine if we are dealing with *Botryopshaera/Neofusicoccum, Lasiodiplodia, Diplodia*, or *Neoscytalidium* (which the new name of the *Hendersonula toruloidea* that causes branch wilt of walnut). These genera can be disstinguished with the compound micrsocope. c) if needed (when there are no sporulating structures, isolations are made on a general **A**cidified **P**otato **D**extrose **A**gar (APDA). For lesions on leaf, fruit, and spurs without any sporulating structures: a) small pieces of the infected parts are cut at the interface of killed tissues and healthy tissues and surface sterilized in 10% bleach (5.25% NaHCl) solution for 2-4 mintues. b) after sterilization the pieces are placed on sterile towels to remove excess moisture; c) then plated on APDA plates, usually 10 pieces per large (10 cm) or 3 pieces per small (5 cm) petri plate; d) the plates are incubated at 27°C for about 4-5 days and then the fungi growing are identified. Once the putative pathogen is identified, the results of the diagnosis are communicated back to the interested parties either by a telephone call, or an e-mail message. In some instance, formal diagnostic letters are written after a specific request usually by a foreman who wants to convince his boss on a specific disease management practice.

2) Determine the time and mode of infection of stems, branches, and fruit by Botryosphaeriaceae fungi.

To determine when hulls are infected, first immature and then maturing fruit were inoculated in the field with *Lasiodiplodia citricola* (isolate 6-I34) and *Neofusicoccum parvum* (isolate 1-L87), using 10 fruit per isolate. Buds were also inoculated simultaneously with the fruit. Inoculations were done every 3 weeks, beginning May 15, 2014 with the last inoculation done Sept 19, 2014. Shoots with fruit were sprayed with a 10,000-50,000 spores/ml spore suspension of *Lasidiplodia citricola*, *Neofusicoccum parvum*, or water for the control treatment. The inoculations were performed in late afternoon, bagged with a plastic bag to maintain humidity, and covered in a white paper bag to prevent sunburn and overheating. Bags were removed early the following morning. At harvest time (Sept 25, 2014), blighted fruit were recorded for each treatment. Blighted fruit were collected on 17 Nov 2014 and isolations made from them to determine if the pathogen used for inoculation could be recovered. Lengths of cankers produced in inoculated shoots were also recorded at the time fruit were collected. Infection of buds will be recorded in February to March 2015, before bud dormancy break.

3) Determine infection of fruit/husks in the field at harvest and postharvest.

Husks left on the trees after commercial harvest time, were inoculated with a spore suspension of *L. citricola* and *N. parvum.* A spore suspension of 10,000 spores per ml was used and it was sprayed on the husks until run off. One batch of husks was covered with a plastic bag for 24 hours and another batch of husks was inoculated and left uncovered. A third batch of husks was not inoculated and instead sprayed with sterile water and served as controls. A plastic net was placed over each husk to collect them in case they fall during winter.

In another experiment, husks were collected from a commercial orchard, brought to the laboratory and inoculated with a drop of spore suspension of *L. citricola* or *N. parvum* as above. Two batches of husks were used a) without any surface sterilization; and b) after surface sterilization with a 10% chlorine bleach solution. The husks were arranged on a plastic screen in containers with about 200 ml water on the bottom to increase humidity and incubated at 27°C (81°F) for 2 weeks. Infection of husks was determined by the presence of pycnidia formation, an indication that the pathogens had grown and reproduced on the husks.

4) Determine whether and how long major pruning wounds are susceptible to infection by *Botryosphaeriaceae* fungi.

To determine whether and how long pruning wounds are susceptible to infection, the two most aggressive (virulent) species, *Lasiodiplodia citricola* and *Neofusicoccum parvum*, were used for this experiment. Three walnut cultivars (Chandler, Tulare, and Vina) at the Kearney Agric. Research & Extension Center experimental field were pruned as follows: A total of 60 shoots per cultivar of 1/2 inch in diameter were pruned and flagged on 7 Feb 2014. The pruning wounds of 10 shoots for each pathogen were inoculated immediately after pruning, and 10 each after 3, 7, 14, 21, and 28 days following pruining. Ten pruning wounds on each cultivar were not inoculated and served as the "non-inoculated control" treatment. Inoculations were done using a 50,000 spores/ml suspension of each species placed with a brush directly on the surface of the cut. The cut was covered with Parafilm to create favorable conditions for infection. Cankers and discolored tissues were evaluated 3 to 4 months after each inoculation, depending on the development of cankers. Pruned shoots were collected on 3 Dec 2014 and canker lengths measured. Results will be compared to determine differences in infection among time of inoculation, walnut cultivar, and fungal species. (Note: 60 shoots × 3 cultivars × 2 fungal pathogens + 30 control shoots = a total of 390 shoots were used in this experiment.)

5) Determine efficacy of fungicides to control Botryosphaeriaceae and anthracnose of walnut.

San Benito County trial - Anthracnose. One row of a commercial walnut orchard in San Benito County was used for this study. The orchard was irrigated using sprinklers. Treatments consisted of three single-tree (cv. Serr) replications using a randomized complete block design. The trade names, active ingredients, and class of the fungicides used in these trials are listed in Table 1. The fungicide treatments, rate of each fungicide, and dates of spray are listed in Table 2. Three sprays were applied with a handgun sprayer using 400 gallons of water per acre from April 8 to June 3, 2014. Surfactants and wetting agents used are listed in Table 2. On August 15, we recorded the incidence of blighted fruit and leaves with lesions. Although this is a trial to determine efficacy of fungicides against anthracnose, because there is plenty of *Botryosphaeria* inoculum in this orchard, spurs and buds will be collected in February/March to determine the long term effect of these fungicides against Botryosphaeria/Phomopsis canker and blight disease.

Butte County trial – Botryosphaeria canker and shoot blight. Two rows of a commercial walnut orchard in Butte County were used for this study. The orchard was irrigated using sprinklers. Although rain was sparse this growing season, about 0.5-inches of rain fell on the orchard during an August 5 storm. Treatments consisted of three single-tree (cv. Chandler) replications using a randomized complete block design. The trade names, active ingredients, and class of the fungicides used in these trials are listed in Table 1. The fungicide treatments, rate of each fungicide, and dates of spray are listed in Table 3. The bloom spray on April 6 was applied with a handgun sprayer at rates recommended by the manufacturer using 100 gallons of water per acre. After bloom time, sprays were applied with a handgun sprayer using 400 gallons of water per acre. All the remaining treatments consisted of three applications which were applied from May 8 to July 10. Surfactants and wetting agents used are listed in Table 3. On November 7, we recorded the incidence of blighted spurs out of 100 current season shoots bearing fruit per replicate tree.

Trials in Colusa and Sutter Counties – Botryosphaeria canker and shoot blight. Treatments are replicated in a randomized, complete block design applied with the grower's airblast sprayer. Each replicate is a row with a row of buffer trees on each side. Half of the speed sprayer nozzles are off and half directed to the treated row which is sprayed on both sides. The sprays were applied at 100 gallons per acre by the grower under the direction of the farm advisor Janine Hasey (UC Cooperative Extension, Colusa, Sutter, & Yuba counties).

RESULTS AND DISCUSSION

1) Continue surveying orchards and diagnosing walnut samples brought to the laboratory by growers, farm advisors, and pest control advisers.

In 2014, a total of 275 samples were diagnosed of which 135 were samples of walnuts, representing the following cultivars: Chandler, Howard, Tulare, Vina, Hartley, Eureka, and Serr. The counties where the samples originated from included: Tehama, Glenn, Butte, Colusa, Sutter, Yuba, Yolo, Solano, San Joaquin, Stanislaus, Contra Costa, Merced, Fresno, Kings, Tulare, Kings, Kern, and San Benito. Of these, the majority of the samples were diagnosed bearing *Botryosphaeria*, or *Botryosphaeria* and *Phomopsis*, and a few having cankers and blight due to branch wilt pathogen *Neoscytalidium dimitiatum* (a synonym of *Hendersonula toruloidea*). In at least 5 of these samples both the pycnidial and the sexual (pseudothecia) stages of Botryosphaeriaceae and Diaporthaceae (*Phomopsis* spp. were present. The presence of these spore producing structures in walnut tissues suggests that these fungi can spread by two kinds of spores: a) water splashed spores and b) spores spread by wind. This efficient means of dispersal may explain why the disease has spread to so many orchards. The diagnosis of all these samples were communicated either by phone or e-mail messages to farm advisors, pest control advisers, growers, and representatives of chemical companies.

2) Determine the time and mode of infection of stems, branches, and fruit by Botryosphaeriaceae fungi.

Inoculation and infection of immature fruit. These inoculations were done periodically, and all inoculated fruit were left on the trees. Isolations from kernels of the nuts inoculated with *Lasiodiplodia citricola* (*L.c*.) showed that *L. citricola* was isolated from all inoculation dates and the proportion of the infected kernels increased for inoculations in August and September. However, *Neofusicoccum parvum* (*N.p.*) was not isolated from any of the fruit of any of the inoculation dates (Figure 1).

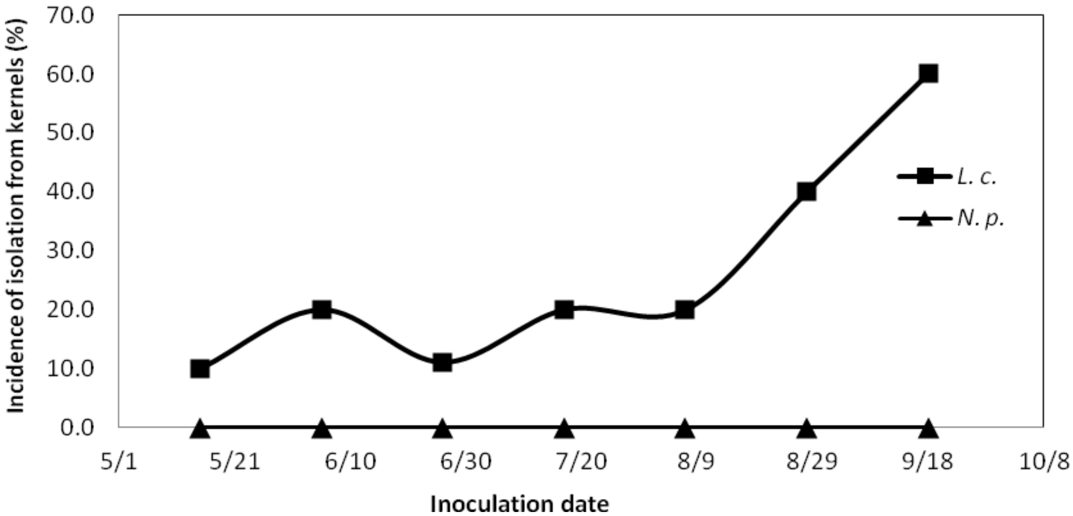

Figure 1. Incidence of *Lasiodiplodia citricola* and *Neofusicoccum parvum* isolations from kernels of immature walnuts inoculated periodically with spore suspensions of *L. citricola* and *N. parvum.*

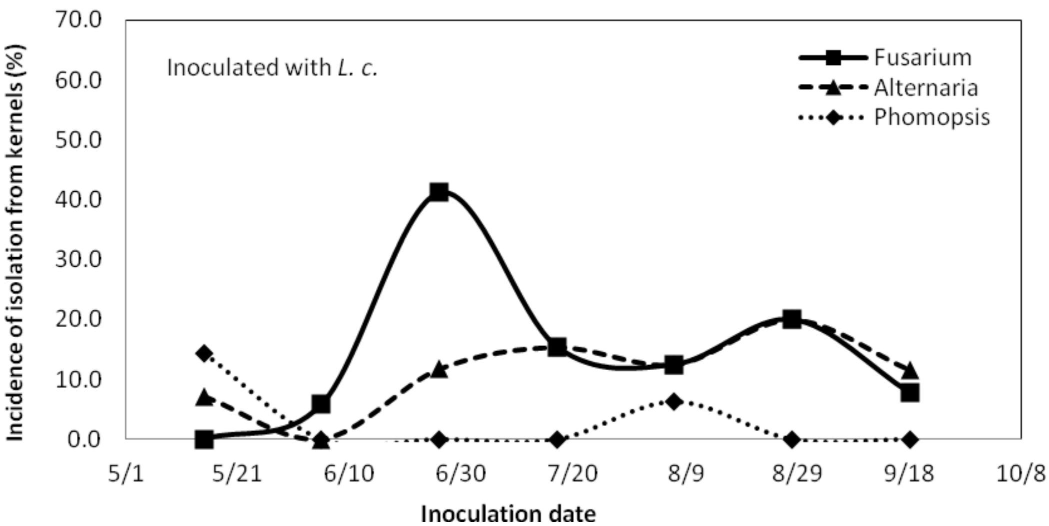

Figure 2. Incidence of *Fusarium, Alternaria,* and *Phomopsis* species isolated from kernels of immature walnuts inoculated periodically with a spore suspension of *Lasiodiplodia citricola.*

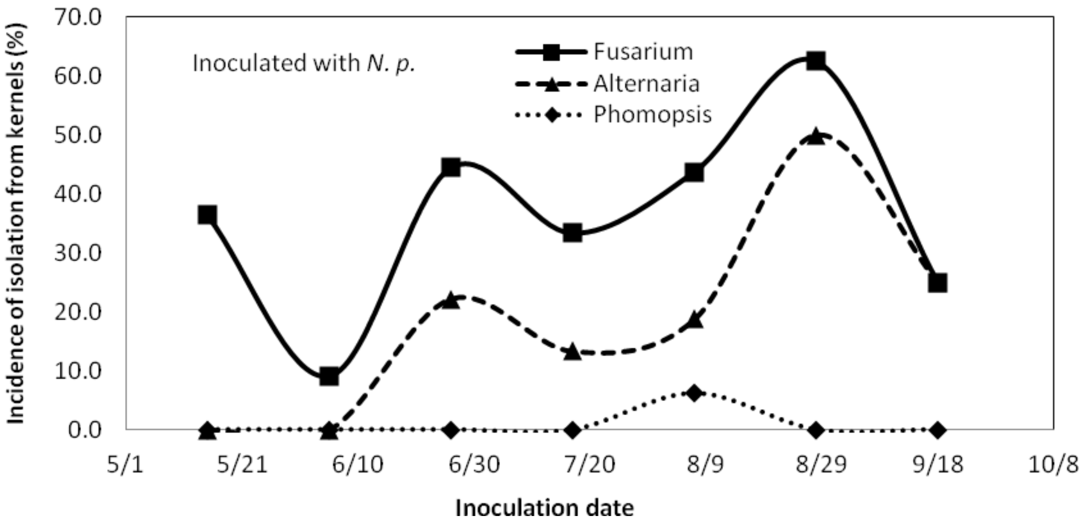

Figure 3. Incidence of *Fusarium, Alternaria,* and *Phomopsis* species isolated from kernels of immature walnuts inoculated periodically with a spore suspension of *Neofusicoccum parvum.*

Other fungi isolated from blighted fruit from periodic inoculations included *Fusarium, Alternaria,* and *Phomopsis* species. Although no distinct pattern of isolations was observed for inoculations with *L. citricola* (Figure 2), when fruit were inoculated with *N. parvum*, the order of isolation was mainly *Fusarium*, less *Alternaria*, and just a little *Phomopsis* (Figure 3).

L. citricola (*L.c.*) resulted in 10-20% infections of kernels from May through early August, but levels of infections increased by the end of August though middle of September (Figure 1). These results suggest that infections that occur later in the season (August and September) will result in more damage to the nuts due to decay of kernels than infections in the spring. Also, this serial inoculation of immature fruit experiment shows that latent infections (infections with no symptoms) could occur throughout the season, if conditions were right (Figure 1). Furthermore, these results suggest that sprays done during spring and summer should provide protection of walnuts against Botryosphaeria blight.

Development of cankers in spurs. Spurs of serial inoculations were collected on Nov 17, 2014 and recorded for canker development. The incidence of canker formation for inoculations in May was very low, and there were no significant differences for the incidence and average canker length from inoculations during June to end of August (Figure 4). For inoculations in mid-September, only *Neofusicoccum parvum* caused cankers while the aggressive *Lasiodiplodia citricola* was not able to move into the spur and cause cankers. The results suggest that infections of fruit during the season lead to canker formation in the spurs, although in the fall, only the *N. parvum* was able to move into the spur (Figure 4 A & B). Infections by *N. parvum* in May did not move into the spur while those by *L. citricola* caused cankers moving into the spurs. Infections from inoculations in May caused significantly shorter lesions than those from other inoculation dates. The maximal lesion length was as long as over 4 inches (10 cm; data not shown).

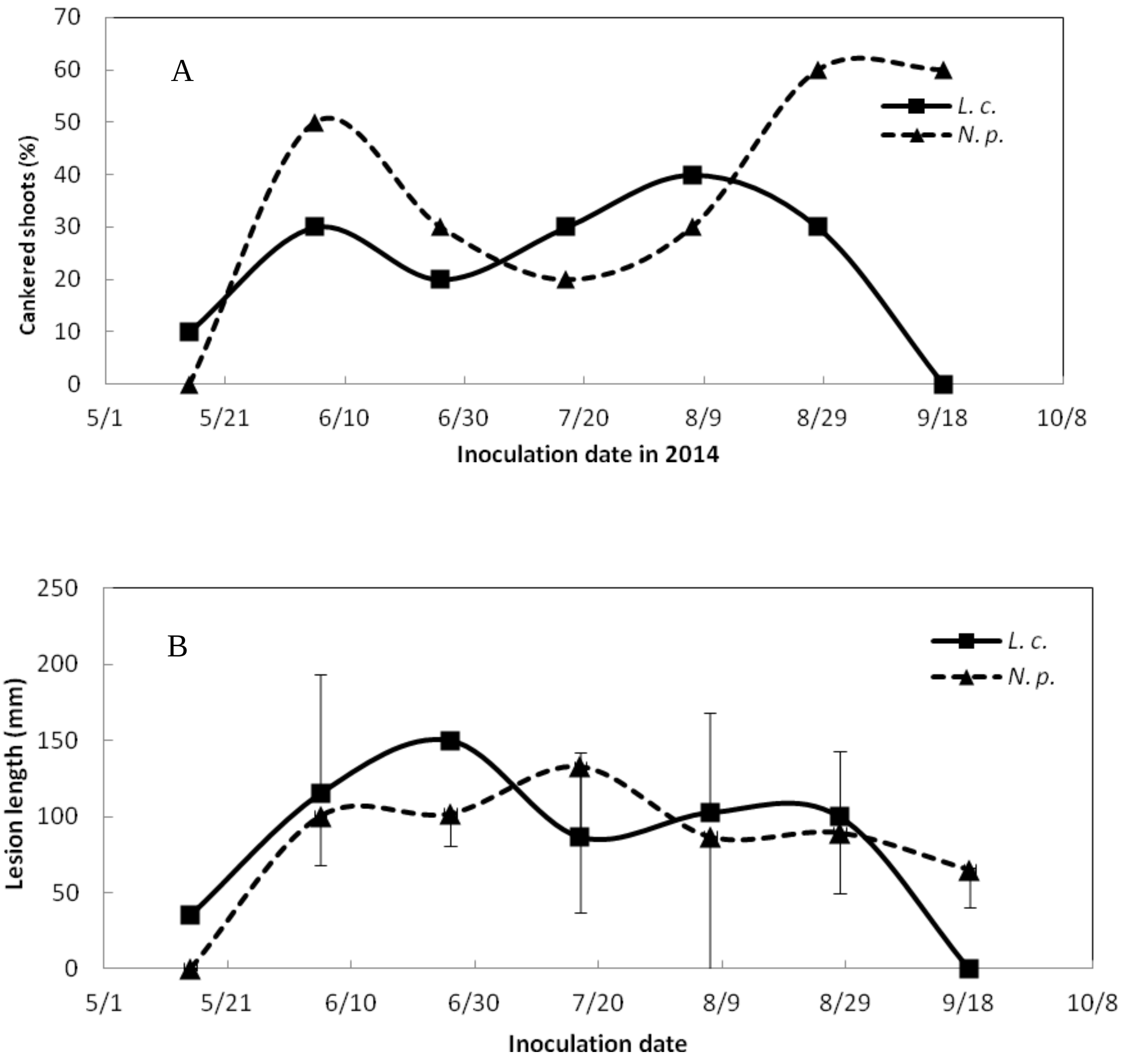

Figure 4. Incidence of cankered spurs (**A**) and length of cankers (**B**) developed after inoculation of fruit periodically during the growing season in 2014 with a spore suspension of *Lasiodiplodia citricola* and *Neofusicoccum parvum.*

3) Determine infection of fruit/husks in the field at harvest and postharvest.

The field experiment is still in progress. In the laboratory inoculations of husks collected from a walnut orchard without surface sterilization and after surface sterilization with a 10% chlorine bleach solution are shown in Figure 5. The husks not only were infected but the pathogens were able to reproduce on them. Significantly more husks were infected after surface sterilization than without surface sterilization when they were inoculated with *L. citricola* (Figure 5) but differences were not significant for *N. parvum.* Up to 70% of husks after sterilization produced pycnidia of *L. citricola.* Regardless of the species, 15 to 25% of the husks were infected and produced pycnidia in those that were not surface sterilized, suggesting that the husks can provide sufficient source of nutrients for the Botryosphaeriaceae fungi to reproduce on them as long as the husks remain on the trees.

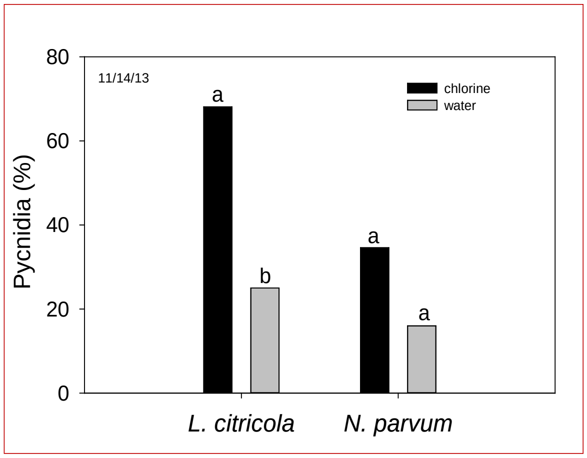

Figure 5. Incidence of pycnidia development of pycnidia on the surface of sterilized with 10% bleach solution and non-sterilized husks after inoculation with *Lasiodiplodia citricola* and *Neofusicoccum mediterraneum* in the laboratory after 2 weeks incubation at 27°C (81°F).

4) Determine whether and how long major pruning wounds are susceptible to infection by *Botryosphaeriaceae* fungi.

Results of infection of pruning wounds were very similar among the three cultivars used and between the two species that were used in these inoculations (Figure 6, A-C). Almost all of the pruning wounds were infected even 4 weeks after pruning and regardless of the walnut cultivar. The range of infection was 89 to 100% for *L. citricola* and 42 to 100% for *N. parvum* (data not shown). The results suggest that the pruning wounds can remain susceptible at least for 4 weeks after pruning, an indication that walnut wounds take a long time to heal. The results support observations in the field where cankers initiated from pruning cuts were up to 3 inches (75 mm). In our inoculation experiments cankers up to 10 inches (250 mm) were recorded from inoculations immediately after pruning for Vina and Chandler cultivars. However, the cankers on Tulare trees were smaller for the initial inoculation for both pathogens (Figure 6C). The patterns of canker development after infection of pruning wounds seem very similar among cultivars and pathogens with no significant differences. No differences in canker length were observed for the inoculations dates or for the two pathogens (Figure 6A-C). However, large variations in canker length were observed among the 10 samples.

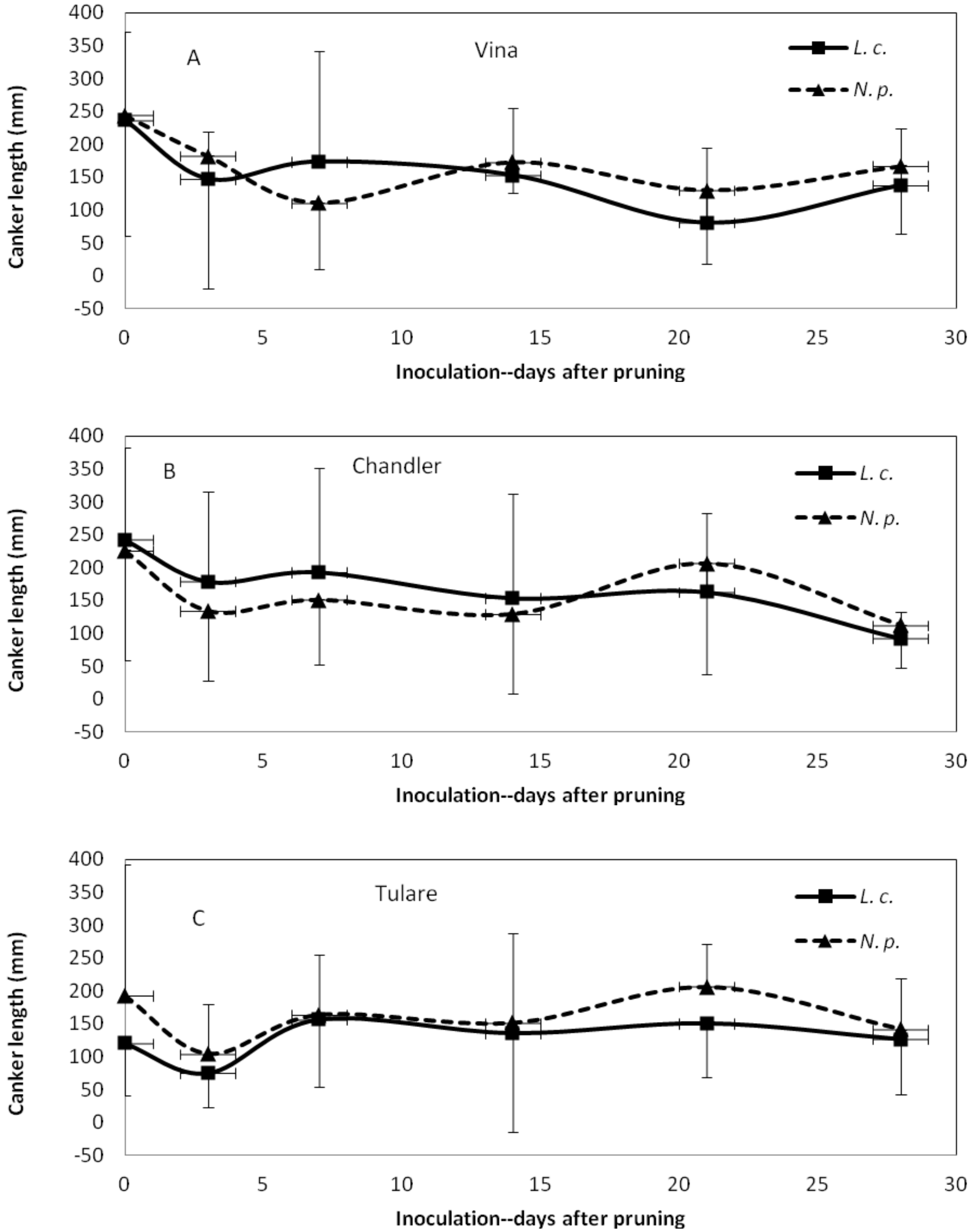

Figure 6A-C. Susceptibility of pruning cuts of three walnut cultivars as measured by the length of canker developed after inoculation with *Lasiodiplodia citricola* or *Neofusicoccum parvum.*

5) Determine efficacy of fungicides to control anthracnose and Botryosphaeria canker and blights in walnut.

San Benito County trial - Anthracnose. Similarly to 2013, no anthracnose developed on any of the trees in 2014 (**Table 2**). However, because this is a replicated and randomized trial, it will be evaluated for Botryosphaeria canker and blight in Feb/March 2015 by recording cankers in spurs and collecting dormant buds to perform BUDMON (to monitor Botryosphaeriaceae in the buds).

Table 1. Trade name, active ingredient, and class of fungicides used in 2014 trials to control anthracnose and Botryosphaeria canker and blight of walnut.

Trade name	**Active ingredient**	**Class of fungicide**
Badge*	Copper	Inorganic
Fontelis*™	Penthiopyrad (20%)	Carboxamide
K-Phite® 7 LP*	Mono-and di-potassium salts of phosphorous acid (56%)	Inorganic salt
Luna® Experience SC400*	Fluopyram (17.6%) Tebuconazole (17.6%)	Carboxamide DMI-Triazole
Luna® Sensation 500 SC*	Fluopyram (21.4%) Trifloxystrobin (21.4%)	Carboxamide Strobilurin
Manzate Prostick Manzate Prostick *	zinc ion and manganese ethylenebisdithiocarbamate	Carbamate
Merivon®*		
Ph-D®*	Polyoxin D zinc salt (11.3%)	Peptidyl pyrimidine nucleoside
Pristine®*	Pyraclostrobin (12.8%) Boscalid (25.2%)	Strobilurin Carboxamide
Quadris Top*™	Azoxystrobin (18.2%) Difenoconazole (11.4%)	Anilinopyramidine
Quash® 50 WG*	Metacolazole (50%)	DMI-Triazole
Tebucon® 45 WP*	Tebuconazole (45%)	DMI-Triazole

*Currently registered for use on walnuts.

Table 2. Efficacy of fungicides against **anthracnose** of walnuts caused by *Marssonina juglandis* in a Serr orchard in San Benito County – 2014.

Trt #	Treatment - fungicide(s)	Rate	Spray dates			Fruit or leaves with lesions[1] (%)
			April 8	May 1	June 3	
1.	Fontelis[4]	20 fl oz	Fontelis	Fontelis	Fontelis	0 a[2]
2.	Fontelis +OR 009	20 fl oz 0.3%	Fontelis+ OR 009	Fontelis+ OR 009	Fontelis+OR 009	0 a
3.	Fontelis[4] + Tebucon 45DF	20 fl oz 8 oz	Fontelis+ Tebucon	Fontelis+ Tebucon	Fontelis+ Tebucon	0 a
4.	EXP 1[4]	5.14 fl oz	EXP-1	EXP-1	EXP-1	0 a
5.	EXP 1[4] EXP 2	3.43 fl oz 5.48 fl oz	EXP 1 EXP 2	EXP 1 EXP 2	EXP 1 EXP 2	0 a
6.	EXP 1[4] EXP 3	3.43 fl oz 4.57 fl oz	EXP 1 EXP 3	EXP 1 EXP 3	EXP 1 EXP 3	0 a
7.	Pristine[4]	14.5 oz	Pristine	Pristine	Pristine	0 a
8.	Merivon[4]	6.8 fl oz	6.8 fl oz	6.8 fl oz	6.8 fl oz	0 a
9.	Quadris Top[4]	14 fl oz	Quadris	Quadris	Quadris	0 a
10.	Viathon 4.1 FS[4]	4 pints	Viathon	Viathon	Viathon	0 a
11.	Luna Sensation[4]	5 fl oz	Luna Sen 5	Luna Sen 5	Luna Sen 5	0 a
12.	Luna Sensation[4]	7.6 fl oz	Luna Sen 7.6	Luna Sen 7.6	Luna Sen 7.6	0 a
13.	Luna Experience[4]	6 fl oz	Luna Exp 6	Luna Exp 6	Luna Exp 6	0 a
14.	Luna Experience[4]	10 fl oz	Luna Exp 10	Luna Exp 10	Luna Exp 10	0 a
15.	K Phite 7LP	3 quarts	K Phite	K Phite	K Phite	0 a
16.	K Phite 7LP + Latron B-1956[3]	3 quarts 4 fl oz	K Phite + Latron	K Phite + Latron	K Phite + Latron	0 a
17.	Badge[4] + Manzate ProStick	4 lbs 2.4 lbs	Badge+ Manzate	Badge+ Manzate	Badge+ Manzate	0 a
18.	Quash[4]	3.5 oz	Quash	Quash	Quash	0 a

19.	Ph-D[4]	6.2 oz	PH-D 6.2	PH-D 6.2	PH-D 6.2	0 a
20.	Ph-D[4] + Tebucon 45	6.2 oz 4 oz	PH-D 6.2 Tebucon	PH-D 6.2 Tebucon	PH-D 6.2 Tebucon	0 a
21.	Control	Untreated				0 a

[1] 100 leaves and fruit for each of 3 replicated trees were recorded on August 15, 2014.
[2] Numbers followed by different letters are significantly different according to the LSD test at $P = 0.05$. Statistical analysis was performed on arcsine transformed data. Values presented were back transformed from the means for the arcsine transformed data.
[3] The surfactant Latron 1956 at 0.0625% vol./vol. was added.
[4] The surfactant Induce at 0.25% vol./vol. was added.

Butte County trial - Botryosphaeria. We observed 12% blighted shoots in the unsprayed control (**Table 3**). The best treatment was Merivon® with 1.6% blighted shoots (Trt 8). However, this was the only treatment that also included a bloom spray. Next came three treatments Ph-D® + Tebucon® (trt #20), K-Phite (trt 15), and Fontelis + OR 009. (trt #2). The results suggest that sprays applied around mid-May, mid-June, and mid-July have a significant effect in reducing Botryosphaeria infection of nuts and development of cankers in spurs. These results are in agreement with the preliminary results of non-replicated 2013 trials done by PCA in commercial orchards in Butte and Tehama Counties.

Table 3. Efficacy of fungicides against **Botryosphaeria canker and shoot blight** of walnuts (cultivar Chandler) caused by Botryosphaeriaceae and *Phomopsis* in Butte County – 2014.

Trt #	Fungicide Treatment	Rate	Spray dates				Blighted spurs (current season) (%)[1]
			Bloom April 6	May 8	June 12	July 10	
1.	Fontelis[4]	20 fl oz		Fontelis	Fontelis	Fontelis	5.6 abc[2]
2.	Fontelis +OR 009	20 fl oz 0.3%		Fontelis+ OR 009	Fontelis+O R 009	Fontelis+O R 009	2.4 a
3.	Fontelis[4] + Tebucon 45DF	20 fl oz 8 oz		Fontelis+ Tebucon	Fontelis+ Tebucon	Fontelis+ Tebucon	4.0 ab
4.	EXP 1[4]	5.14 fl oz		EXP-1	EXP-1	EXP-1	3.0 ab
5.	EXP 1[4] EXP 2	3.43 fl oz 5.48 fl oz		EXP 1 EXP 2	EXP 1 EXP 2	EXP 1 EXP 2	2.9 ab
6.	EXP 1[4] EXP 3	3.43 fl oz 4.57 fl oz		EXP 1 EXP 3	EXP 1 EXP 3	EXP 1 EXP 3	2.7 ab
7.	Pristine[4]	14.5 oz		Pristine	Pristine	Pristine	3.6 ab
8.	Merivon[4]	6.8 fl oz	Merivon	Merivon	Merivon	Merivon	1.6 a

9.	Quadris Top[4]	14 fl oz		Quadris Top	Quadris Top	Quadris Top	5.6 abc
10.	Viathon 4.1 FS[4]	4 pints		Viathon	Viathon	Viathon	6.0 abc
11.	Luna Sensation[4]	5 fl oz		Luna Sen 5	Luna Sen 5	Luna Sen 6	8.6 bc
12.	Luna Sensation[4]	7.6 fl oz		Luna Sen 7.6	Luna Sen 7.6	Luna Sen 7.6	3.0 ab
13.	Luna Experience[4]	6 fl oz		Luna Exp 6	Luna Exp 6	Luna Exp 6	4.3 ab
14.	Luna Experience[4]	10 fl oz		Luna Exp 10	Luna Exp 10	Luna Exp 10	3.9 ab
15.	K Phite 7LP	3 quarts		K Phite	K Phite	K Phite	2.2 a
16.	K Phite 7LP + Latron B-1956[3]	3 quarts 4 fl oz		K Phite 7LP + Latron	K Phite 7LP + Latron	K Phite 7LP + Latron	2.7 ab
17.	Badge[4] + Manzate ProStick	4 lbs 2.4 lbs		Badge+ Manzate ProStick	Badge+ Manzate ProStick	Badge+ Manzate ProStick	4.5 ab
18.	Quash[4]	3.5 oz		Quash	Quash	Quash	2.0 a
19.	Ph-D[4]	6.2 oz		PH-D 6.2	PH-D 6.2	PH-D 6.2	4.7 ab
20.	Ph-D[4] + Tebucon 45	6.2 oz 4 oz		PH-D 6.2 Tebucon	PH-D 6.2 Tebucon	PH-D 6.2 Tebucon	2.0 a
21.	Control	Untreated					11.9 c

1 100 current season shoots for each of 3 replicated Chandler walnut trees were recorded on November 7, 2014.

2 Numbers followed by different letters are significantly different according to the LSD test at $P = 0.05$. Statistical analysis was performed on arcsine transformed data. Values presented were back transformed from the means for the arcsine transformed data.

3 The surfactant Latron 1956 at 0.0625% vol./vol. was added.

4 The surfactant Induce at 0.25% vol./vol. was added.

Colusa County trial - Botryosphaeria. There were sprays done during the season as well as postharvest for some of the treatments in this orchard. All the fungicide treatments significantly reduced cankers in the spurs (Table 4) and also infections of nuts (Figure 7), suggesting that, even when the disease is low in a dry year like 2014 was, sprays during the growing season can reduce Botryosphaeria canker and blight of walnut. These results support the results recorded in the Butte County trial (**Table 3**). To determine differences in the number of blighted nuts after natural infection in the field, two treatments were evaluated only: the untreated control had an average of 38 fruit per tree and those sprayed with Merivon had 21 black fruit per tree. (All the black nuts of each of 10 trees were counted in each of the four replications per treatment.) This difference was significant ($P < 0.05$). When the black and brown nuts were plated to isolate Botryosphaeriaceae spp., we also isolated *Alternaria, Fusarium, Nigrospora, Phomopsis* and *Aspergillus niger*. These fungi are very common also in walnut orchards and orchards of other nut crops grown in California.

Table 4. Fungicide efficacy trial against **Botryosphaeria canker and shoot blight** in Chandler orchard in Colusa County - 2014.

Trt #	Fungicide Treatment	Rate	Spray dates				Blighted spurs (current season) (%)[1]
			Mid-May (May 16)	Mid-June (June 16)	Mid-July (July 16)	Post-harvest (Oct 15)	
1.	Control						11.9 b[2]
2.	Merivon	6.5 fl oz	Merivon	Merivon	Merivon	---	6.2a
3.	Luna Experience	10 fl oz	Luna Experience	Luna Experience	Luna Experience	---	7.4 ab
4.	Fontelis + Tebucon	20.0 fl oz 8.0 oz	Fontelis + Tebucon	Fontelis + Tebucon	Fontelis + Tebucon	---	6.1 a
5.	Quadris Top	14 fl oz	Quadris Top	Quadris Top	Quadris Top	---	7.0 ab
6.	Merivon	6.5 fl oz	Merivon	Merivon	Merivon	Merivon	6.2 a
7.	Luna Experience	10 fl oz	Luna Experience	Luna Experience	Luna Experience	Luna Experience	3.8 a
8.	Fontelis + Tebucon	20.0 fl oz 8.0 oz	Fontelis + Tebucon	Fontelis + Tebucon	Fontelis + Tebucon	Fontelis + Tebucon	5.2 a
9.	Quadris Top	14 fl oz	Merivon	Merivon	Quadris Top	Quadris Top	3.7 a

[1]100 current season spurs for each of 4 replicated Chandler walnut tree rows were recorded on November 13, 2014.

[2]Numbers followed by different letters are significantly different according to the LSD test at $P = 0.05$. Statistical analysis was performed on arcsine transformed data. Values presented were back transformed from the means for the arcsine transformed data.

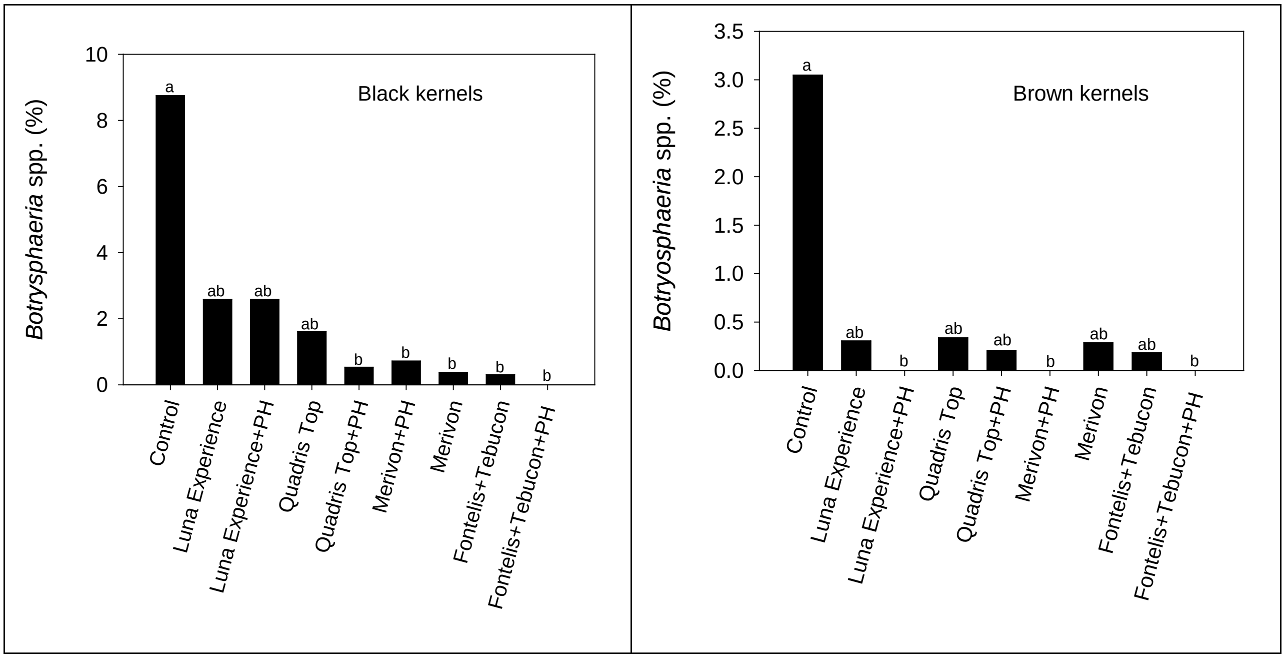

Figure 7. Isolations of Botryosphaeriaceae from black and brown kernels of walnuts collected after shaking the trees for harvest the walnuts from a fungicide efficacy trial on a Chandler orchard in Colusa County (2014).

Sutter County trial – Botryosphaeria. In the Howard walnuts, only bloom, bloom + postharvest, and only postharvest treatments were replicated and tested. Only the trees sprayed at bloom and postharvest (trt #4) showed a trend in some reduction of incidence of cankered spurs but there were no significant differences between the untreated trees and those sprayed at bloom only or only postharvest (**Table 5**).

Sutter County trials – Botryosphaeria. In the first Chandler walnuts (Block 4A), where the mid-May, mid-June, and mid-July sprays were applied, there was a significant reduction of disease in comparison with the untreated control (**Table 6**). These results agree with the results obtained in Butte (Table 3) and in Colusa (Table 4). Therefore, sprays done during the season (May through July) are necessary for controlling Botryosphaeria canker and blight disease of walnut.

Table 5. Fungicide efficacy trial against **Botryosphaeria canker and shoot blight** on Howard walnuts in Sutter County - 2014.

Trt #	Fungicide treatment	Rate	No. of sprays	Timing	Blighted spurs (current season) (%)[1]
1.	Untreated check				20.4 ab[2]
2.	Merivon	6.5 fl oz	1	Bloom	21.7 ab
3.	Merivon	6.5 fl oz	1	Postharvest	26.2 b
4.	Merivon	6.5 fl oz	2	Bloom + Postharvest	17.1 a
5.	Luna Experience	10.0 fl. oz	1	Bloom	22.0 ab
6.	Luna Experience	20.0 fl. oz	1	Postharvest	23.2 ab

1 100 current season spurs for each of 4 replicated Howard walnut tree rows were recorded on December 9, 2014.

2 Numbers followed by different letters are significantly different according to the LSD test at *P* = 0.05. Statistical analysis was performed on arcsine transformed data. Values presented were back transformed from the means for the arcsine transformed data.

3 Label rate is 17.0 fl. oz./acre.

Table 6. Fungicide efficacy trial against **Botryosphaeria canker and shoot blight** on Chandler walnuts in Sutter County - 2014 (Block 4A).

Trt #	Fungicide treatment	Rate	No. of sprays	Timing	Blighted spurs (current season) (%)[1]
1.	Untreated check				12.4 b[2]
2.	Merivon	6.5 fl oz	3	mid-May, mid-June, mid-July	5.6 a
3.	Luna Experience	10 fl oz	3	mid-May, mid-June, mid-July	7.0 ab
4.	Fontelis + Tebucon	20 fl oz 8 oz	3	mid-May, mid-June, mid-July	5.9 a
5.	Quadris Top	14 fl. oz	3	mid-May, mid-June, mid-July	5.0 a

1 100 current season spurs for each of 4 replicated Chandler walnut tree rows were recorded on December 9, 2014.

2 Numbers followed by different letters are significantly different according to the LSD test at *P* = 0.05. Statistical analysis was performed on arcsine transformed data. Values presented were back transformed from the means for the arcsine transformed data.

Sutter County trials – Botryosphaeria. In the second Chandler walnut (Block 1), only the Merivon treatment significantly reduced the incidence of cankered spurs in comparison with the untreated control (Table 7). All the other treatments showed a trend in reducing disease (Table 7) as compared to the untreated control. Interestingly, the incidence of the disease in this orchard was about half of that recorded in the Chandler Block 4A (Table 6), probably because the trees were of different age and had different levels of spore inoculum.

Table 7. Fungicide efficacy trial against **Botryosphaeria canker and shoot blight** on Chandler walnuts in Sutter County - 2014 (Block 1).

Trt #	Fungicide treatment	Rate	No. of sprays	Timing	Blighted spurs (current season) (%)[1]
1.	Untreated check				6.3 b[2]
2.	Merivon	6.5 fl oz	3	mid-May, mid-June, mid-July	4.7 ab
3.	Luna Experience	10 fl oz	3	mid-May, mid-June, mid-July	4.2 ab
4.	Fontelis + Tebucon	20 fl oz 8 oz	3	mid-May, mid-June, mid-July	2.9 ab
5.	Quadris Top	14 fl. oz	3	mid-May, mid-June, mid-July	2.8 ab
6.	Merivon	6.5 fl oz	4	mid-May, June, July + bloom	4.9 ab
7.	Luna Experience	10 fl oz	4	mid-May, June, July + bloom	2.3 ab
8.	Fontelis + Tebucon	20 fl oz 8 oz	4	mid-May, June, July + bloom	3.9 ab
9.	Quadris Top	14 fl. oz	4	mid-May, June, July + bloom	2.7 ab
10.	Merivon	6.5 fl oz	4	mid-May, June, July + PH	1.1 a
11.	Luna Experience	10 fl oz	4	mid-May, June, July + PH	5.6 b
12.	Fontelis + Tebucon	20 fl oz 8 oz	4	mid-May, June, July + PH	4.9 ab
13.	Quadris Top	14 fl. oz	4	mid-May, June, July + PH	4.8 ab

[1] 100 current season shoots for each of 3 replicated Chandler walnut tree rows were recorded on December 9, 2014.

[2] Numbers followed by different letters are significantly different according to the LSD test at $P = 0.05$. Statistical analysis was performed on arcsine transformed data. Values presented were back transformed from the means for the arcsine transformed data.