Instruction for Pine-Wood Nematode Sampling and Assay

Weimin Ye (Ph. D)
Nematologist

N.C. Department of Agriculture & Consumer Services, Agronomic Division, Raleigh, NC

Physical Address: 4300 REEDY CREEK ROAD, RALEIGH NC 27607-6465
Phone: (919) 733-2655; FAX: (919) 733-2837
Email: Weimin.Ye@ncagr.gov

http://www.ncagr.com/agronomi/
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

http://permaculturinginportugal.net/blog/pine-wilt-nematode/
# Hosts of pine wood nematodes

- 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
Hypergeometric table

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

Hypergeometric table

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

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

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

Sampling

The PWN samples used were extracted from exported pine-wood logs. USDA/APHIS/PPQ officers followed the protocol provided in their Export Program Manual (http://www.aphis.usda.gov/import_export/plants/manuals/domestic/downloads/xpm.pdf) when inspecting and sampling PWN in pine-wood logs slated for export. The number of units to inspect was 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, were 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 were mixed together, and a minimum 200 g of wood shavings were collected as one lab sample. Samples were shipped overnight to NCDA&CS for nematode analysis.
Prepare nematode submission on-line

http://www.ncagr.gov/agronomi/PALS/Pages/Utilities/OnlineAccount/

On-line data entry instruction

Pine wood sample shipment

- Collect 200g to 300g of pinewood chips and place in a gallon zip-lock bag. Be sure to seal the bag to retain moisture in the bag.
- Write the sample ID on each bag with a sharpie marker or use a label.
- Print the online submission form and put this form in a separate plastic bag to keep it dry.
- Place this form inside the shipping box

Pine wood nematode assay fee:
$10 per sample in-state (North Carolina)
$20 per sample out-of-state

- Print on-line submission form, place in a plastic bag, and put in the shipment box with the samples. Do not put the sheet with the sample in the same bag.
- Ship the samples on the same day when samples are collected.
- Use a private carrier (FedEx, UPS, etc.) and choose express mail overnight service.
- Leave the samples indoor, keep moisture, avoid heat.
- Only live nematodes can be detected in the lab.

Shipping address:
Nematode Lab, Agronomic Division
NC Department of Agriculture & Consumer Services
PINEWOOD ASSAY
4300 Reedy Creek Road, Raleigh, NC 27607-6465
Phone: (919) 733-2655; FAX: (919) 733-2837

http://www.ncagr.gov/agronomi/nemhome.htm

Warning: Do not use the US Postal Service. Do not use this address - 1040 Mail Service Center, Raleigh, NC 27699-1040. This is in a different location and significant delivery delays may be expected. We do not receive mail on the weekend and holiday.

Go to PALS to check if your shipment is received the next day. It will take at least another day to receive the report. Email notification will be sent to you once report is completed.
Nematode extraction

NCDA&CS Nematode assay lab: extraction of PWN
Soak the wood sample in water in funnel overnight to get the nematodes
Nematode extraction and identification

Sample is weighed
Soak in water

Collect nematode extraction in a cup the next day
Pour in a counting dish

Identify nematodes in inverted microscope
Micrographs of PWN from Japanese black pine (*Pinus thunbergii*) in Carteret County, N.C. (Lab ID: 12-19124)
Sample submission form

http://www.ncagr.gov/agronomi/uyrnem.htm


<table>
<thead>
<tr>
<th>Boxes</th>
<th>Sample boxes are not available by mail. Pick them up at your county Cooperative Extension office or at the Agronomic Division office in Raleigh.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brochures</td>
<td>Nematode Assay</td>
</tr>
<tr>
<td>Instructional Presentation</td>
<td>Nematode Management</td>
</tr>
</tbody>
</table>

**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: Citrus 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
- Note 17: Managing Root-knot Nematode Species
- Note 18: Pine wood Nematode Sampling & Assay
- Note 19: Soybean Cyst Nematode (Heterodera glycines) Distribution in North Carolina, USA
- Note 20: List of Plant-Parasitic Nematodes Found in North Carolina

**Sample Information Forms**
- Routine samples (form AD-3) Updated
- Continuation page for routine samples (form AD-3+) Updated
- Diagnostic (problem) samples (form AD-2) Updated
- Continuation page for problem samples (form AD-5+) Updated
- Phytophrotectant certification (form AD-14) Updated

**Sampling Instructions & Protocols**
- Sampling for Plant-Parasitic Nematodes
- Submitting Samples for Problem Diagnosis
- Pine Wood Nematode Sampling and Assay Protocol
- Instructions for Online Data Entry for Pinewood Samples
- Instructions for Online Data Entry for Predictive Nematology Samples

Use form AD-5 and AD 5+
Use this form for your own record, prepare this document on-line and print this form for NCDA&CS Nematode Lab use.

---

Use it for tracking this report

Industry’s contact and email

APHIS’ contact and email

---

### NEMATODE PROBLEM-DIAGNOSIS INFORMATION

**NCDA&CS Agronomic Division Nematode Assay Section**

Mailing Address: 1040 Mail Service Center, Raleigh, NC 27699-1040

Physical Address (UPS/FedEx): 4300 Reedy Creek Road, Raleigh, NC 27607

Phone: (919) 733-2655

Web Address: www.ncagr.gov/agronomi

---

### SAMPLE INFORMATION

**SAMPLE ID** (5 characters only)

**CURRENT CROP** (include variety, if known)

**CROP LAST YEAR** (include variety, if known)

**NEMACIDE APPLIED LAST YEAR**

**SOIL TYPE**

**PLANT APPEARANCE** (check any that apply)

**SYMPTOM DISTRIBUTION**

<table>
<thead>
<tr>
<th>Serial</th>
<th>ID</th>
<th>CURRENT CROP</th>
<th>CROP LAST YEAR</th>
<th>NEMACIDE APPLIED LAST YEAR</th>
<th>SOIL TYPE</th>
<th>PLANT APPEARANCE</th>
<th>SYMPTOM DISTRIBUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>Pine</td>
<td>Pine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Pine</td>
<td>Pine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Pine</td>
<td>Pine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Pine</td>
<td>Pine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>Pine</td>
<td>Pine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>Pine</td>
<td>Pine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

*Thank you for using agronomic services to manage nutrients and safeguard environmental quality — Steve Troxler, Commissioner of Agriculture*
<table>
<thead>
<tr>
<th>SHEET</th>
<th>LAB NUMBER (leave blank)</th>
<th>SAMPLE ID (include variety, if known)</th>
<th>CURRENT CROP (include variety, if known)</th>
<th>CROP LAST YEAR (include variety, if known)</th>
<th>NEMATICIDE APPLIED LAST YEAR</th>
<th>SOIL TYPE</th>
<th>PLANT APPEARANCE (check any that apply)</th>
<th>SYMPTOM DISTRIBUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ID</td>
<td>Pine</td>
<td>Pine</td>
<td>Pine</td>
<td></td>
<td></td>
<td>Normal, Stunted, Yellow, Dead</td>
<td>Field, Patches</td>
</tr>
<tr>
<td></td>
<td>ID</td>
<td>Pine</td>
<td>Pine</td>
<td>Pine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ID</td>
<td>Pine</td>
<td>Pine</td>
<td>Pine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ID</td>
<td>Pine</td>
<td>Pine</td>
<td>Pine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Thank you for using agronomic services to manage nutrients and safeguard environmental quality. — Steve Troxler, Commissioner of Agriculture
Log in as grower (exporter) or advisor (USDA/APHIS/PPQ)
Use the same name in information sheet, no password is required
Pine wood nematode (Bursaphelenchus xylophilus) was found from 5 out of 29 samples submitted.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Lab ID</th>
<th>Number of Pine Wood Nematodes (PWN)</th>
<th>Sample Weight (g)</th>
<th>PWN / gram Wood</th>
<th>Number of Fungivores</th>
<th>Number of Bacterivores</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N000402</td>
<td>0</td>
<td>276</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>N000403</td>
<td>0</td>
<td>283</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>N000404</td>
<td>0</td>
<td>267</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>N000405</td>
<td>0</td>
<td>264</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>N000406</td>
<td>28</td>
<td>288</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>N000407</td>
<td>0</td>
<td>328</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>N000408</td>
<td>640</td>
<td>225</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>N000409</td>
<td>1336</td>
<td>268</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>N000410</td>
<td>54</td>
<td>294</td>
<td>1</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>N000411</td>
<td>0</td>
<td>215</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>N000412</td>
<td>0</td>
<td>242</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>N000414</td>
<td>0</td>
<td>282</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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.