

Case studies in ascospore survival in ground (chipped) hazelnut prunings, 2004.

The OSU Extension Service has for years received questions about the effectiveness of grinding or chipping EFB infected prunings. In the early 1990’s most of the questions had to do with movement of chips out of EFB quarantine areas for use as landscape mulch. Today, the questions deal with chipping as an alternative to burning for getting rid of infected branches. Our main objective was to determine if EFB cankers and ascospores would survive the chipping process. Our investigation focused on 3 situations where EFB infected branches or trees were ground up using various chipping machines.

Case Study #1 (Canby area)

On 20 Feb 04, 120 hazelnut branches infected with EFB, approximately 1 meter in length, were ground with a pto-driven wood chipper. Branches were selected that had obvious EFB cankers with many stroma. Half of the resulting chip pile was spread out to cover a 3x5 ft area 4-5 in deep near a commercial hazelnut orchard located near Canby, OR. The other half was spread out in a field of young ‘Ennis’ trees at the NWREC. Chip samples (enough to fill a 3 liter bag) were collected from the Canby site on 20 Feb, 25 Mar, 7 May, and 30 Jul while chip samples were only collected from the NREWC site on 25 Mar and 7 May. Samples were examined for EFB cankers and intact stroma. Chips with stroma (6-10) were placed into 700 ml distilled water for 3 days. Drops from this solution were placed on a hemacytometer and examined at 400X. The number of ascospores per 5 microscope fields was then determined. Stroma were also removed from chips, crushed in a mortar with 10 ml of distilled water and examined for ascospores. Bucket spore traps were placed within the pile area at Canby and 4 m to the west of the pile area on 3 Mar. These 6L plastic buckets had a wire screen on top and remained open to collect rain water. Buckets were also buried such that the top was 4 in above the ground. Water samples were removed from each bucket on the 14 Apr and 12 May. The rainwater was filtered first through a 20 um sieve then through a cellulose nitrate filter with 0.8 um pore size. This filter paper was placed on a microscope slide, stained with 0.05% (v/v) trypan blue in lactoglycerine. The number of ascospores on filters was then determined using a light microscope at 400X.

Numerous large (1-1.5 x 6-7 cm) and small (0.5 x 1 cm) chips were easily found with intact stroma at all collection times at each location. Ascospores were found at each chip collection time by water extraction or by grinding. Similar numbers of ascospores were found in spore trap buckets whether located within or outside of the chip pile at the Canby location. The greater number from the second collection was due to a greater amount of rainfall during the collection period.

Table 1. Ascospores from cankers that survived chipping.

Date Collected	Number of ascospores – Canby Site		Number of ascospores – NWREC site	
	Water Extraction	Grinding	Water Extraction	Grinding
20 Feb.....	4	69	--	--
25 Mar.....	0	6	9	3
7 May.....	3	6	0	24
30 Jul.....	1	0	--	--

Table 2. Ascospores from rainwater collected in buckets.

Date Collected	Number of ascospores	
	Within Chip Pile	Outside of Chip Pile
14 Apr.....	1	1
12 May.....	8	7

Case Study #2 (Dever-Conner area)

A 5-10 acre Ennis orchard found lightly infected with EFB was cut down and chipped from 14 to 29 Oct 03. Chips were spread out in this former orchard area to a depth of 2-3 in. Similar spore trap buckets described above were placed within the chipped area and 1 m to the west on 10 Mar. Buckets were also buried such that the top was 4 in above the ground. Water samples were removed from each bucket on the 9 Apr and 12 May. The rainwater was filtered first through a 20 um sieve then through a cellulose nitrate filter with 0.8 um pore size. This filter paper was placed on a microscope slide, stained with 0.05% (v/v) trypan blue in lactoglycerine. The number of ascospores on filters was then determined using a light microscope at 400X.

No chips could be found with intact stroma, however, ascospores were found in rainwater collection buckets. More ascospores were found in the spore trap bucket located within the chipped area than in the bucket located outside of this area to the west.

Table 3. Ascospores from rainwater collected in buckets.

Date Collected	Number of ascospores	
	Within Chip Area	Outside of Chip Area
9 Apr.....	2	0.3
12 May.....	7	1

Case Study #3 (Newburg area)

EFB infected prunings from a Barcelona/Duchilly orchard located near Newburg, OR were ground up in the orchard on 12 Feb and spread out in 10 x 10 ft area 6 in deep. Chip samples (enough to fill a 3 liter bag) were collected on 24 Feb, 25 Mar, and 7 May. Samples were stored in the freezer for 2 days to 2 months before extraction. Samples were examined for EFB cankers and intact stroma. Chips with stroma (6-10) were placed into 700 ml distilled water for 3 days. Drops from this solution were placed on a hemacytometer and examined at 400X. The number of ascospores per 5 microscope fields was then determined. Stroma were also removed from chips, crushed in a mortar with 10 ml of distilled water and examined for ascospores.

Large (1.5-2 x 9-10 cm) and small (0.5-1 x 3-4 cm) chips were found with intact stroma. These chips were difficult to locate among the rest of the chipped material. Ascospores were found at each chip collection time by water extraction or by grinding.

Table 4. Ascospores from cankers that survived chipping.

Date Collected	Number of ascospores – NWREC site	
	Water Extraction	Grinding
24 Feb.....	0	30
25 Mar.....	2	2
7 May.....	0	2

Conclusions – EFB cankers on hazelnut branches can survive various grinding, shredding and chipping machines with intact stroma. In addition, ascospores appear to survive the chipping process and can be liberated when hydrated or ground. It is not known if ascospores were viable or not during this investigation. How much inoculum might come from chipped sources is unknown but appears to be greater than zero. The importance of this inoculum source may change depending on the location of the orchard and the background inoculum available in the general vicinity. Further study is needed in low inoculum areas to determine the potential impact this practice might have on the overall EFB epidemic.