Band Canker Detection in Young Almond Trees Before Planting or Just After Planting in the Field Before Disease Symptoms Appear

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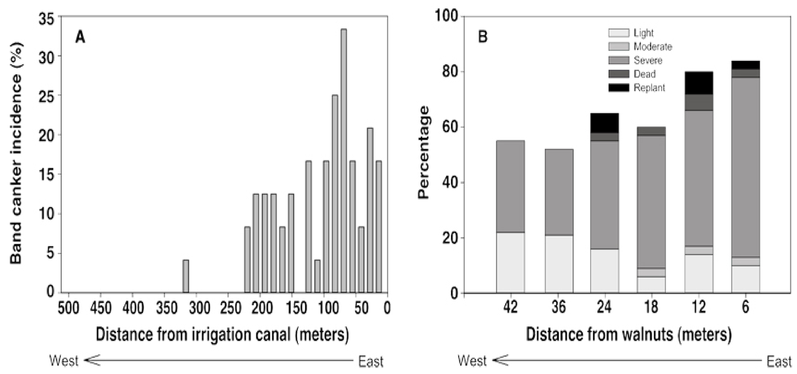
Background:
Band canker of almond is caused by seven different fungal species in the family of Botryosphaeriaceae. During the 2004 to 2007 studies of the band canker disease of almond, we determined the spread of the disease from an external source. For instance, in a 3-year-old Nonpareil/Padre orchard in Fresno County, the incidence of band canker disease was higher closer to the source of inoculum (riparian trees along an irrigation canal) than far from the canal (**Figure 1A**). In fact, no disease was found in any of the trees beyond 325 meters (1,066 feet). Botryosphaeriaceae species were abundant in the riparian trees and bushes as well found along the irrigation canal.

In a second 5-year-old Nonpareil/Aldrich/Peerless orchard in Butte County, band canker disease was more abundant and more severe closer to a 15-year-old walnut orchard loaded with dead branches which were covered with both pycnidia (producing water-splashed spores) and pseudothecia (producing airborne ascospores) of *Botryosphaeria dothidea* (**Figure 1B**). These patterns of disease spread in these orchards suggest an external source of spore inoculum.

Recently though, we found young almond orchards with band canker that developed in a uniform pattern in the orchard (**Figures 2 and 3**), suggesting that there was no external source of inoculum in these orchard, instead the disease developed uniformly throughout the orchard. This uniform pattern of Botryosphaeria disease appearance in these young orchards suggests that perhaps the inoculum was brought into the orchard on the trees themselves. To test this hypothesis we developed a qPCR technique to test whether the propagules (or latent infection) of Botryosphaeriaceae fungi were present in very young symptomless (healthy looking) trees.



A, 3-yr-old Nonpareil/Padre; inoculum source: riparian trees and water canal

B, 5-yr-old Nonpareil/Aldrich/ Peerless; source: a 15-yr-old walnut orchard.

Figure 1. Spread pattern of band canker from an external source of pathogen's inoculum.

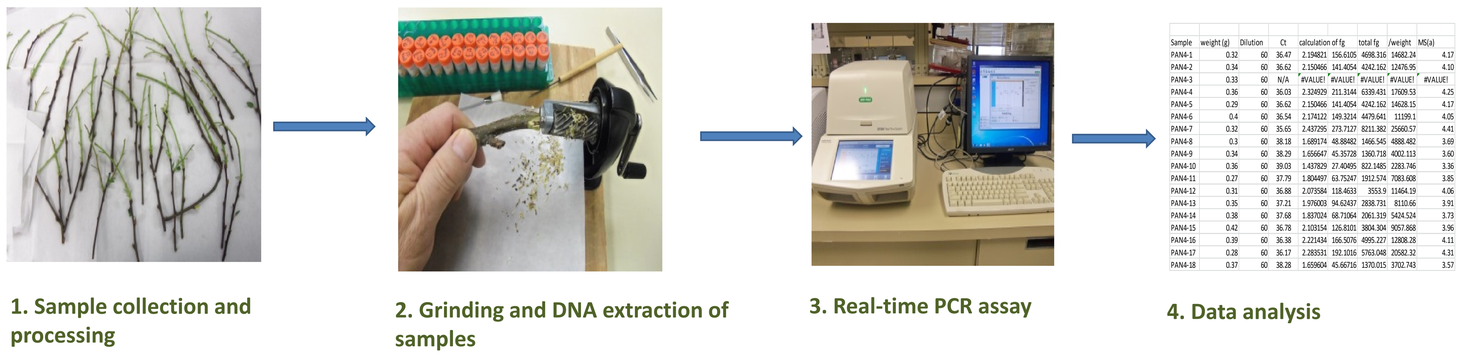
Figure 2. Overview of a 2nd-leaf almond orchard with gaps due to tree removal because of band canker (Glenn County).

Figure 3. Overview of a young almond orchard showing tree gaps because of infection by band canker that developed in a uniform pattern (orchard in Glenn County).

Research approach:
1. Three orchards (1st leaf, 2nd leaf, and 3rd leaf) were selected.
2. The new emerged and 1-year-old (2nd leaf and 3rd leaf) shoot samples were randomly collected on 15 June 2017.
3. Molecular primers were designed to target 6 canker-causing pathogen groups: *Phomopsis* spp., *Botryosphaeria dothidea, Lasiodiplodia* spp., *Cytospora* spp., *Neofusicoccum* spp., and *Diplodia* spp.
4. The *q*PCR was applied to quantify the latent infection level.
5. Latent infection quantification was expressed in three ways as follows:

a) Incidence of latent infection (I): I = The number of samples showing positive / the total number of samples × 100.
b) Molecular Severity (MS): MS = $\log_{10}$(P/H), where
P = the weight of the pathogen's DNA in femtograms (fg) from the standard curve; [1 femtogram (fg) = 0.000000000000001 g]
H = the weight of shoot in grams (g). The range of MS value is 0–15.
c) Index of latent infection (ILI): ILI = Incidence (I) × MS / 100

Figure 4 Shows the working approach for this project.



Sample	weight (g)	Dilution		Ct	calculation of fg	total fg	/weight	MS(a)	
PAN4-1	0.32		60	36.47	2.194821	156.6105	4698.316	14682.24	4.17
PAN4-2	0.34		60	36.62	2.150466	141.4054	4242.162	12479.95	4.10
PAN4-3	0.33		60	N/A	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
PAN4-4	0.36		60	36.03	2.324929	211.3144	6339.431	17609.53	4.25
PAN4-5	0.29		60	36.62	2.150466	141.4054	4242.162	14628.15	4.17
PAN4-6	0.4		60	36.54	2.174122	149.3214	4479.641	11199.1	4.05
PAN4-7	0.32		60	35.65	2.437295	273.7127	8211.382	25660.57	4.41
PAN4-8	0.3		60	38.18	1.689174	48.88482	1466.545	4888.482	3.69
PAN4-9	0.34		60	38.29	1.656647	45.35728	1360.718	4002.113	3.60
PAN4-10	0.36		60	39.03	1.437829	27.40495	822.1485	2283.746	3.36
PAN4-11	0.27		60	37.79	1.804497	63.75347	1912.574	7083.608	3.85
PAN4-12	0.31		60	36.88	2.073584	118.4633	3553.9	11464.19	4.06
PAN4-13	0.35		60	37.21	1.976003	94.62437	2838.731	8110.66	3.91
PAN4-14	0.38		60	37.68	1.837024	68.71064	2061.319	5424.524	3.73
PAN4-15	0.42		60	36.78	2.103154	126.8101	3804.304	9057.868	3.96
PAN4-16	0.39		60	36.38	2.221434	166.5076	4995.227	12808.28	4.11
PAN4-17	0.28		60	36.17	2.283531	192.1016	5763.048	20582.32	4.31
PAN4-18	0.37		60	38.28	1.659604	45.66716	1370.015	3702.743	3.57

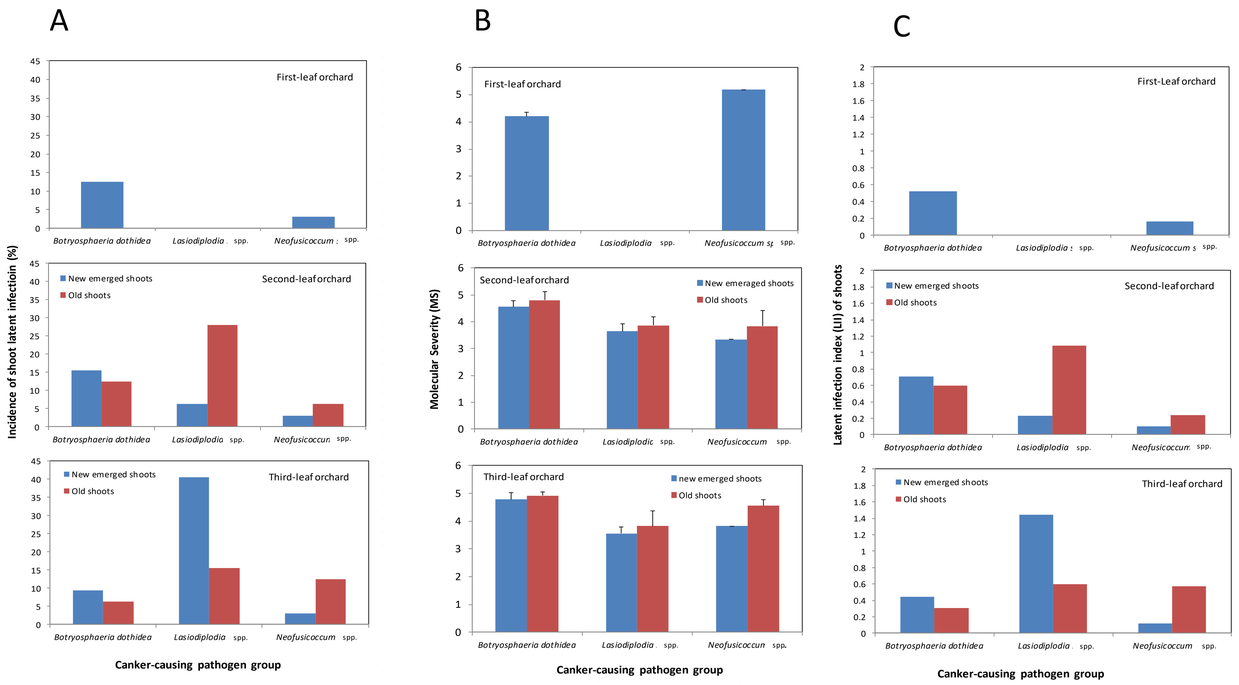


Figure 5. Incidence (**A**), molecular severity (**B**) and index of latent infection (**C**) of canker fungi in newly emerged and 1-year-old symptomless almond shoots collected from one each first-leaf, second-leaf, and third-leaf orchards located in Glenn County.

Conclusions:
1) Two band canker pathogens, *Botryospaeria dothidea and Neofusicoccom* spp. were detected in newly-emerged shoots of the first-leaf orchard with incidences of 12.5% and 3.13%, respectively.
2) Three canker pathogens, *Botryosphaeria dothidea, Lasiodiplodia* spp. *and Neofusicoccom* spp. were detected in both newly-emerged and 1-year-old shoots in the second-leaf and third-leaf orchards, while incidences varied.
3) Regardless of the orchard age, the Molecular Severity levels ranged from 4.0 – 5.0, suggesting low levels of infection.
4) The levels of index of latent infection were below 1.0 for all the samples, except for the *Lasiodiplodia* spp. (1-year-old shoots in the second-leaf orchard and in the newly-emerged shoots in the third-leaf orchard.
5) Although it may take some time to detect the pattern of accumulation of latent infection in trees of different age, the three band canker pathogen groups were consistently detected in newly-emerged and 1-year-old shoots of very young symptomless almond trees.