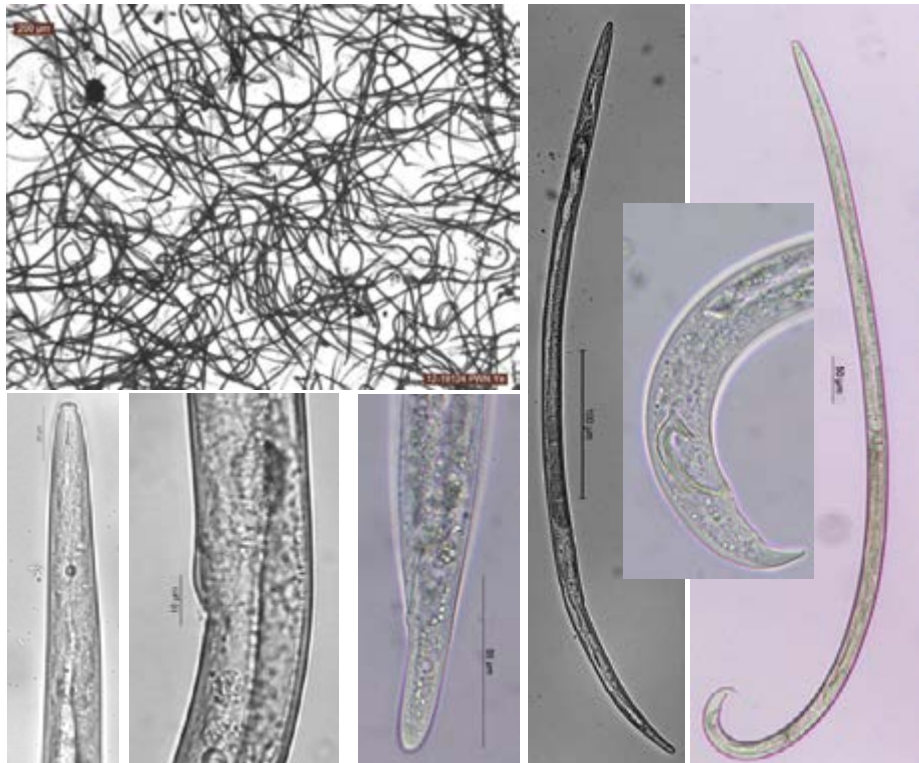


Pinewood Nematode Sampling & Assay

www.ncagr.gov/agronomi/uynem.htm



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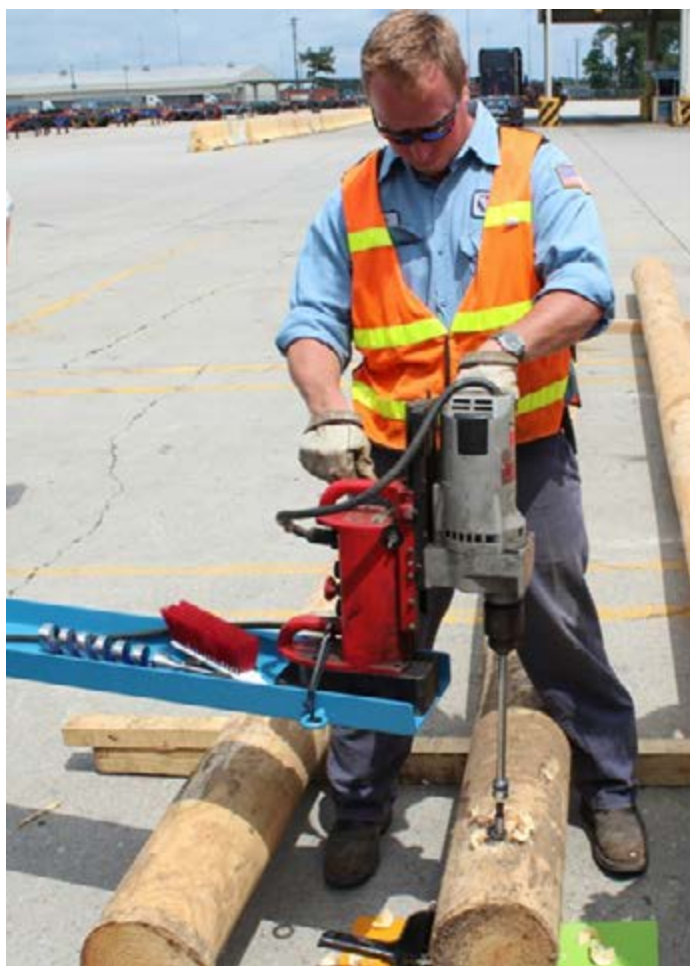
STEVE TROXLER
AGRICULTURE COMMISSIONER

Pinewood nematode (PWN) is the causal agent of pine wilt disease, one of the most damaging emerging pest problems to forests around the world. The international spread of PWN occurs mainly through the movement of infested logs, untreated wood products and wood-packaging material. PWN is native to North America where it causes relatively minor damage to native conifers. However, in many countries, it is a regulated pest and subject to quarantine because of its destructive potential. Exports of wood logs and commodities with softwood packaging materials now require a lab test for the presence/absence of this nematode species. This note provides Agronomic Division guidelines about sample collection, submission and report retrieval.

Before shipping any samples to the NCDA&CS Nematode Assay Section, you must call the Agronomic Division office and set up an escrow account to cover payment of fees (\$10/sample). Be ready to provide the following information: 1) desired account name, 2) address, 3) contact person, 4) phone number, and 5) source of funds to establish the account [cash, check, money order or credit card (MasterCard or Visa only)]. For additional information, visit www.ncagr.gov/agronomi/pdf/escrow.pdf.

Sampling Protocol for Pine-wood Logs Slated for Export

Contact the USDA Animal and Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ) program before sampling. According to their *Export Program Manual*, the number of units to inspect is based on one of two hypergeometric tables at 95% confidence of detecting a 10% or 5% infestation with 100% efficacy, depending on the state from which the logs will be shipped (Tables 1 & 2). Two holes, up to six inches (15 cm) deep, are drilled per log at six inches (15 cm) from both ends using a 2.125-inch (5.4-cm), self-feeding-wood bit. The wood shavings from two logs are mixed together. From this mixture, a minimum of 200 g of wood shavings are placed in a plastic bag to comprise one sample.



With an indelible marker, label each bag with a unique sample identifier. Use the same identifier(s) when filling out the nematode assay sample information form (www.ncagr.gov/agronomi/pdf/files/isnempd.pdf). If you want to be able to identify each shipment of samples to the lab, place a unique identifier in the Farm ID field of the sample information form. Samples and forms should be overnight-shipped to our lab for nematode assay.

View www.ncagr.gov/agronomi/PALS to search for completed reports. In the **Report Quick Search** box, type the "Grower" name you provided on the sample information form. No password is required.

Hypergeometric Tables for Random Sampling *

TABLE 1. Sampling requirements for N.C., S.C., and Va. [LOGS ONLY]		TABLE 2. Sampling requirements for all other states	
Total # of inspectional units	Randomly select this number of units to inspect	Total # of inspectional units	Randomly select this number of units to inspect
1–24	inspect all units	1–13	inspect all units
25	24	14–15	13
26	25	16–17	14
27	26	18–19	15
28	27	20–22	16
29	28	23–25	17
30	29	26–28	18
31	30	29–32	19
32–41	31	33–38	20
42–46	35	39–44	21
47–51	39	45–53	22
52–82	42	54–65	23
83–104	45	66–82	24
105–242	51	83–108	25
243–352	54	109–157	26
353–453	55	158–271	27
454–699	56	272–885	28
700–1,000	57	886–200,000	29
1,001–4,999	58		
5,000 and up	59		

* 95% confidence level of detecting a 5% infestation with 100% efficacy
www.aphis.usda.gov/import_export/plants/manuals/domestic/downloads/xpm.pdf

For More Information

- Contact the Agronomic Division Nematode Assay Section in Raleigh (919-733-2655).
- Refer to *Instructions for Pinewood Nematode Sampling and Assay* — www.ncagr.gov/agronomi/pdffiles/PWNprotocol.pdf.
- Refer to *Molecular characterization and development of real-time PCR assay for pine-wood nematode *Bursaphelenchus xylophilus* (Nematoda: Parasitaphelenchidae)* — www.ncagr.gov/agronomi/pdffiles/2013Ye.pdf.
- Refer to the USDA/APHIS/PPQ Export Program Manual — www.aphis.usda.gov/import_export/plants/manuals/domestic/downloads/xpm.pdf.