BLACK WALNUT (*Juglans nigra*)
Black Walnut Decline; *unknown*

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Investigation of Black Walnut Decline, 2008.

Our goal is to characterize and understand what might be causing the unknown decline of black walnuts in Oregon. Black Walnut Decline is a progressive dieback of the upper crown of mature black walnut trees. Trees may die in 2 to 5 years once symptoms are noticed. It was first detected in Oregon during the early 1990's but there were few complaints about this problem until the mid 2000's when this dieback occurred in a wide variety of locations. Early investigations could not pinpoint any particular cause.

Pathologists in Colorado have also observed a similar decline and dieback of black walnuts during the mid 2000's (Tisserat, unpublished). Trees there were of smaller diameter and die more quickly, usually within one year of first symptom observation. They have implicated several factors to the decline including drought, temperature extremes, poor sites, walnut twig beetle (WTB, *Pityophthorus juglandis*), and discolored cambium around the trunks associated with fungi (including *Fusarium solani* and/or *Geosmithia sp.*). The working name for this disease in Colorado is "thousand canker disease".

Several theories and ideas have been proposed to explain this decline in Oregon including drought, cohort death, genetic vulnerability, WTB (which was identified in 2007 for the first time in Oregon) and a phytoplasma. The phytoplasma explanation has been rumored more extensively than many of the others but is based on meager evidence.

Disease Characterization

The disease was initially defined as a progressive dieback of the upper crown of mature black walnut trees. Branches may leaf out very weakly in the spring or die anytime during the summer. Generally dead leaves do not remain on declining branches. Dieback always appears to start in the upper crown and progresses to lower branches in succeeding years. Some trees appear to have top dieback appearing almost concurrently with spotty limb dieback at various places throughout the crown.

Interviews with tree owners in 08 indicated that a dominate symptom included branches that failed to leaf out in the spring. Owners removing trees in 08 indicated there was disease progression from 2007 to 2008. Owners also indicated spending thousands of dollars with tree care service companies to prune out dead wood only to have more limbs die later in the year. Evidence of these events was observed in several locations both in the Willamette Valley, Mosier and The Dalles.

A group of 51 trees, from 5 locations in the Willamette Valley were monitored during the 2008 growing season. This group of trees had 46 with dieback symptoms and 5 that appeared healthy in 2007. The amount of canopy with dieback symptoms was recorded in Sep 07 and again in Sep 08. Canopy dieback in 07 ranged from less than 1% to 95%. Some active dieback was observed during the 2008 growing season but only a few small branches on a few trees. There was no significant progression of symptoms during the 2008 growing season. In this same group of trees there are 2 trees in each of 2 locations (for a total of 4 trees) that were topped or pruned hard due to their location under power lines. These trees looked healthy in 07 and remained so in 08.

Phytoplasma Testing

Shoots were collected from trees with and without symptoms of dieback for phytoplasma testing. Total nucleic acids were extracted and used for analysis. Reverse transcription with reverse # 1 was preformed for ribosomal RNA products. Samples were then amplified using PCR with primer pairs P1/P7. Then nested PCR was done with primer pair R16F2n/R16R2. Samples were then loaded onto an agarose gel, stained with ethidium bromide and DNA bands visualize with UV light to estimate their size. Correct sized fragments were then sequenced to confirmed presence of phytoplasma nucleic acid.

The first set of samples was collected on 14 Jun 08 from 10 symptomatic trees and 5 trees without symptoms of dieback. Samples were held several days in a cold room before processing. Extensive smearing indicated rapid sample degradation and inconclusive results. The next set of samples from different trees was collected from 16 to 18 Aug 08 from 6 symptomatic trees and 3 trees without symptoms of dieback. Samples were processed right away and tested twice at three different DNA concentrations. Phytoplasmas were not detected in these samples.

No witches' brooms, a symptom of bunch disease caused by a phytoplasma, have been observed on declining black walnut trees in Oregon or on any other walnut species. Black walnut (*J. nigra*) is considered tolerant of bunch disease and may not show symptoms until it is cut down when stumps re-sprout with witches' broom-like growth. Based on these observations and negative testing it is thought that this decline is not due to a phytoplasma induced disease.

Walnut Twig Beetle - *Pityophthorus juglandis*

The walnut twig beetle was first discovered in association with declining trees in late 2007. Examination of past samples indicated the beetles have been around since 1997, first sent in from Multnomah (1997) and then Wasco (The Dalles – 2000 and 2005) counties.

Many trees were examined throughout the Willamette Valley, Mosier and The Dalles for presence and activity of the walnut twig beetle (WTB) during the 2008 growing season. The WTB was found in association with declining as well as healthy trees in all locations. Beetle activity was always associated with dead twigs, branches and large limbs. A dead branch sample collected from Marion county on 27 Aug contained numerous beetles excavating new galleries. Eggs, larvae, callow adults and mature adults were all found in this sample. It indicated that adults emerge, mate and lay eggs from Aug to Sep.

Beetle galleries were found in association with living cambium in only a few cases. In The Dalles, beetle galleries were found only in the phloem of what appear to be naturalized, shrubby trees. Beetles were found in both healthy appearing and declining trees in this area on 19 Sep. Galleries were found extensively in the thick bark near live cambial tissue of a mature declining tree on 27 Aug. Galleries came near and touched the cambium, but did not extend into the xylem. Necrotic phloem tissue extended away from these galleries. On another tree observed on 16 Sep, galleries, including adults, were seen to extend into living xylem. Beetle gallery holes were observed in the center of isolated, oval, necrotic tissue in the cambium. This isolated necrotic tissue was found well below the margin of dead wood above.

Fungal Isolations and Observations

Traditional isolations from the margin of the necrotic/living tissue interface yielded a variety of fungi most of which were not pathogens. Some isolations detected *Fusarium* or *Rhizoctonia* species. Isolations for *Geosmithia* sp. were positive from all locations from Corvallis north to Portland and in the east side cities of Moiser and The Dalles. The fungus *Geosmithia* sp. was associated with beetle galleries and not found in areas without galleries. *Geosmithia* sp. was isolated from the oval, necrotic tissue in the cambium associated with WTB holes.

Case Studies

There were only 3 cases where trees could be thoroughly examined. These trees were removed due to extensive dieback (40 to 50%) and sold for lumber since they were of large size. Cases 1 and 2 were single trees while case 3 was a set of 4 trees growing together in a line. *Geosmithia* sp. and WTB were found in all three cases. WTB activity was only on dead wood in case 1, more extensive in case 2 and throughout all trees in case 3 including living cambium. Roots in both case 1 and 2 were rotted with confirmed *Armillaria* root rot in case 2.

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Case # (code and date of removal)	Walnut Twig Beetle	Fungal Isolations		Phytoplasma Testing	Comments
		Geosmithia	Other Pathogens		
Case 1 (MBW - 14 Jun)	+	+	None Found	Inconclusive	Extensive root rot noted but directly observed. WTB only active on dead branches.
Case 2 (BD – 27 Aug)	+	+	None Found	Not Done	Confirmed *Armillaria* root rot. WTB on dead branches and near live cambium.
Case 3 (KG – 16 Sep)	+	+	Not Done	negative	Root ball not examined. WTB activity extensive throughout trees.

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