

## Isolation of *Nectria galligena* from Cankers on Sweet Birch

Sandra L. Anagnostakis and Francis J. Ferrandino, The Connecticut Agricultural Experiment Station, New Haven 06504

### ABSTRACT

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The fungus *Nectria galligena* infects many species of hardwood trees, causing "perennial canker," "European canker," or "target canker." Cultures from ascospores produced in stromata on the cankers have confirmed the pathogen species, but the fungus has rarely been successfully isolated from inside the cankers. Concern for the sweet birch trees affected by this disease in Connecticut prompted us to try isolations, using a method successful in isolating chestnut tree pathogens. Small pieces of cankered bark were stabbed into Granny Smith apples and, after incubation in boxes in the laboratory, pure cultures of *N. galligena* were easily removed as the pathogen slowly grew out, unchallenged, into the tissue of the apples. This technique will now be used to sample the *N. galligena* population in the birch populations being studied.

Additional keywords: *Betula lenta*, European canker, Nectria canker, perennial canker, target canker

Arthur H. Graves (3) was the first person to describe Nectria canker on sweet birch (*Betula lenta*) in the United States. Writing in 1919, he said that G. P. Clinton, from The Connecticut Agricultural Experiment Station, had found cankered birch trees in New Haven in 1906, and Graves found the disease in Orange in 1909 and in Milford, Connecticut in 1918, as well as in New York City (3). In Kienholz and Bidwell's 1938 survey of tree diseases in Connecticut forests, they reported that, of 813 plots where *B. lenta* was found, 314 of the plots had *B. lenta* with cankers. The average percentage of cankered trees ranged from 2 to 53%, and there was an average of 4.6 cankers per tree.

Early workers found it difficult to isolate the pathogen from canker tissue, and usually used easily obtained ascospore isolates. Ashcroft (1) studied the pathology of this fungus using 28 isolates from perithecia but had only 1 (from *Pyrus malus*) from the wood of a canker. Plante and Bernier (6) recently reported that Golden Delicious apple fruit can be used as "host" for virulence tests with isolates of *N. galligena*. This reminded us that Crandall, Gra-

vatt, and Ryan (2) had successfully isolated *Phytophthora cinnamomi* from roots of chestnut trees with Ink Disease using the method of C. M. Tucker, in which slivers of wood and inner bark from infected tissue were forced into an apple.

"Granny Smith" apples are available year-round in markets in Connecticut. Ap-

ples were carried to the forest during survey trips, and cankers from marked *B. lenta* trees were sampled. A pocket knife blade was sterilized in 95% ethanol and used to scrape away the bark surface. The knife blade and our fingers were dipped in ethanol again and used to remove small pieces of cankered bark. The pieces were stabbed into the apple flesh, and the apple skin was marked with the tree number (Fig. 1). Back in the laboratory, the apples were placed in clear plastic boxes with lids, and incubated in the dark in a cabinet at room temperature. Dark discoloration of the apple tissue expanded very slowly from the bark chip (Fig. 2). After 10 to 15 days, pure cultures of *N. galligena* were recovered by peeling away the skin, carefully removing a bit of the discolored tissue with a scalpel, and placing it on a petri plate containing potato dextrose agar (Difco Laboratories, Detroit). Other organisms occasionally grew out from the bark into the apple tissue, causing rapidly spreading, water-soaked lesions. Isolations from such areas usually yielded bacteria or rapidly growing fungi, including *Pestalotiopsis*.

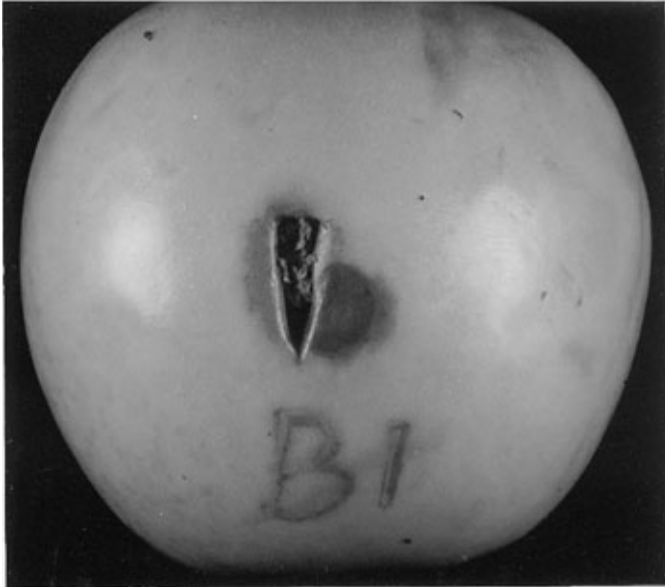


**Fig. 1.** F. J. Ferrandino in a Connecticut forest with a cankered sweet birch tree (*Betula lenta*). He has just removed a piece of canker tissue and forced it into a Granny Smith apple to isolate the canker pathogen.

Corresponding author: Sandra L. Anagnostakis  
E-mail: slanagan@caes.state.ct.us

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**Fig. 2.** A Granny Smith apple with a bark sample from sweet birch tree (*Betula lenta*) number B1. The dark circular spot in the apple tissue is caused by 12 days' growth of the fungus *Nectria galligena* from the cankered bark.

We have collected bark samples from a total of 107 cankers from five sites in Connecticut and, using this apple method, isolated *N. galligena* from 38 of them for a study of diversity of the pathogen. We are currently evaluating field inoculations (5) of these isolates and of morphological mutants produced from three of our early isolates.

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