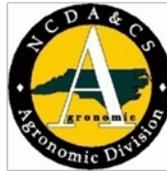


Instructions for Pine-Wood Nematode Sampling and Assay

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The 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. The Agronomic Division of the N.C. Department of Agriculture & Consumer Services operates a high-throughput and publicly operated nematode assay lab. Recently, due to strict regulations on PWN, an increasingly large number of pinewood samples have been submitted to our lab. This document provides general guidelines about sample collection, submission and report retrieval.

Southern Yellow Pine

1. Loblolly pine

2. Longleaf pine

3. Shortleaf pine

4. Slash pine



Eastern White Pine

Pinus strobus



Symptom



Hosts of Pine Wood Nematode

- Red silver fir
- Balsam fir
- Grand fir
- Atlas cedar
- Deodar cedar
- European larch
- Tamarack
- Western larch
- Engelmann spruce
- White spruce
- Black spruce
- Red spruce
- Sitka spruce
- Armand's spruce
- Jack pine
- Arolla pine
- Sand pine
- Shore pine
- Lodgepole pine
- Japanese red pine
- **Shortleaf pine**
- **Slash pine**
- Limber pine
- Aleppo pine
- Jeffrey pine
- Korean pine
- Sugar pine
- Western white pine
- Mountain pine
- Bishop pine
- Austrian pine
- **Longleaf pine**
- Maritime pine
- Ponderosa/Yellow pine
- Colorado pine
- Monterey pine
- Red pine
- Northern pitch pine
- Pond pine
- Southwestern white pine
- **Eastern white pine**
- Scots pine
- Chinese pine
- **Loblolly pine**
- Japanese black pine
- Virginian pine
- Douglas fir
- Mountain hemlock



Sampling Tools



18-Volt Compact Drill/Driver



2 1/8" Self-feed Bit



Sampling Protocol for Pine-wood Logs Slated for Export



USDA/APHIS/PPQ Export Program Manual
www.aphis.usda.gov/import_export/plants/manuals/domestic/downloads/xpm.pdf

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. 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, and a minimum 200 g of wood shavings are collected as one lab sample. Samples are shipped overnight to NCDA&CS for nematode assay.

Refer to the hypergeometric tables on the following page.

Hypergeometric Table for Random Sampling

Total number of Inspectional units:	Randomly select this number of units to inspect:
1-13	Inspect all units
14-15	13
16-17	14
18-19	15
20-22	16
23-25	17
26-28	18
29-32	19
33-38	20
39-44	21
45-53	22
54-65	23
66-82	24
83-108	25
109-157	26
158-271	27
272-885	28
886-200,000	29

95
percent
confidence
level

www.aphis.usda.gov/import_export/plants/manuals/domestic/downloads/xpm.pdf

Hypergeometric Table for Random Sampling

Sampling requirements for N.C., S.C., and Va.* [LOGS ONLY]

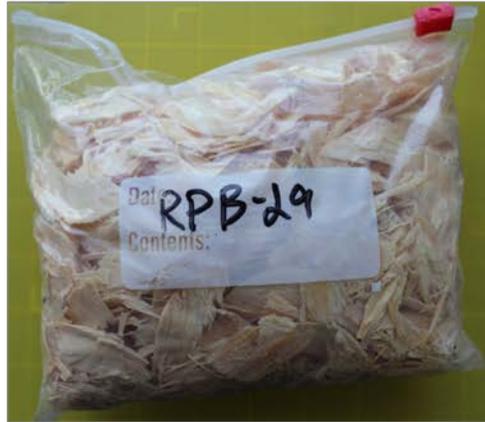
HYPERGEOMETRIC TABLE RANDOM SAMPLING	
Total number of Inspection units:	Randomly select this number of units to inspect
1 - 24	Inspect all units
25	24
26	25
27	26
28	27
29	28
30	29
31	30
32-41	31
42-46	35
47-51	39
52-82	42
83-104	45
105-242	51
243-352	54
353-453	55
454-699	56
700-1,000	57
1001-4,999	58
5,000 and up	59

95
percent
confidence
level

* 95 % confidence of detecting a 5% infestation with 100% efficacy.

www.aphis.usda.gov/import_export/plants/manuals/domestic/downloads/xpm.pdf

Pine-wood Sample



- Submit at least 200 g per sample.
- Include fee: \$10 per sample.
- Please create escrow account before shipping!

To establish an escrow account, please call the Agronomic Division at 919-733-2655. You will need to provide the following information:

- Desired Account Name
- Address
- Contact Person
- Phone Number
- Funds to establish the account [cash, check, money order or credit card (Visa/MasterCard only)].

For additional information, visit

www.ncagr.gov/agronomi/pdf/escrow.pdf.

Fill Out a Sample Information Form

www.ncagr.gov/agronomi/uyrnem.htm

www.ncagr.gov/agronomi/pdffiles/isnempd.pdf

Staff Virtual Tour Instructional PowerPoints Related Sites Agronomic Site Map	Brochures	Nematode Assay
	Instructional PowerPoint	Nematode Management
	Nema Notes:	Note 1: Root-Knot Nematodes: Biocontrol with French Marigold Note 2: Nematode Management in Soybeans (2-1 through 2-6) Note 3: Root-Knot Nematodes on Tobacco (3-1 through 3-10) Note 3-11: Lesion Nematodes on Tobacco Note 4: Sampling Problems (4-1 through 4-3) Note 5: Control of Plant-Parasitic Nematodes on Strawberry Note 6: Nematode Problems in the Urban Landscape Note 7-1: Nematode Parasites of Corn Note 7-2: Nematode Parasites of Turf Grass Note 7-3: Sting Nematodes Note 8: The B Recommendation for This Field Note 9: Root-Knot Nematodes on Irish Potatoes Note 10: Nematode Problem Diagnosis (10-1 through 10-2) Note 11: Nematode Management in Cotton Note 12: Root-knot Nematodes on Vegetables Note 13: Apple Nematodes in Western North Carolina Note 14: Nematodes on Sweetpotato Note 15: Nematode Management on Peach Note 16: Nematode Management on Peanut
	Sample Information Forms Print, Fill Out by Hand & Submit via Mail	Routine samples (form AD-3) Continuation page for routine samples (form AD-3+) Diagnostic (problem) samples (form AD-5) Continuation page for problem samples (form AD-5+)
	Sampling Instructions	Sampling for Plant-Parasitic Nematodes

Use form AD-5 and AD 5+

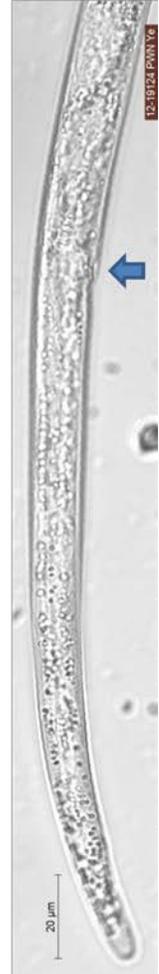
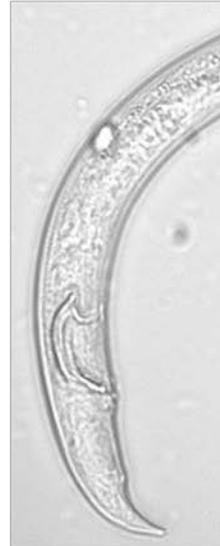
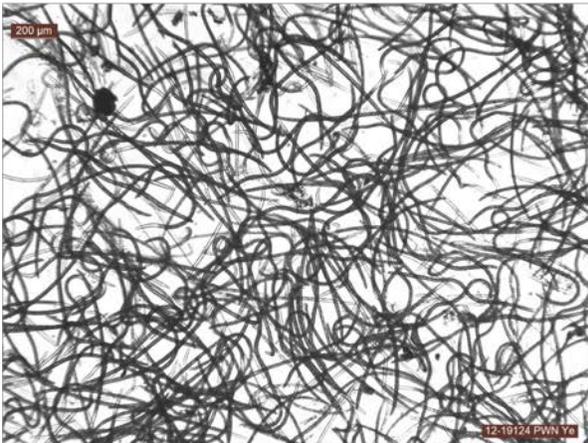
See examples on next page.



**Ship samples with information forms
via carrier (DHL, FedEx, UPS) to**

Weimin Ye, Ph.D., Nematologist
Nematode Assay Section
NCDA&CS Agronomic Division
4300 Reedy Creek Road
Raleigh, NC 27607-6465

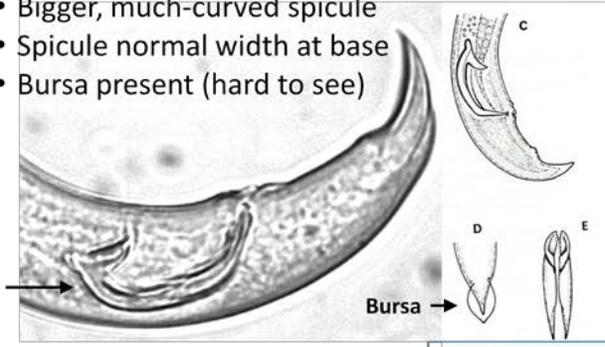
Typical Assay Results and Report



Micrographs of PWN from Japanese black pine (*Pinus thunbergii*) in Carteret County, N.C. (Lab ID: 12-19124)

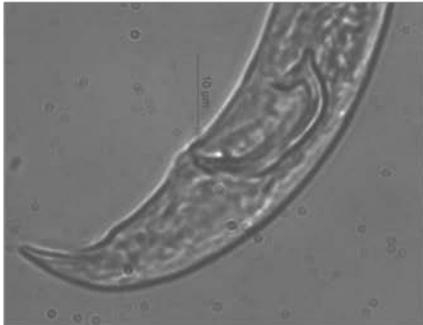
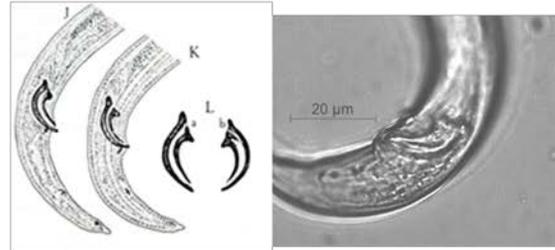
Pine wood nematode

- Bigger, much-curved spicule
- Spicule normal width at base
- Bursa present (hard to see)

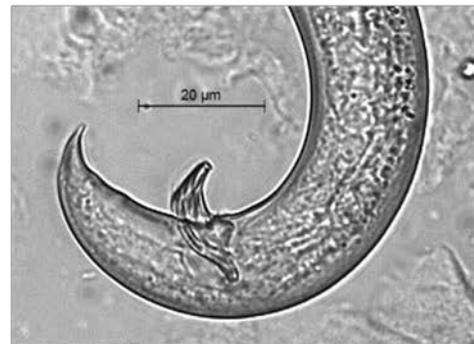
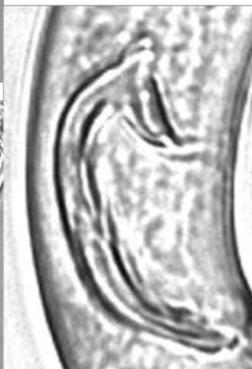
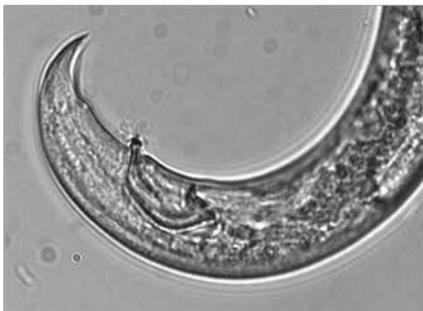
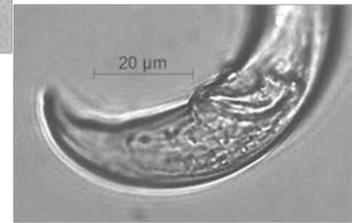
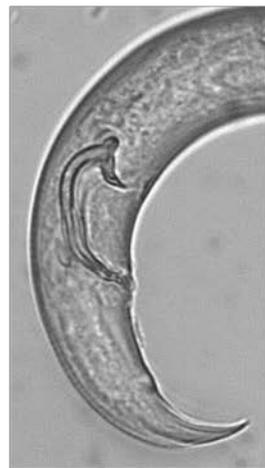
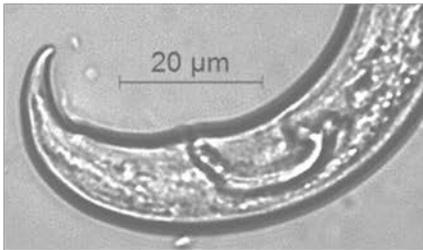
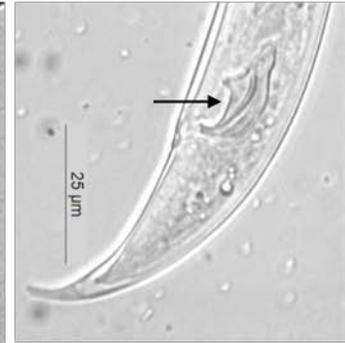
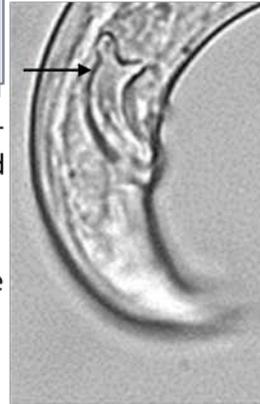


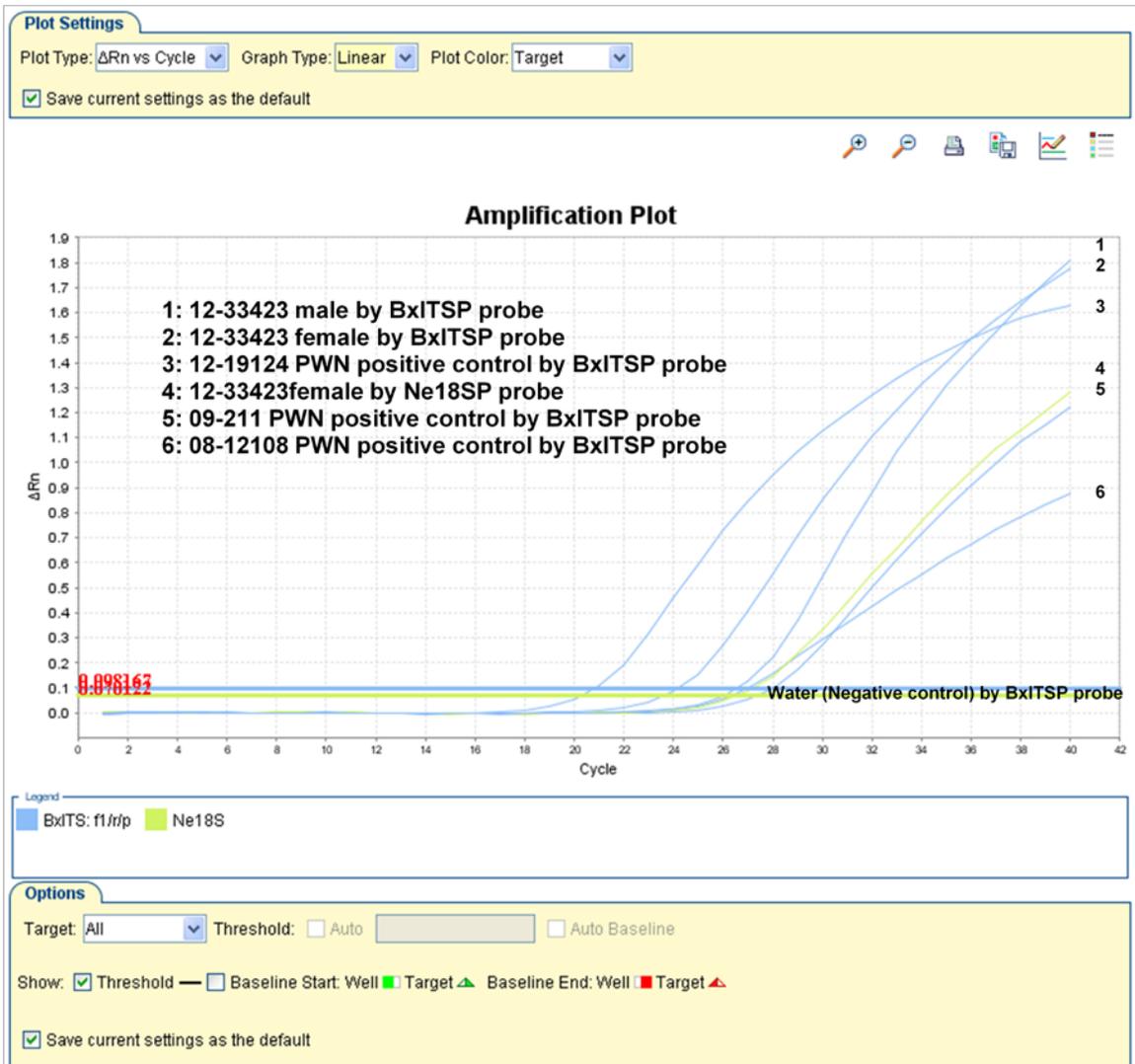
Male

Aphelenchid (fungivore)



- Smaller, rose-thorn-shaped spicule
- Spicule much wider at base (see arrow)
- No bursa





Example of a real-time PCR result for testing sample 2013-33423 by PWN-specific- and nematode-universal- primer/probes

Access report online: www.ncagr.gov/agronomi/PALS.

In the Report Quick Search box, enter the same name as written on the sample information form; no password is required.

Report Search Result for Daewoo GTL

Customer: Daewoo GTL
12750 Center Court DR #460
Cerritos

Client Advisor Agronomist

From: 2011 To: 2013

39 report(s) found. [Download CSV](#) [Download XML](#)

Fiscal Year	Report	Lab	Report Type	Status	Status Date	Farm ID	Number of Samples	PDF File	Spreadsheet	Select All
2013	N001009	Nemato...	Pine Wood	Released	2012/10/29	ILM-T2-2012	4	New Report!	Download Data	<input type="checkbox"/>
2013	N001008	Nemato...	Pine Wood	Released	2012/10/29	ILM-T2-2012	25	New Report!	Download Data	<input type="checkbox"/>
2013	N000798	Nemato...	Pine Wood	Released	2012/10/11	SAA 016	4	View Report	Download Data	<input type="checkbox"/>
2013	N000797	Nemato...	Pine Wood	Released	2012/10/11	ILM S2 2012	14	View Report	Download Data	<input type="checkbox"/>

NCD&CS Agronomic Division		Phone: (919) 733-2655	Website: www.ncagr.gov/agronomi/		Report No. FY14-N000061	
 <p>Pinewood</p>	Client: Northern Forest Product 800 Davis Dr, Blacksburg, VA 24061		Advisor: Neil Skaggs 4601 Research Way Room 1 Tifton, GA 31794			
	Nematode Report		County: OUT OF STATE		Links to Helpful Information	
Sampled:	Received: 07/15/2013	Completed: 07/15/2013	Farm: Sun 02			
Nematologist's Comments: Pine wood nematode (<i>Bursaphelenchus xylophilus</i>) was found from 5 out of 29 samples submitted.						
Sample Information		Results				
Total # Samples on Report: 29						
Sample ID	Lab ID	Number of Pine Wood Nematodes (PWN)	Sample Weight (g)	PWN / gram Wood	Number of Fungivores	Number of Bacterivores
1	N000402	0	276	0	0	2
2	N000403	0	283	0	0	6
3	N000404	0	267	0	0	0
4	N000405	0	284	0	0	0
5	N000406	26	288	1	0	4
6	N000407	0	328	0	0	2
7	N000408	640	225	3	0	0
8	N000409	1336	268	5	0	0
9	N000410	54	294	1	2	8
10	N000411	0	215	0	0	0
11	N000412	0	242	0	0	2
12	N000413	0	282	0	0	0
13	N000414	12	250	1	0	0


 Reprogramming of the laboratory-information-management system that makes this report possible is being funded through a grant from the North Carolina Tobacco Trust Fund Commission.

Thank you for using agronomic services to manage nutrients and safeguard environmental quality.
 - Steve Troxler, Commissioner of Agriculture.