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The Trouble with Butternuts

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Butternut trees have not always been valued in Connecticut. In 1906, Experiment Station forester Austin Hawes said "…hemlock has come up under worthless butternut. The latter should be cut to allow better growth of the hemlock..." (Hawes, 1906). However, nut growers and wood carvers have always liked butternut trees, and over the years there have been several diseases that threaten these trees. More recently, there has been a growing concern about one disease, butternut canker, which I will discuss in more detail in this article.

A black canker disease caused by *Melanconis juglandis* (*Diaporthe juglandis*) was described by Arthur Graves in Connecticut in 1923 (Graves, 1923). This fungus can be walled off by the tree and remain alive inside a branch or trunk (Fig. 1), only to break out and start growing rapidly when the tree is stressed. Known as a "slow killer," this pathogen is certainly capable of killing butternut trees. The asexual spores (conidia) are formed in curled ribbons of black, exuding from lenticels (pores) in the bark. The sexual spores (ascospores) are shot out of the long black necks of the perithecia that also emerge through lenticels in the bark (Fig. 2). The conidia are black, and large, averaging 22 microns X 10 microns (Fig. 3). On Petri plates of potato dextrose agar (Difco, PDA), the cultures are light yellow and fairly flat.

In 2006, I found another canker disease. *Phomopsis oblongata* (*Diaporthe eres*) had been reported on two species of *Juglans* in Japan, but never found in the USA. I found cankers caused by this pathogen on small hybrid butternut trees that had been given to me by Scott Schlarbaum (University of Tennessee), and planted in a forest opening in central Connecticut (Anagnostakis, 2007). This fungus does not push its conidia out through the lenticels, but produces small, blistered spots that rupture as the conidia are ready for release. The conidia are distinctive, as two kinds are formed: Alpha conidia are a short ellipse, single-celled, and 8 microns X 2 microns, and Beta

conidia are long and thin, and single-celled, 22 microns X 1 micron in size (Fig. 4). On Petri plates of PDA, the fungus is white and fluffy, and forms black lumps where conidia are produced.

The disease that has caught the attention of foresters and nut growers is butternut canker disease, caused by the fungus *Ophiognomonia clavigignenti-juglandacearum* (formerly *Sirococcus clavigignenti-juglandacearum*). This pathogen forms black pegs on the cankers, and these lift up the outermost layer of the bark giving it a lacy, or frayed appearance (Fig. 5). Next to the pegs, conidia are extruded in clear curls from black pycnidia (Fig. 6). These conidia are two-celled, and 12 microns X 2 microns in size (Fig. 7). On Petri plates of PDA, the fungus forms light to dark brown mycelium (Fig. 8). Earline Holmes and Michael Ostry in Wisconsin sent us isolates from the northern U.S., and Scott Schlarbaum sent us cultures from the south. When we grew them on plates of PDA at six different temperatures, the more southern isolates grew better at 30^{o}C than the northern isolates, and two cultures grew very poorly at 30^{o}C (Fig. 9).

Looking for reasons to explain the poor growth of these strains when compared to the others, we extracted the nucleic acids from each of the isolates, separated the nucleic acids of the different strains in gels using electrophoresis, and stained the gels using dye that attaches to nucleic acid. We found that the two strains that grew the least at 30^{o}C had the double stranded Ribose Nucleic Acid (dsRNA) of fungal viruses. Such viruses had been reported in this pathogen by Spain, Schlarbaum, and McElreath (1999). Since the dsRNA viruses in the chestnut blight fungus can used as a biological control of the disease, I plan to study this *Ophiognomonia* virus further to see whether it can significantly reduce the severity of butternut canker disease and whether strains with the virus can be used as a biological control.

Next we went out looking for butternut trees to see whether butternut canker disease was wide-spread in Connecticut. First, I had to make sure I could recognize a butternut. It's not easy! I had the photograph taken by Richard Jaynes (McDaniel, 1979) that showed nuts from different species of *Juglans* (Fig. 10). A nut grower gave me the hint that one-year-old twigs split in half would reveal the wide-chambered, light colored pith of *Juglans ailantifolia*, the Japanese walnut, or the closely-chambered, very

dark colored pith of *Juglans cinerea*, the true butternut (Fig. 11). Unfortunately, these two species hybridize readily, and the hybrids are not easy to identify from the pith characteristics. My assistant, Pamela Sletten, then noticed that butternut leaflets drooped down from the petiole, while Japanese walnut leaflets stood nearly straight out from the petiole (Fig. 12). That also was not reliable, so I tried an enzyme test that I have found to be helpful in distinguishing species of chestnut. We ground up dormant buds, extracted the proteins, used gel electrophoresis to separate the proteins by size, and stained the gel for the presence of the enzyme peroxidase. This enzyme usually has several size forms, and the sizes of the molecules present are consistent by species. Using proteins from butternut 'Buckley,' Japanese walnuts 'Wright' and 'Rhodes,' Persian walnuts 'Somers' and 'Hansen,' and a black walnut, we found patterns of peroxidase that could be used to distinguish the four species. When we used proteins from some of our Connecticut "butternuts," only two of them had patterns like that of butternut 'Buckley' (Fig 13).

I wanted a better test. Fortunately, Dr. Jeanne Romero-Severson and her assistants at the University of Notre Dame in Indiana have developed molecular markers to identify butternut and Japanese walnut trees with a high degree of accuracy. When I asked her to check my conclusions from the peroxidase test, she said I was "mostly right." As we sent her more and more samples to test, I learned that there are very few pure butternuts in Connecticut: most were hybrids. In fact, the "National Champion Butternut," which grows in Chester, Connecticut, is actually a hybrid whose mother was a Japanese walnut (Fig. 14). This is a very large tree, so clearly hybrids can grow well and easily compete with other trees (Fig. 15). The next question was whether the Japanese walnut parents might confer some resistance to butternut canker disease.

Scott Schlarbaum sent me six families of half-sib (same mother, unknown father) seedling trees, with 25 from each family. We planted them at the Experiment Station research farm in Hamden, CT and sent samples to Jeanne Romero-Severson, who checked them with her molecular markers. She reported that we had one row of 22 butternuts, three rows each with 24 Japanese walnuts, and one row of 25 mixed hybrids. When these trees were four years old, we inoculated them with the two

Ophiognomonia strains that we had isolated from trees in Connecticut (Figure 16). One of the strains has the dsRNA virus and the other does not. The average canker sizes are shown in Figure 17. The strain with the virus did not produce cankers as large as the other strain, but more tests will be necessary to check the significance of this difference. Not all of the Japanese walnut families had more resistance to the fungus than the butternut family, and some seemed to have much less. There was variation in the sizes of the cankers formed by the virus-free strain, so perhaps individual trees might be identified that could contribute resistance in a breeding program to produce butternut trees that are resistant to this pathogen.

Efforts to identify such trees are now in progress as the result of a multi-state project that involves Tom Hall in Pennsylvania, Dale Bergdahl in Vermont, and Aaron Flickinger in Ohio looking for surviving trees, Jeanne Romero-Severson in Indiana checking the species of the trees, and Mark Coggeshall in Missouri grafting scions of the trees onto black walnut rootstocks. Grafted clones of these trees will be sent to me to check their resistance by inoculating them with *Ophiognomonia*, and perhaps also with *Melanconis*, and resistant clones can then be put into a repository for breeding better butternuts for our forests and orchards.

References:

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Graves, A. H. 1923. The Melanconis disease of the butternut (Juglans cinerea L.). Phytopathology 13:411-437.

Hawes, A. F. 1906. Chestnut in Connecticut and the improvement of the woodlot. Bulletin 154, CT Agric. Exp. Stn., 41 p.

McDaniel, J. C. 1979. Other walnuts including butternut, heartnut, and hybrids. p98-110 IN: Nut Tree Culture in North America, R. A. Jaynes, ed., The Northern Nut Growers Association, Inc.

Spain, P., Schlarbaum, S., and McElreath, S. Detection of dsRNA in the butternut canker fungus. Phytopathology 89:S74

Figure Legends:

Figure 1. Black staining of a butternut stem where the fungus *Melanconis juglandis* is present.

Figure 2 Asexual spores (conidia) of *Melanconis juglandis* have come out of the bark of a butternut stem through the lenticels in curly ribbons (left arrow). The long necks of the perithecia have also burst through lenticels (right arrow), and sexual spores (ascospores) will be shot out from the perithecia under the bark through the necks.

Figure 3. Conidia of *Melanconis juglandis* are distinctive by their dark color and large size, 22µ x 10µ.

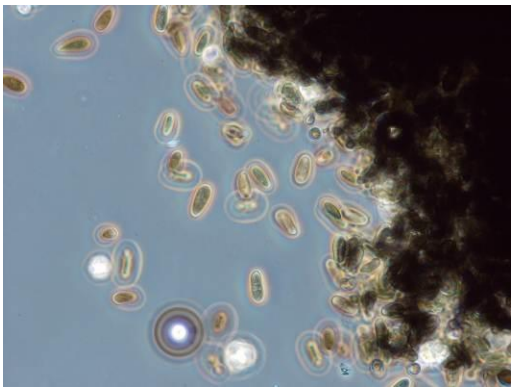

Figure 4. Two kinds of conidia are formed by *Phomopsis oblongata*, one long and thin (22µ x 1µ) and one short and fat (8µ x 2µ). Both are single cells.

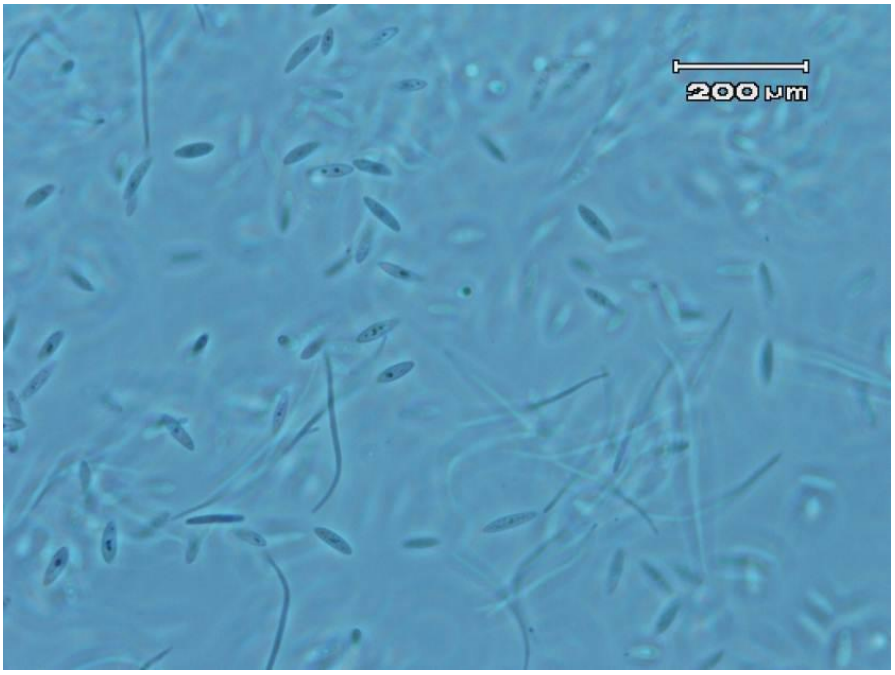

Figure 5. A canker caused by *Ophiognomonia clavigignenti-juglandacearum* on a butternut stem. Black hyphal pegs have pushed up a thin outer layer of bark exposing the pycnidia underneath.

Figure 6. Pycnidia of *Ophiognomonia clavigignenti-juglandacearum* next to the hyphal pegs are extruding ribbons of clear conidia. The hyphal pegs themselves produce no spores.

Figure 7. Conidia of *Ophiognomonia clavigignenti-juglandacearum* are two-celled (12µ x 2µ), and could easily be confused with the short conidia of Phomopsis until the cell wall is formed and easily visible.

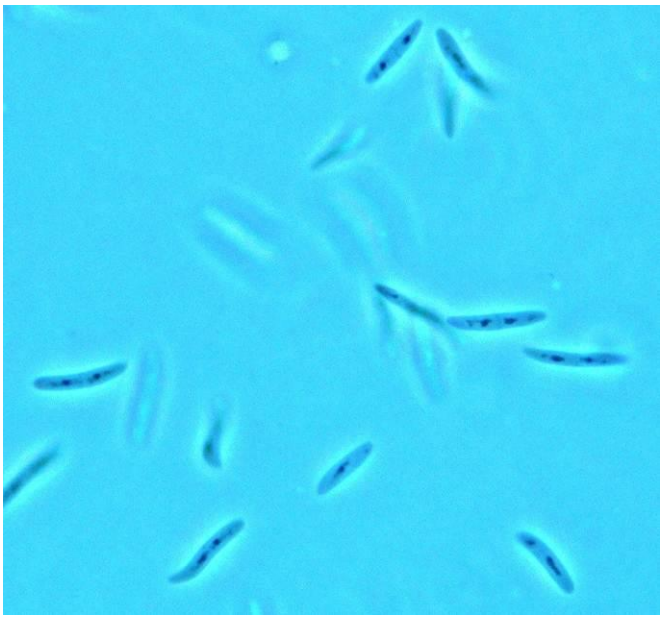

Figure 8. Two strains of *Ophiognomonia clavigignenti-juglandacearum* from Connecticut (growing on PDA) have slightly different morphology. CT1 on the left has a double stranded RNA virus, and CT 11 on the right does not.

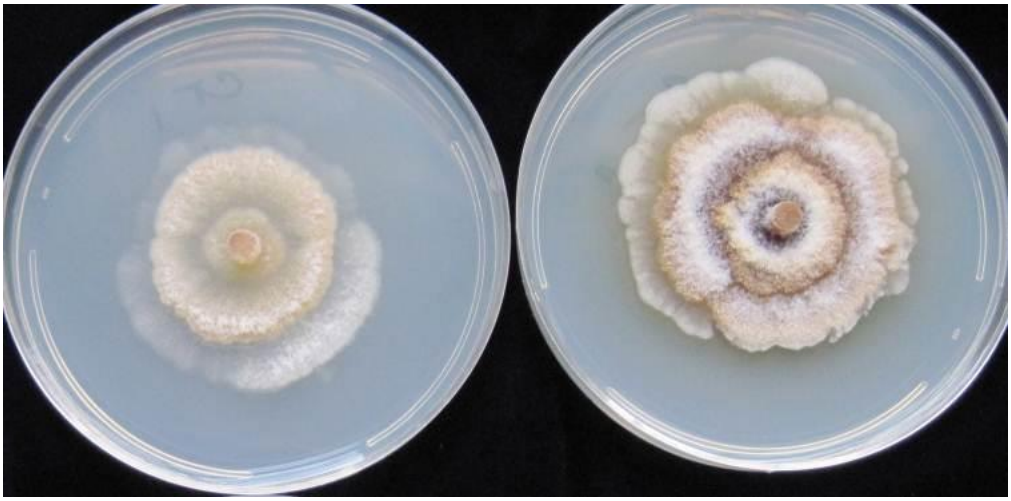

Figure 9. Ten different strains of *Ophiognomonia clavigignenti-juglandacearum* were grown on PDA at six different temperatures. Strains from Arkansas and Tennessee grew the most at 30*C, North Carolina, Wisconsin, and Minnesota strains grew less, and two strains with dsRNA viruses grew very poorly at this high temperature.

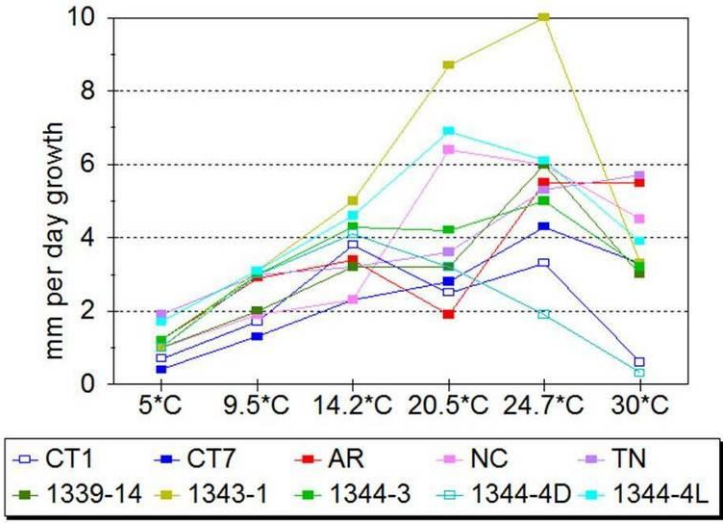

Figure 10. Nuts of butternut, Japanese and Manchurian walnut and a hybrid. Photo by R. A. Jaynes.

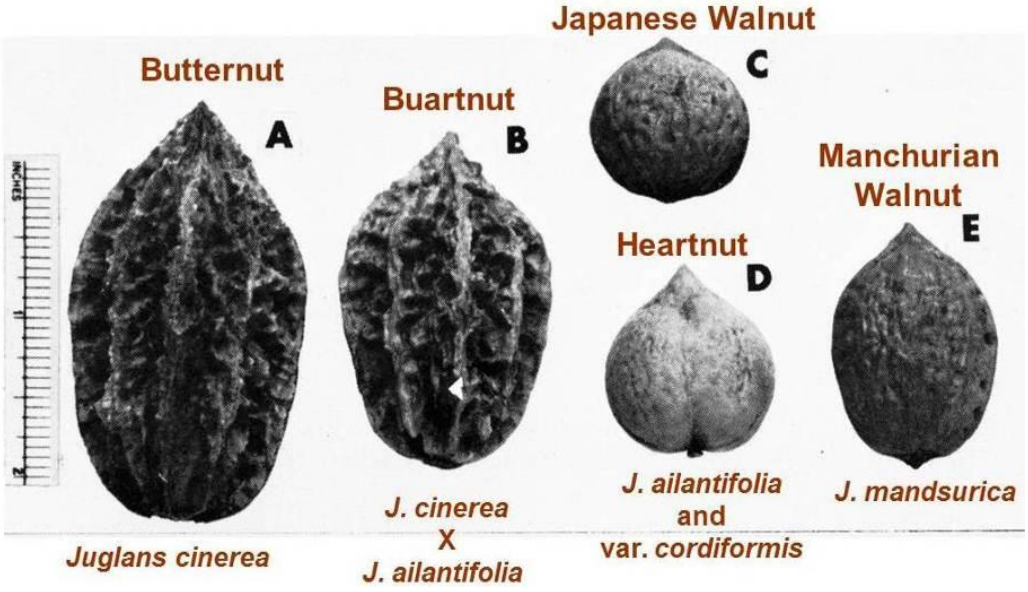

Figure 11. The pith of one-year-old twigs looks quite different in pure butternut and pure Japanese walnut, but hybrids are not easily distinguished by this feature.

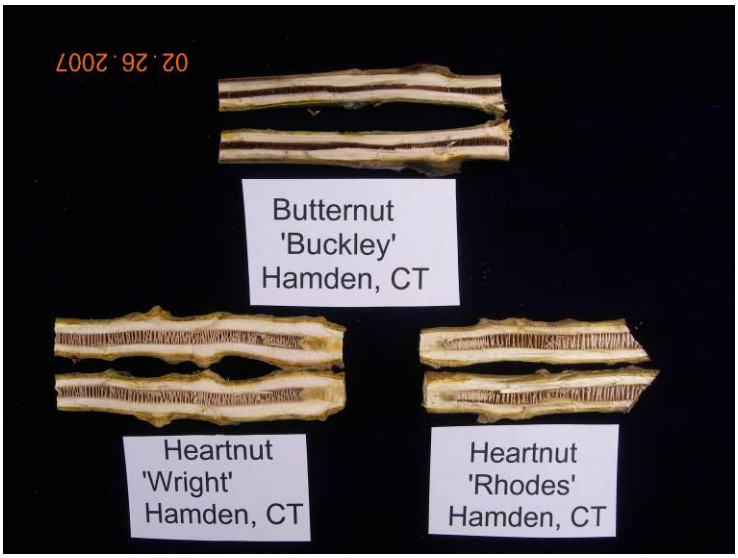

Figure 12. The leaflets of Japanese walnut trees (left) stand out nearly straight from the petiole, while those of butternut trees (right) droop down.

Figure 13. When proteins from dormant buds are separated on gels by electrophoresis, and then stained for the activity of the enzyme peroxidase, repeatable differences are seen in the bands of different *Juglans* species. Butternut has a single small molecule (left side of the gel) and other species have different patterns. We can use this test as a rough discriminator of species of *Juglans*.

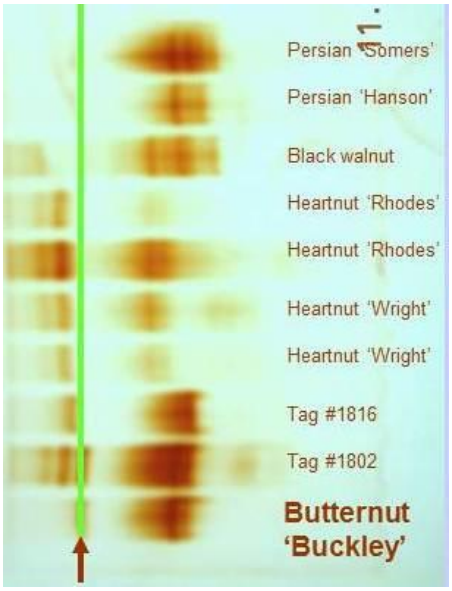

Figure 14. The former National Champion Butternut, whose mother was a Japanese walnut. This tree is growing in Chester, CT.

Figure 15. A view of the former National Champion Butternut showing the whole tree.

Figure 16. Pamela Sletten inoculating a small Juglans tree with a culture of *Ophiognomonia clavigignenti-juglandacearum.*

Figure 17. Mean diameter of *Ophiognomonia clavigignenti-juglandacearum* cankers on four families of Japanese walnut, one family of butternut, and one group of mixed hybrids. Two of the Japanese walnut families had smaller cankers than the butternut family, but two families had larger cankers. The two strains used were both from Connecticut, and CT 1 has a dsRNA virus while CT 11 does not. The strain with the virus was not as virulent on any of the trees as the strain with no virus.