Investigating Incidence and Type of Wood-Decay Fungi Associated With Windfall in Almond

Project No.:   **16-PATH11-Rizzo**

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Objectives:

1. Identify the main fungi associated with windfall caused by wood-decay diseases of almond in California
2. Determine patterns of infection in orchards and possible sources of inoculum
3. Deploy taxonomic specific primers for improved diagnostics and early detection of decay fungi in standing trees.
4. Assess rootstock resistance and susceptibility.

Interpretive Summary:

Butt rot is the most poorly understood of the wood decay types associated with almond and perhaps the most important. Butt rot fungi decay occurs on the tree from the bottom up as well as from the inside out, often causing no obvious external symptoms on the host. Windfall related to butt rot is one of the factors limiting almond orchard age to 20-25 years. Butt rot has long been recognized in California orchards; but was usually considered a problem of older orchards, often associated with weak or already diseased trees. Recent observations by growers suggests loss of younger, apparently healthy trees due to windfall is becoming a problem in some locations. Surveys for wood decay fungi in almond orchards began in late 2015 and identified *Ganoderma* species as the primary cause of decay in windfall trees.

- Two *Ganoderma* species were identified in California almond orchards, *G. brownii* and *G. adspersum.*

- *G. brownii* is endemic to California and seems to be less aggressive. Infection was limited to a few trees in older orchards, or in trees already suffering from some other disease.
- *G. adspersum*, previously unreported in California, is native to Europe and is more aggressive than *G. brownii*. It has been found in orchards as young as 7 years old and incidence of infection tends to be high.
- *G. adspersum* has only been identified from orchards in the middle to southern San Joaquin Valley.
- *G. adspersum* infections are confirmed in 5 counties, with an average orchard age of 14.
- Significant tree loss from *G. adspersum* has resulted in the complete removal of almond orchards as young as 10 years old.
- The site of initial infection is typically at or below the soil line and most likely corresponds to unseen damage caused by shaking during harvest.
- The primary inoculum source for *Ganoderma* infections is most likely airborne spores from fruiting bodies (conks) formed on the stem of infected trees. Sporulating conks have been observed year-round in infested almond orchards including during harvest season.
- All confirmed reports to-date of *G. adspersum* are on almonds planted on peach rootstock and in sandier soils.
- In laboratory tests, *G. adspersum* decays wood significantly faster than *G. brownii*. *G. adspersum* tended to decay rootstocks with almond parentage more slowly than those with peach or plum parentage.
- *G. adspersum* mycelia was viable in woody debris with a volume of 0.91 L and larger after 16-months buried in the soil.
- Sonic tomography offers an efficient and non-invasive method for screening standing trees for decay.

Materials and Methods:

Identify the main fungi associated with windfall caused by wood-decay diseases of almond in California

We carried out orchard surveys from the Fall of 2015 through the Fall of 2017. Windfall trees were sampled as they became known. Windfall trees in 34 orchard blocks in 8 counties were sampled. As a result of early findings and substantial grower response our focus in 2017 largely shifted to understanding the current and potential impact of *Ganoderma* root and butt rot in almond orchards.

Sampling in an orchard includes collection of fungal fruiting bodies and decayed wood from windfall and the lower stem of standing trees. Wood decay or fungal fruiting body context tissue are plated (2mm x 2mm pieces) onto Malt Extract Agar (MEA) amended with benomyl (4µg a.i./ml) and streptomycin sulfate (100 µg/ml) and sub-cultured onto MEA after incubation at room temperature for 7-14 days. Fungal species are first identified to the genus level based on morphological characteristics of fruiting bodies and growth in culture. DNA is extracted from

pure cultures using Prepman Ultra Sample Preparation Reagent followed by PCR amplification of the ITS region. Isolates are sequenced and identified using BLASTn searches in GenBank.

Determine patterns of *Ganoderma* infection in orchards and possible sources of inoculum
Patterns of *Ganoderma* infection are being investigated on three scales:
- The tree scale
- The orchard scale
- Landscape scale

Tree scale
Sampling of windfall trees entailed visual inspection for fruiting bodies and any accompanying symptoms followed by destructive sampling. Trees were assigned a decay severity rating at each of four cross-sectional cuts made to the scaffold branches, 18 inches above the graft, directly above the graft, directly below the graft, and to the root mass as a whole. Decay severity ratings were (0-4) with 0 being no visible decay and 4 being complete decay. Presence or absence of crown gall symptoms were also noted. Nearby standing trees were also visually inspected for any signs or symptoms characteristic of wood decay.

Several root excavations were carried out using a backhoe, but this was limited in its usefulness, as it required the destruction of a tree and significant effort on the part of the grower. However supersonic air excavation, used by commercial arborists, and previously used to investigate *Armillaria* in pears, grapes, and other perennial crops proved to be less invasive and more time efficient. Partial excavations of windfall and standing trees using supersonic air as described by Rizzo et al. (1998) allowed for evaluation and sampling of the belowground portion of the tree, without causing significant damage to trees. During the Spring and Summer of 2017, we excavated portions of root systems to determine the initial site of infection and investigate the link between infection and possible trunk symptoms. However, we found that excavation with the airspade was much more efficient when soil moisture was high thus limiting the usefulness to the winter months.

In January 2018, in collaboration with the Gilbert lab at UC Santa Cruz, we employed an Argus Electronic PiCUS 3 Sonic Tomograph to non-invasively estimate the extent of decay inside symptomatic and asymptomatic standing trees in an 11-year-old orchard infected with *G.adspersum* in Madera County. Sonic tomography utilizes measurements of the sound velocity traveling between known points around the circumference of the trunk of a tree. The velocity at which sound travels through wood is partially a function of the wood density, with higher velocities in denser wood and slower velocities through less dense decayed wood. Sonic tomography provides a non-invasive method to estimate and quantify decay within standing trees and could prove useful in evaluating orchards for early evidence of infection (Gilbert et al., 2016). We scanned a total of 18 trees, two of which were scanned at multiple depths, all others were scanned at a depth of 10cm below the soil line. Scanned trees fell into 3 categories, asymptomatic, symptomatic, and known infected trees. Asymptomatic trees appeared healthy and had no obvious signs of disease. Symptomatic trees had a flat strip up the trunk, a cleft at the graft union, and the grower reported it exhibited a general decline. Known infected trees were similar to symptomatic trees except they also had a *Ganoderma* fruiting body near the soil line.

Soil was excavated from around the base of the tree to a depth of approximately 15cm. Following measurements of circumference, nine or ten furniture tacks were inserted through the bark into solid wood at equal intervals around the circumference. The PiCUS 3 was used to map the contour of the trunk and then was connected to the furniture tacks. Scans were made according to operating protocol and heat maps of trunk density were digitally generated. Samples of decay were collected from scanned trees for isolation in the laboratory.

Orchard Scale
Understanding the spatial and temporal progression of infection within an orchard is vital to understanding the epidemiology of the disease and forecasting potential impacts. Mapping infection in the orchard was carried out utilizing several methods. Grower interviews provided information on rootstock, irrigation, cultural practices and site history. In some orchards each individual tree within the orchard was assessed for evidence of infection and isolates from infected trees were collected. In three orchard blocks scheduled for removal, a subsample of 100 trees in each was destructively sampled. Historical aerial images accessed through Google Earth were utilized to record and map tree mortality over time for individual orchard blocks. Each orchard block with a confirmed *Ganoderma* infection as well as orchards for which the status of *Ganoderma* infection was unknown were mapped. We recorded locations of missing tree and replanted trees for each year for which a high-quality image was available and generated an orchard decline curve.

Somatic compatibility groupings are a common way to determine if two *Ganoderma* isolates are the same or different individuals and suggests whether the infection was a result of spore-borne inoculum or the pathogen is spreading via root to root contact.

Sixty isolates of *G. adspersum* were obtained from windfall trees at the Hanford site and were used to determine somatic compatibility groups. We selected 10 of these isolates that originated from trees adjacent to or near each other within the orchard. Each isolate was paired with all others and itself. Plugs of mycelia were placed 1cm apart on a common plate of MEA and incubated at 25°C in the dark. After 14 days, isolates had either fused or developed a barrage line. Isolates that fuse when they meet are assumed to be the same individual, whereas isolates that are antagonistic and formed a barrage line are assumed different individuals. The experiment was repeated twice, and twice again with a different set of 10 isolates.

The length of time mycelia persists on woody debris in the soil and remain infectious varies greatly and is influenced by a few factors including, soil moisture, size of debris, type of debris and species of fungi. The Almond Board of California has funded research to explore orchard recycling at the end of orchard life, where trees are ground up in place and incorporated into the soil instead of removing the entire orchard biomass. If mycelia in woody debris can serves as a source of inoculum, understanding how long mycelia can survive and remain infectious once incorporated into the soil will help a grower decide how to proceed with tree removal if there is a high incidence of *Ganoderma* root and butt rot.

Thirty *G. adspersum* colonized stumps from recently toppled 10-year-old trees from Kings County, were collected for use in an inoculum survival study. Each stump was cut at the graft

union and below the graft union to a length of 9 inches, each was then cut longitudinally into four quarters. Two quarters were kept whole and two quarters from each stump were further subdivided into six size classes of decreasing volume. Following isolation from each piece, the pieces from each stump were placed into two separate five-gallon buckets with drainage holes in the bottom, for a total of 60 buckets. Each bucket contained seven size classes with average volumes, 1.82, 0.91, 0.46, 0.23, 0.11, 0.06, and 0.03 L. The wood pieces were buried in the buckets taking care to adequately surround each wood piece with soil, and the buckets were buried below the soil line. Buckets and wood pieces from one half of each stump were removed after 7 months while the other half was removed after 16 months. Isolations were attempted on each wood piece. Successful re-isolation confirmed the potential for infectious inoculum to remain in the soil following orchard removal.

Landscape Scale
In addition to *Ganoderma* samples collected from orchards, *Ganoderma* samples have been collected from non-agricultural areas throughout California. *Ganoderma* isolates are plotted on a map of California. Our *Ganoderma* sequences and *Ganoderma* sequences from GenBank were used to construct a Maximum Likelihood phylogenetic tree of the ITS region.

In addition to collection of *Ganoderma* samples we have begun collaboration with SCANIT Technologies to better understand seasonal and regional patterns of *Ganoderma* spore inoculum. SCANIT has developed an automated airborne particle classification and monitoring system. Unlike conventional spore traps that require substantial labor and time to collect, identify, and quantify spores of interest, their system is designed to remotely classify and report, via a web-based application, inoculum levels in real-time. Particles are imaged with a patent pending light illumination techniques that force unique particle signatures. Once deployed in the field, imaged particles are compared to a cloud-based particle image library and machine learning techniques are used to identify particles. We have provided spores of 5 *Ganoderma* species and SCANIT is currently generating baseline data to add to the particle image library. The first spore trap will be deployed in the field in early Fall 2018.

Deploy taxonomic specific primers for improved diagnostics and early detection of decay fungi in standing trees.
Efficient culture independent diagnostic protocols for identification of wood decay pathogens are being adapted for use in Almond. Guglielmo et al. (2010) optimized a sampling protocol using a small drill bit to collect wood frass from standing trees that minimizes damage to trees. Isolation and identification of fungi from frass samples will remain as described above. Once some of the main fungal associates are identified, a protocol for PCR-based identification like that used by Guglielmo et al. (2007 and 2010) will be developed for almond using the DNA extractions directly from wood frass. We are in the process of developing PCR primers to distinguish between *G. adspersum and G. brownii* without the expense and time of sequencing.

Assess rootstock resistance and susceptibility
Rootstock susceptibility to *Ganoderma* and other wood decay pathogens is largely unknown. Several different approaches to determining susceptibility are being utilized.

Inoculation of Wood Blocks

Two wood decay rate experiments were carried out to ascertain differences in decay rate among various rootstocks by different *Ganoderma* isolates. Each experiment was a full factorial design with each rootstock/isolate combination replicated 10 times. The rootstock varieties included in these experiments were chosen in consultation with and material was provided by Tom Gradziel (TG) and Sierra Gold Nursery (SG). Branch material of different rootstock varieties was used in both experiments. Rootstocks and their parentage are listed in (**Table 1**). Nonpareil was included in both experiments as an almond standard. *Ganoderma* isolates and their sources are listed in (**Table 2).**

Table 1. Rootstock varieties included in wood decay experiments

Rootstock	Parentage	Experiment	Source
Nonpareil	almond	1,2	TG
Hrse-East	almond	2	TG
Hrse-West	almond	2	TG
Arbuckle	almond	2	TG
3-305	almond (*P. scoparia*) x peach	2	TG
1-107	almond (*P. tangutica*) x peach	2	TG
20-192	almond x peach	2	TG
Hansen 536	almond x peach	1,2	SG
Viking	almond x peach x plum x apricot	2	SG
Empyrean 1	peach	2	SG
Nemaguard	peach	1,2	SG
Lovell	peach	1,2	SG
Krymsk 86	peach x plum	1,2	SG
Myrobalan 29c	plum	2	SG
Mariana 2624	plum	1,2	SG

Table 2. *Ganoderma* isolates included in wood decay experiments.

Treatment	Species	Isolate number	Source	Experiment
GA1	*G. adspersum*	BJ161	almond - Kings	1,2
GA2	*G. adspersum*	BJ293	almond - Tulare	1,2
GA3	*G. adspersum*	BJ297	almond - Tulare	2
GR	*G. resinaceum*	BJ286	oak - Yolo	2
GB1	*G. brownii*	BJ88	almond - Solano	1,2
GB2	*G. brownii*	BJ194	almond - Kings	1,2

Experiment 1 included five rootstock varieties and four *Ganoderma* isolates. Branches with a diameter of 1 – 1.5cm were cut into 3cm pieces and fresh mass of each piece was recorded. Ten pieces of each variety were immediately placed in a 60°C drying oven for 48hrs, and their dry masses used to calculate an approximate moisture content. Following surface sterilization each wood piece was placed onto either, a 10-day old culture of *Ganoderma* grown on a plate

of 1.5% MEA or on a fresh plate of 1.5% MEA as a control. Plates were sealed with parafilm and cultures were maintained in the dark for six weeks at room temperature. After six weeks, the wood pieces were removed from the cultures and surface mycelia was removed. Wood pieces were dried at 60°C for 48hrs and dry mass was recorded. Percent moisture content and initial fresh weight were used to calculate predicted initial dry weights for each wood piece.

Experiment 2 included 14 rootstock varieties and six *Ganoderma* isolates and was repeated with fresh and autoclaved wood pieces. Branches with a diameter of 1.5 – 2.5cm after bark removal were cut into 2cm pieces. Fresh mass was recorded for all pieces and the pieces to be autoclaved were dried as described previously and weighed prior to rehydration and autoclaving for 30 minutes twice. Each replicate was randomized, and wood blocks were placed on a wire mesh inside a moist chamber. A plug of mycelia (3mm x 3mm) from 14-day old *Ganoderma* cultures were placed on top of each block. Moist chambers were maintained in the dark at room temperature for 12 weeks. After twelve weeks, surface mycelia were removed, and blocks and were dried and weighed as previously described. As in experiment 1 predicted initial dry weights were calculated for fresh blocks.

In both experiments an Analysis of Variance was performed on mass loss using initial dry mass or predicted initial dry mass as a covariable. Tukey's mean separation of mass loss was performed on *Ganoderma* isolates. in experiment 1, control treatments were removed to determine differences in mass loss among rootstock varieties. Similarly, in experiment 2, all treatments except for *Ganoderma adspersum* were removed for analysis.

Wood block decay experiments are currently being repeated.

Inoculation of Saplings
Potted nemaguard/wood colony seedlings with an approximate diameter at the soil line of 2cm were provided by SierraGold Nursery and repotted into 3 gal pots using UC potting mix. Trees were maintained in a growth chamber at 23C and irrigated once weekly.

Inoculum was prepared by placing twice autoclaved 6.4 mm x 19 mm hardwood dowels on an actively growing culture of *G. adspersum* and incubating in the dark for 6 weeks at room temperature. Twice autoclaved dowels were used for mock inoculations.

Half of the trees were randomly assigned *G. adspersum* while the others received mock inoculation. The soil level in each pot was lowered to slightly expose the uppermost surface roots, and the site of inoculation was surface sterilized with 95% ethanol. An 8 mm diameter hole was drilled 2.5 cm above the soil line and the drill bit was flame sterilized between trees. Dowels were inserted in the holes and wrapped with two layers of parafilm and an outer coating of aluminum foil.

After 12 weeks each tree was cut approximately 5 cm below the inoculation site and 5 cm above. Using a pair of pruning shears, the 10 cm piece of trunk was split longitudinally into 4 sections. Isolations were made from decayed tissue a minimum distance of 0.5 cm from the inoculum and the maximum distance from inoculum from which re-isolation was possible on the decay column was recorded.

SierraGold Nursery provided potted trees of common rootstock varieties and inoculations as described above will take place in the Fall of 2018.

Non-Destructive Sampling of ABC Rootstock Trials
The almond industry has partnered with UC Cooperative Extension on trials to evaluate rootstocks. As these trials age the opportunity arises to evaluate the original treatments for effects on the incidence and severity of root and butt rot. The culture independent developed in objective 3 will be used to non-destructively look for the presence or absence of wood-decay pathogens in these trials.

Results and Discussion:

Objective 1.
Wood decay fungal genera isolated from the lower trunk and roots of windfall trees from Fall 2015 through Fall 2017 are presented in (**Table 3).** *Ganoderma* was the most prevalent genus causing damage in almond orchards, with *Ganoderma adspersum* being the most prevalent species (**Figures 1 and 2**). *Oxyporus laetimarginates* and *Phlebia* sp. also caused severe root and butt rot resulting in windfall but were not widespread within the orchards in which they were found. The tree from which *Oxyporus laetimarginates* was isolated failed 12" above the soil surface at an old shaker wound but had a decay column that extended from the crotch to the butt of the tree, well below the soil line. *Armillaria mellea* did not result in windfall, but killed trees standing, it was widespread in the orchard and exhibited characteristic disease centers. The other wood decay fungi identified are not known to be pathogenic and are acting as saprophytes coming in on dead or previously decayed wood.

Table 3. Summary of wood decay fungi isolated from roots and lower trunks of windfall trees over a two-year period.

Fungus	Tree status[1]	Type[2]	Distribution[3]
Armillaria mellea	S, D	P	1
Coprinellus micaceus	W	N	1
Ganoderma brownii	W, S	P	4
Ganoderma adspersum	W, S	P	28
Oxyporus laetimarginates	W	P	1
Peniophorella pubera	W	N	1
Phlebia sp.	W, S	P	2
Psathyrella sp.	W	N	3
Punctularia sp.	W	N	1
Stereum hirsitum	W	N	3
Trametes versicolor	W, S	N	4
[1] W = windfall, S = standing, D = standing dead. [2]N = nonpathogenic, P= pathogenic. [3] The total number of orchards from which specific fungi was isolated.			

An important finding from these surveys the high prevalence of *G. adspersum*, previously unreported in North America. During the two years of the survey we identified 28 orchard blocks in the San Joaquin Valley with *G. adspersum* infections. *G. adspersum* was shown to be more aggressive than other *Ganoderma* species and can overcome the reaction zone of the tree that generally confines wood decay fungi to the heartwood (Schwarze and Ferner, 2003).

Objective 2.

Tree Scale
Destructive sampling and root excavation revealed that most decay is occurring below the soil surface and that infections are initiated at or below the soil line. Crown gall symptoms were present in some but not all infected and windfall trees. Picus scans at different depths concur with this earlier finding and the amount and severity of decay increased dramatically with only a few centimeters increase in depth (**Figure 5**).

Evidence of *Ganoderma* infection in an orchard include windfall and conks at or near the base of the tree, often accompanied by a flat strip on the trunk of the tree above the conks, presumably above the site of the initial infection (**Figure 3**). Backhoe excavations revealed that windfall trees have few or no healthy roots remaining and that infected trees that are still standing also have substantial loss of healthy roots (**Figure 3**).

We isolated *G. adspersum* from the area directly below the flat strip trunk symptoms (**Figure 4**) on 67% of the trees that were excavated with the airspade, however decay was present in all. Picus scans of asymptomatic, symptomatic and known infected trees revealed that decay was present in all the 12-year-old trees scanned (**Figure 6**). Trees with the flat strip trunk symptom had substantially more decay than asymptomatic trees. Unsurprisingly, known infected trees, those with a conk, contained the greatest area and severity of decay. These same trees will be scanned again during the fall of 2018 and destructively sampled so rates of decay within trees can be determined.

Orchard Scale
Mapping of orchards is ongoing and provides the opportunity to determine a patterns of tree mortality for orchards infected with *G. adspersum* (**Figure 7**). Orchards known to be infested with *G. adspersum* experienced a substantially quicker decline in overall stand density than those that were mapped of unknown infected status (**Figure 9**). As more orchards are mapped utilizing via historical aerial images, differences in decline rates and orchard longevity will begin to emerge and assist growers in long-term orchard decision making.

A 12-year-old orchard block in Madera county 7% losses to windfall in the 10th and 11th year. Surveys of every tree in this block found that 20% of the remaining trees had a *G. adspersum* conk, and an additional 35% had the flat trunk symptom indicative of *Ganoderma* root and butt rot (**Figure 8**)

In the somatic compatibility study, all paired isolates from the Hanford site were antagonistic towards one another, indicating a unique individual and independent spore born infection in

each tree. In several instances, more than one individual was isolated from the same tree. These findings suggest spores are the primary mode of infection. However, root to root spread and inoculum in woody debris in the soil has not been completely ruled out.

In the inoculum survival study, after seven months buried in the soil *G. adspersum* was re-isolated from wood pieces with volume 0.1 L and larger, with the percentage of re-isolation increasing with the size of wood piece. After 16 months in the soil, re-isolation was possible from only the two largest size classes with volume 0.91 and 1.82 L and the percentage of re-isolation was significantly less than after 7 months (**Figure 12**). This finding is significant in that it indicates the ability of *G. adspersum* to survive on woody debris in the soil after orchard removal and must be a consideration when replanting. This is of importance when considering the adoption of whole orchard recycling at removal.

Landscape Scale
The geographic distribution of *Ganoderma* species identified during our surveys is presented in (**Figure 10**). Maximum likelihood phylogenetic tree of the ITS region of *Ganoderma* species collected in California is presented in (**Figure 11**). Of the two species identified in almond orchards *G. brownii* has a much wider host range and distribution. G. *adspersum* is known only on almond, and only in the San Joaquin Valley. Most likely the limited distribution is a product of the site of the initial introduction. Based on the biology of *Ganoderma* there is no reason to assume that it will not spread to the other almond growing regions of California.

Remote spore monitoring is in the primary stages, but the technology should be able to readily distinguish between *Ganoderma* spores of different species.

Objective 3
Existing *Ganoderma* specific primers have been tested but are unable to distinguish between *G. adspersum* and *G. brownii*. Additional molecular methods to more efficiently distinguish between the two species are being explored.

Objective 4
Inoculation of Wood Blocks
Decay experiments agree with previous assessments regarding the aggressiveness of *G. adspersum* compared to other *Ganoderma* spp. G. *adspersum* decayed significantly more wood than did *G. brownii* and *G. resinaceum* (**Figures 13 and 16**). In addition, in experiment two, isolates of G. *adspersum* survived and colonized wood more often than isolates of *G. brownii*. Autoclaved blocks of wood loss more mass than non-autoclaved blocks in most cases.

There are differences among rootstocks in decay rates by *Ganoderma* (**Figures 14 and 17**). However, it is too early to make any recommendations, as decay rate in a block of wood **(Figure 15)** does not directly equate to how decay will progress in a growing tree.

Inoculation of Saplings
12 weeks after inoculation, all nine saplings inoculated with *G. adspersum* showed discoloration and had a column white rot decay, whereas the nine mock inoculated saplings were only discolored **(Figure 18).** Re-isolation of *G. adspersum* was successful in all cases.

The average distance of from the inoculation site from which re-isolation was possible was 1.6 cm, with a minimum and maximum of 0.5 and 2.5 cm respectively.

<u>Non-Destructive Sampling of ABC Rootstock Trials</u>
Surveys of ABC rootstock trials for wood decay has yet to take place. However, the non-destructive survey methods developed as part of Objective 2 should allow for this to take place during late 2018 and early 2019.

Research Effort Recent Publications:

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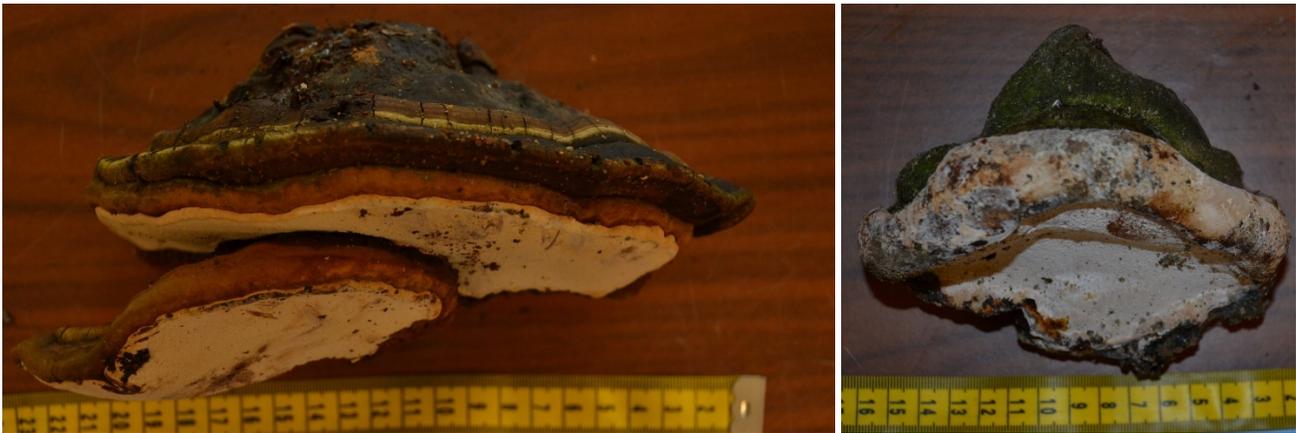
Figure 1. *Ganoderma* basidocarps collected from almond orchards fruiting on the peach rootstock Nemaguard. Left, *G. adspersum* from Tulare County on standing 14-year-old tree. Right, *G. brownii* from Merced County on a 22-year-old tree.

Figure 2. From left. Windfall caused by *Ganoderma* butt rot. Active *Ganoderma adspersum* conk, notice bright white edge and reddish spores covering top surface. Inactive conk and trunk symptom indicated by red arrow.
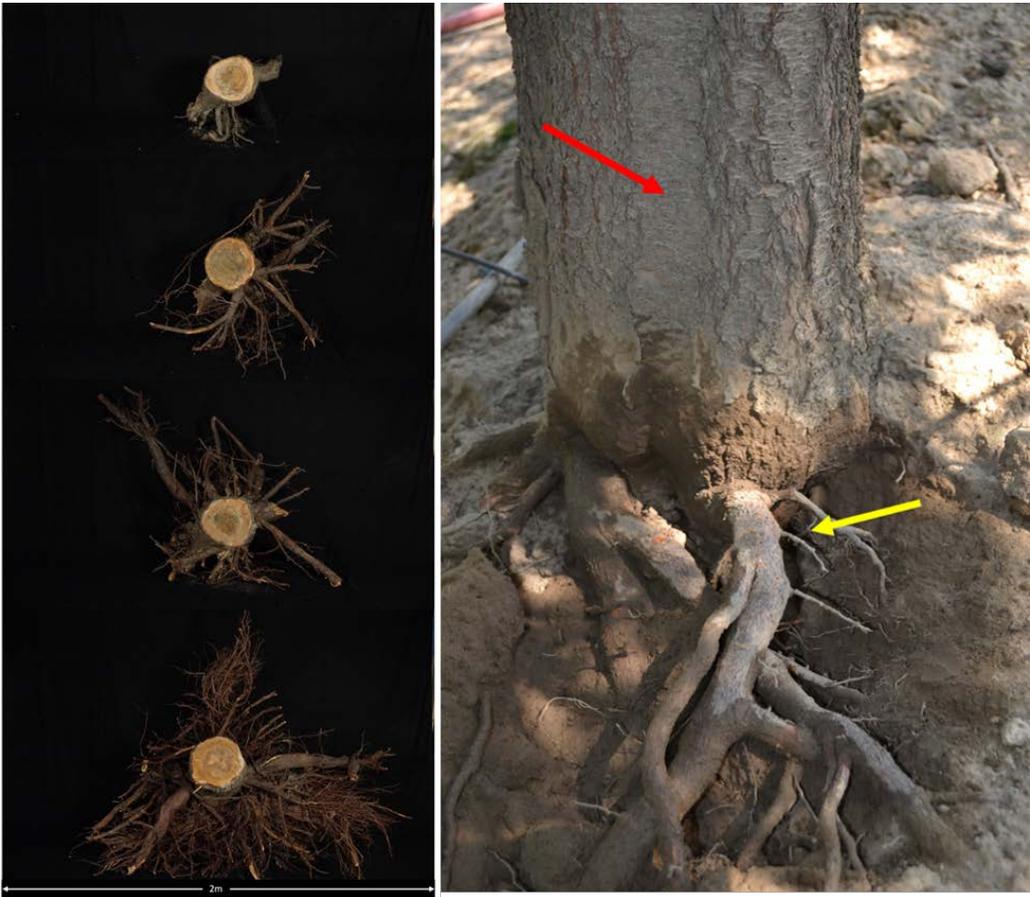
Figure 3. Right, stumps excavated with a backhoe. Scale at bottom is 2m. The top stump was a windfall tree, the other three stmps came from trees that showed no obvious symptoms, but from which *G. adspersum* was **i**solated. Left, red arrow indicates trunk symptom, yellow indicates site of airspade excavation and isolation of *G.*
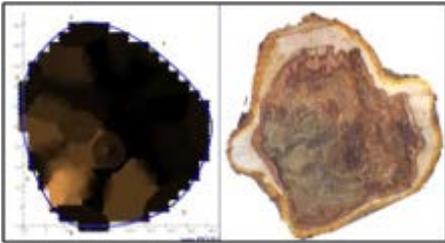
Figure 4. Left, scan of windfall tree just below the graft union. Right, cross section at same level as scan.
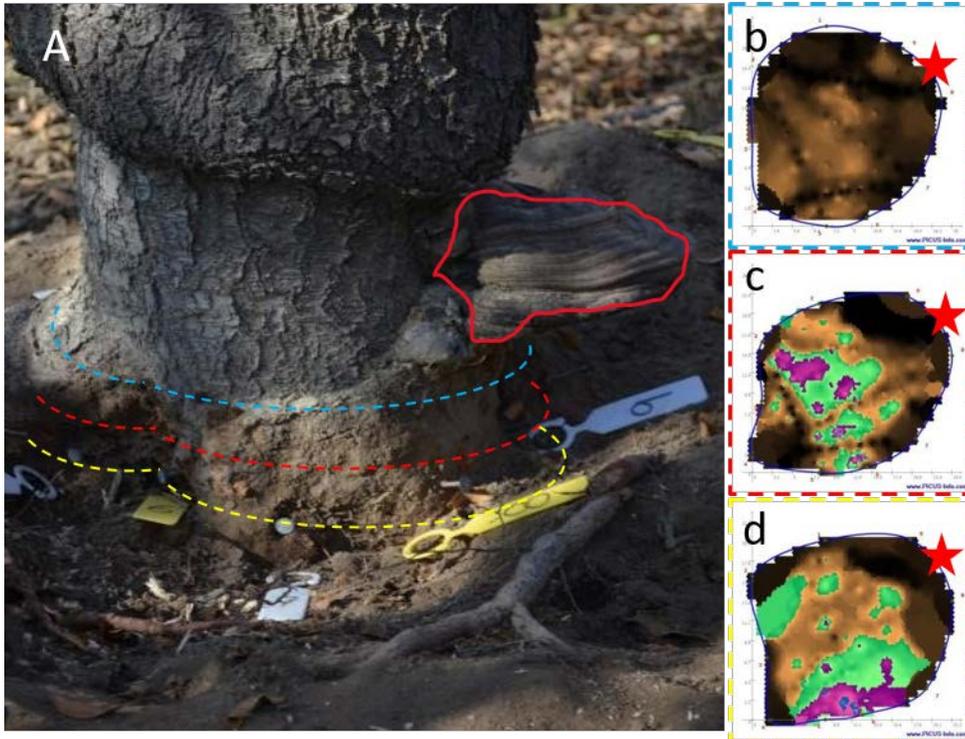
Figure 5. (A) *G. adspersum* infected tree with fruiting body (outlined by red solid line), dashed lines indicate location of sans at different depths, blue- soil line, red – 5cm below soil line, and yellow – 10cm below soil line. (b-d) scans at soil line, 5cm below soil line, and 10cm below soil line respectively, red star indicates location of fruiting body on trunk.
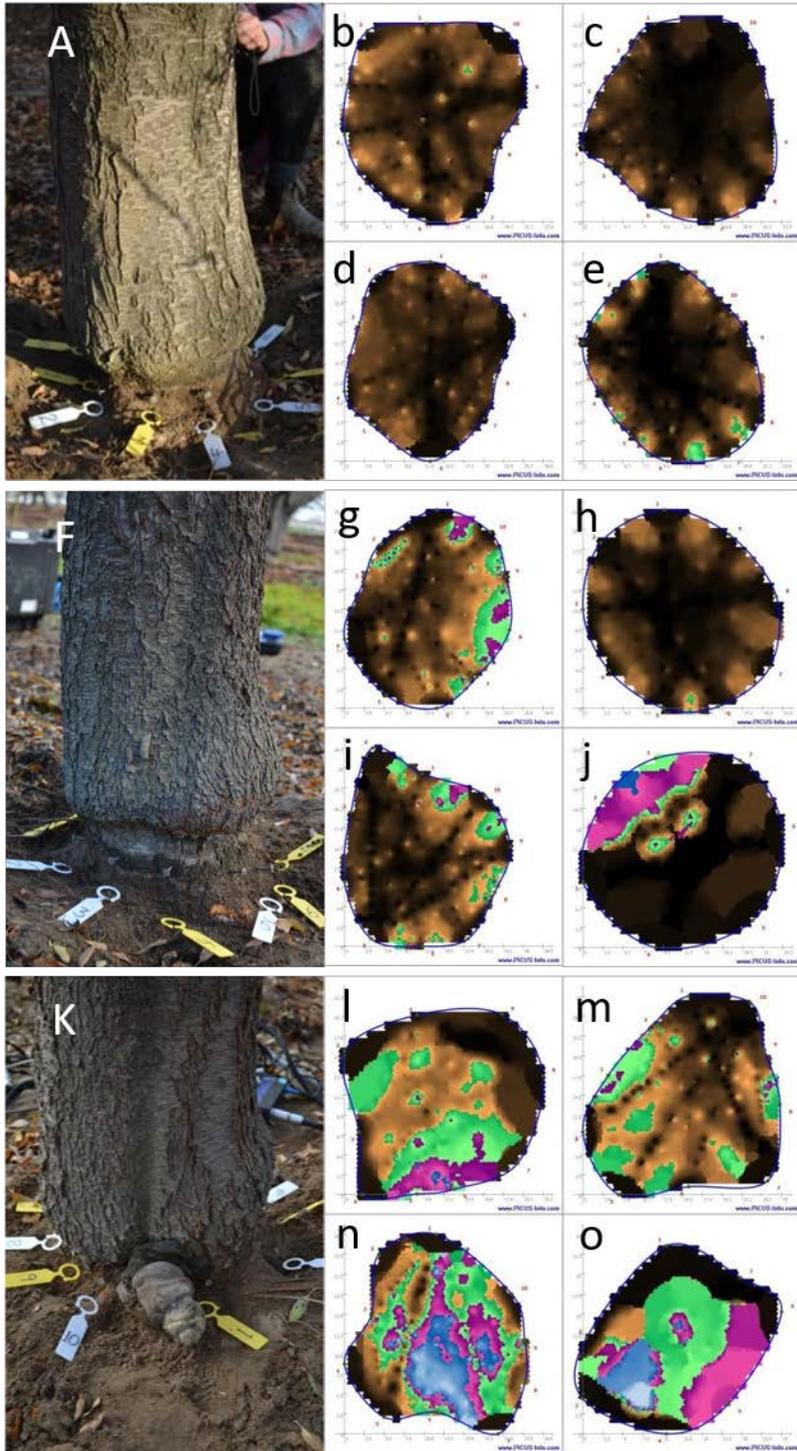
Figure 6. Synopsis of sonic tomography scans. All scans were done 10cm below the soil line. (A) representative asymptomatic tree, (b-e) scans of 4 different asymptomatic trees. (F) representative symptomatic tree, (g-j) scans of symptomatic trees. (K) representative known infected tree with *G. adspersum* fruiting body, (l-o) scans of known infected trees.
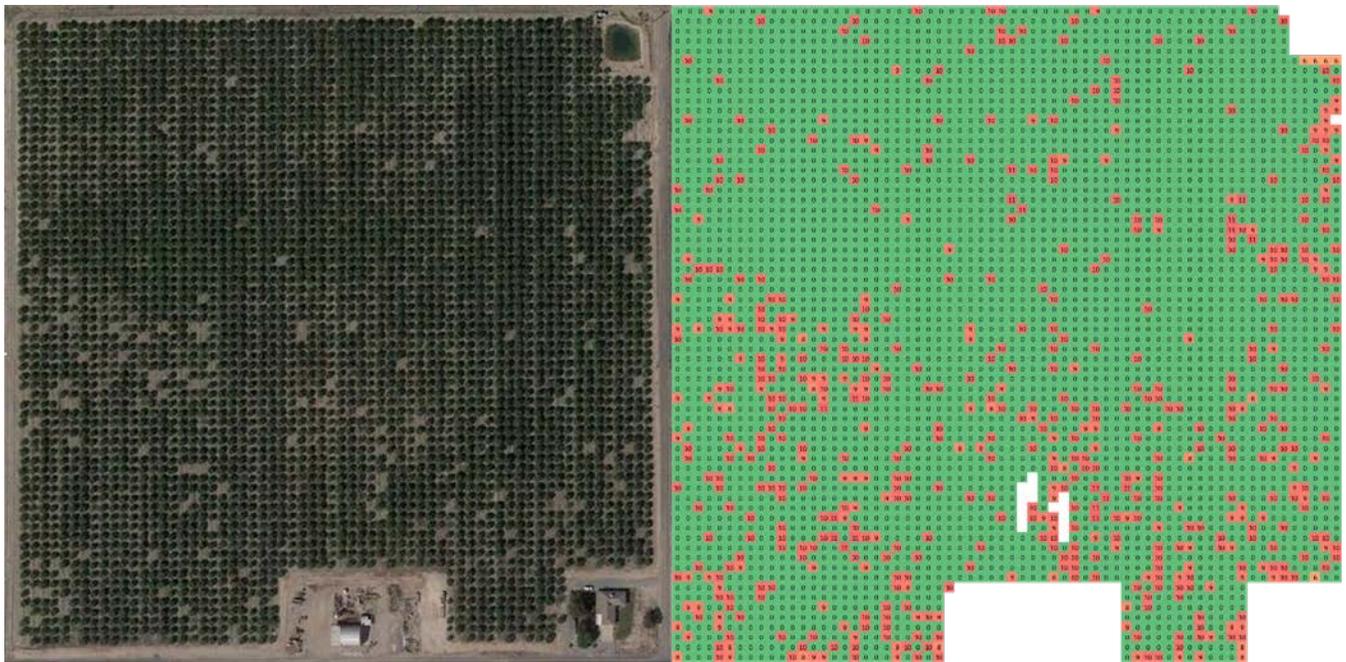
Figure 7. Example of orchard mapping for orchards infected with *G. adspersum*. Left, aerial image of ten-year-old orchard (Google Earth). Right, Spatial Temporal map of tree mortality based on multiple years of aerial imagery

Figure 8. Results of on ground survey of an 11-year-old orchard infested with *G. adspersum*. Red squares represent missing trees, orange squares represent trees with fruiting bodies, and yellow represents trees with other symptoms.
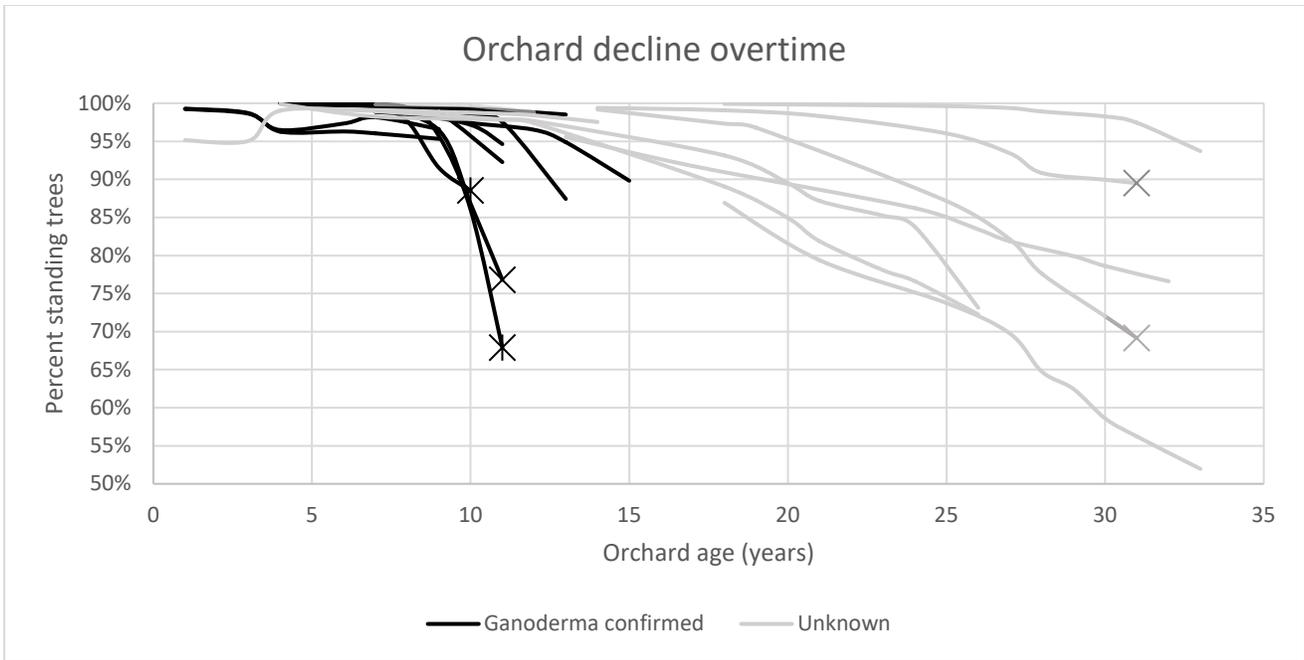
Figure 9. Tree mortality over time for 20 almond orchards.
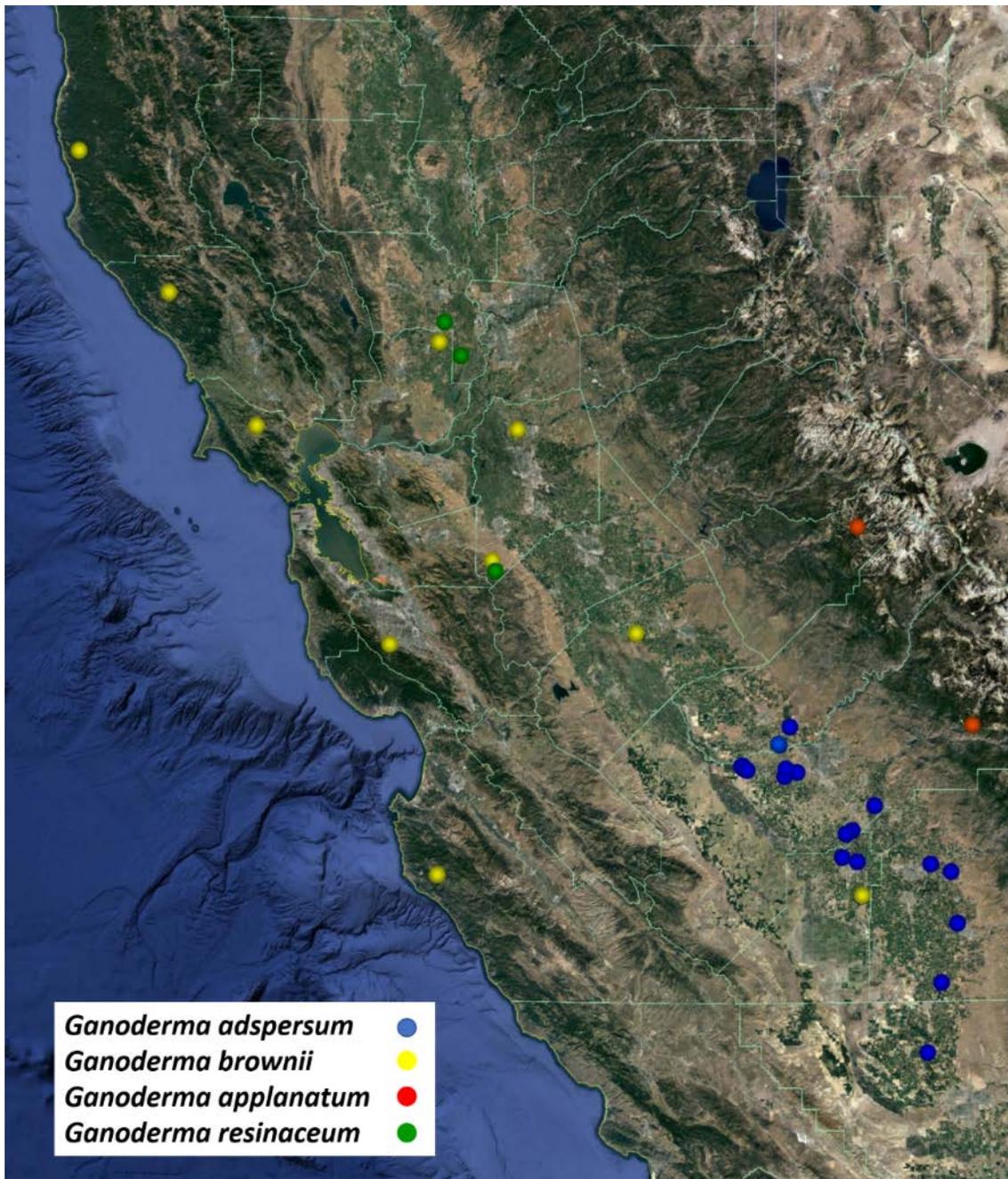
Figure 10. Geographic distribution of *Ganoderma* spp. collected during our surveys.
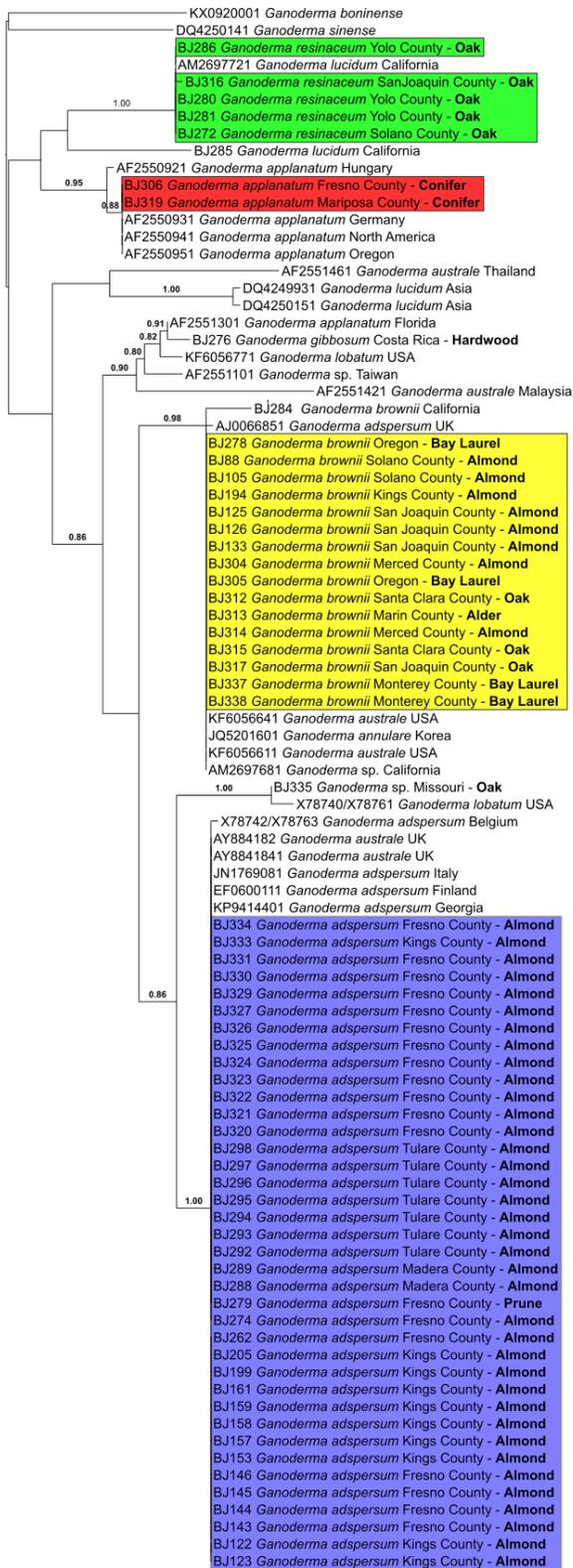
Figure 11. Maximum Likelihood phylogenetic tree of ITS region. Shaded isolates are represented on the map in (**Figure 10)**.
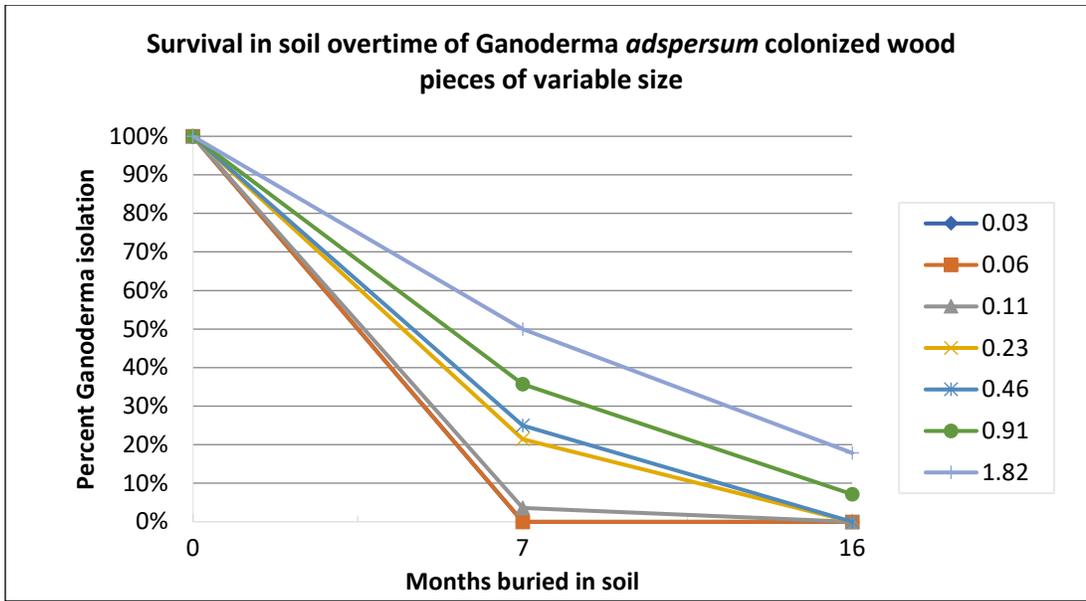
Figure 12. Survival in soil of *G. adspersum* colonized wood pieces of variable size. Legend is volume (L) of wood piece.
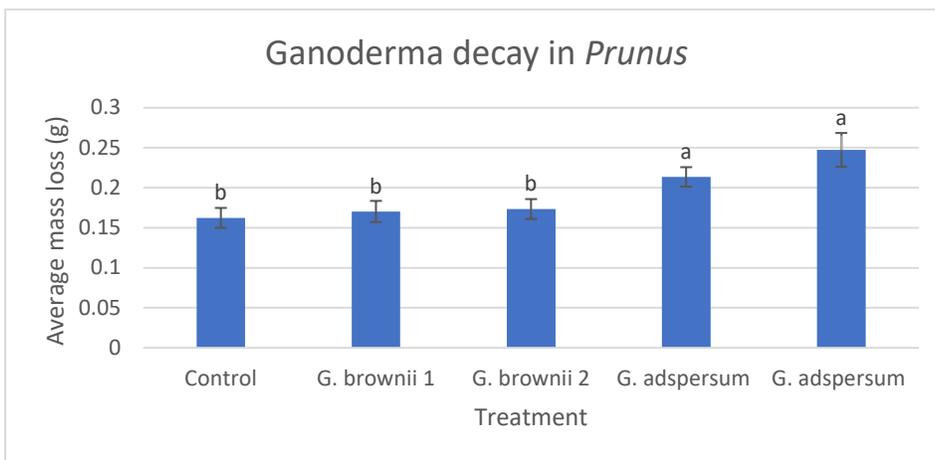

Figure 13. Decay rate of different *Ganoderma* isolates 6 weeks after inoculation. Letters represent tukey's grouping, different letters indicate a significant difference.
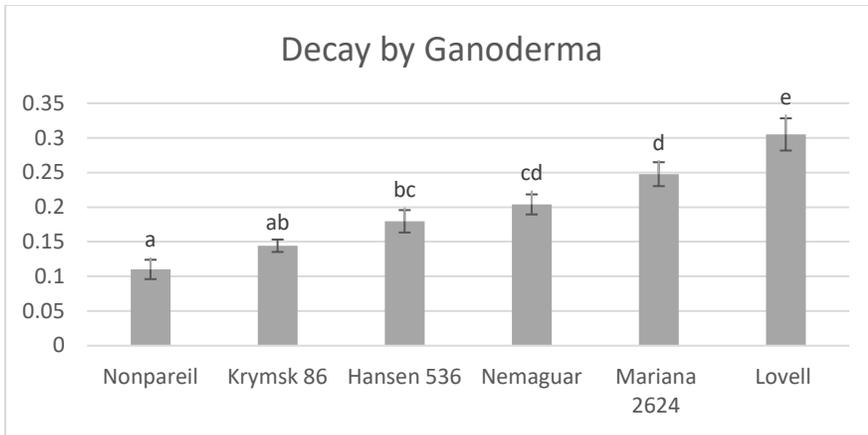
Figure 14. Average decay among different *Prunus* rootstocks 6 weeks after inoculation. Letters represent tukey's grouping, different letters indicate a significant difference.

Figure 15. Blocks of *Prunus* wood colonized by *Ganoderma* spp. From left, control. *G. adspersum*, *G. resinaceum*, *G. brownii.*
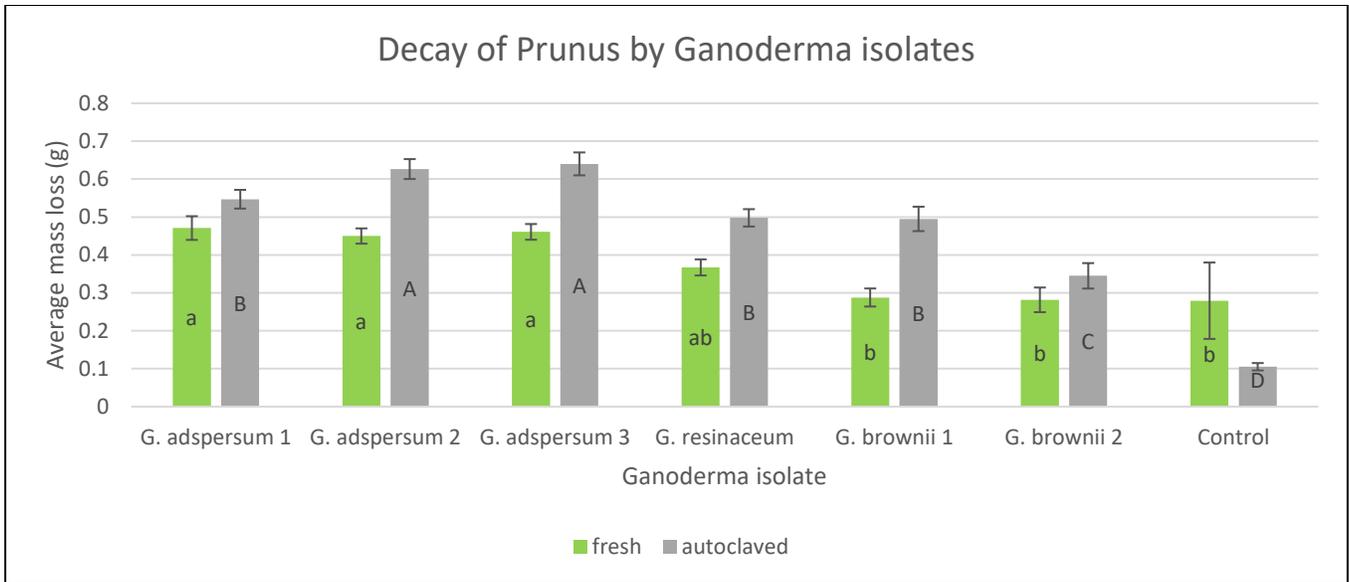
Figure 16. Decay caused by different *Ganoderma* isolates 12 weeks after inoculation. Lower case letters represent tukey's grouping for fresh wood blocks, and uppercase letters represent tukey's grouping for autoclaved wood blocks. Different letters indicate a significant difference.
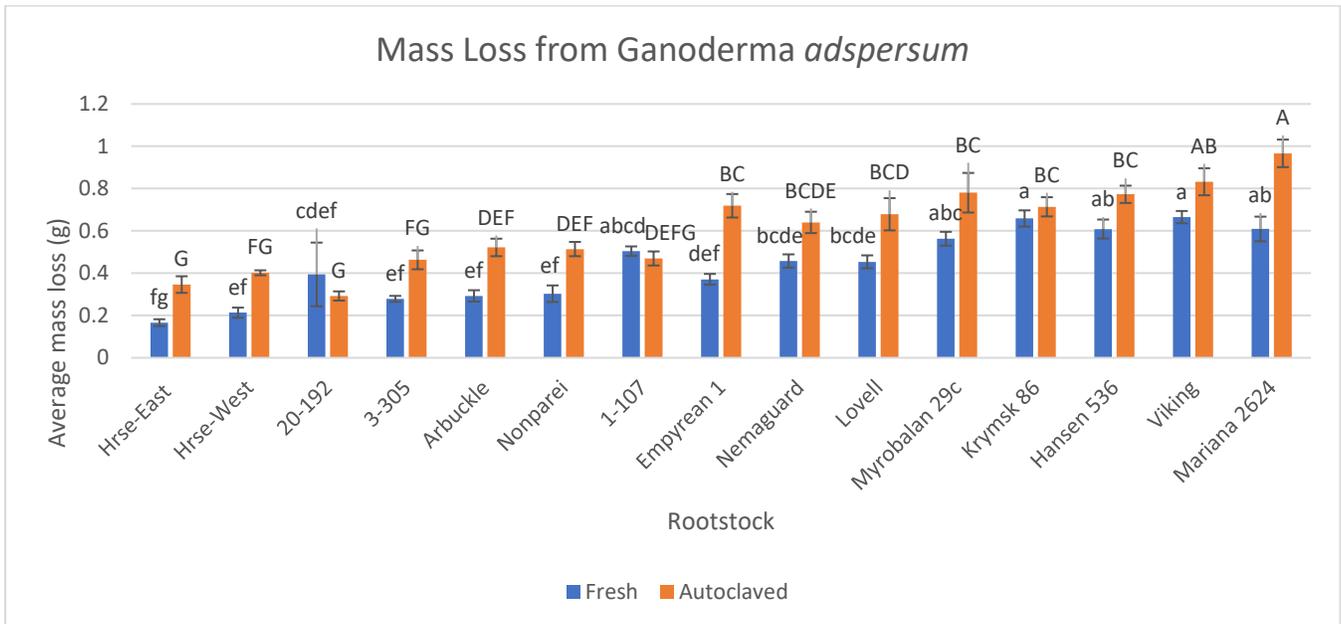

Figure 17. Average decay among different Prunus rootstocks three months after inoculation. Lower case letters represent tukey's grouping for fresh wood blocks, and uppercase letters represent tukey's grouping for autoclaved wood blocks. Different letters indicate a significant difference**.**

Figure 18. Saplings of Nemaguard rootstock 12 weeks after inoculation with either (A) control, non-colonized hardwood dowel or (B) *G. adspersum* colonized hardwood dowel. Notice presence of white rot in *G. adspersum* inoculated saplings.