

National *Dothistroma* collection – Sampling and shipping protocol

The objective of this protocol is to describe the types of sample collections that are requested and the preferred methods of handling samples so that the samples will be optimally useful to assess geographic and host ranges of *Dothistroma* species on conifer needles in the USA, and to minimize the risk of releasing exotic biota. Shipment of samples using the following protocol has been approved by the North Dakota State Plant Health Director, APHIS PPQ under the permit held by the North Dakota State University Plant Diagnostic Laboratory.

Questions or Mailers: If you have questions about collections, or you need mailers, contact:
Jim Walla, j.walla@ndsu.edu, 701-231-7069

Most important:

- 1) Mail the samples the same day as collected or keep them cool after collection in the field, and then freeze them until they are shipped.
- 2) Ship samples only in double tyvek envelopes. The samples can be in other containers, which are then placed within double tyvek envelopes, or the double tyvek envelopes can be shipped inside another container (but pre-addressed tyvek envelopes would not be needed for that).
- 3) The samples should be shipped to the NDSU Diagnostic Lab, not to Jim Walla, because the lab has the permit and the appropriate isolation facilities.

Sample locations: Samples of needles that may have *Dothistroma* needle blight (DNB) are being requested from as many different states as possible to cover the whole geographic range of the USA. In addition, we would like to obtain collections from many different types of sites (e.g., geographic, environmental, stand type) and from as many different host species in each type of site as possible. If you have, can obtain, or can arrange for collection of a sample of DNB, and that sample represents a different state, site type (e.g., altitude, stand type) within a state, or host within a site type, we want it, even if the locations are in close proximity to each other. If you have questions about whether a collection would be appropriate, either make a collection and send it, or contact us with your questions.

Hosts: We want as many host genera and species represented in the sampling as possible within each sampling location. It is likely that every pine species in the USA could have DNB. In addition to pines, DNB has been reported on several spruce species (*Picea*), on Douglas-fir (*Pseudotsuga*), and on larch (*Larix*). The latter hosts are most likely to be infected if there are heavily infected pines nearby.

What and when to collect: Needles with symptoms similar to those caused by DNB (Figure 1) and with fruiting bodies (Figure 2) should be collected. Optimum sample size is about 50 needles, at least some of which have fruiting bodies similar to those of *Dothistroma* (fungal mass/conidiomata pushing up through the needle surface). The needles for a single collection should all be from the same tree, but they can come from different age classes or different branches on the same tree. Needles should be collected during or near the growing season. Samples can be collected at least through Fall, 2012 whenever someone finds a symptomatic tree and needles with fruiting bodies, and they have the means to either collect and mail them the same day or keep the samples in cold storage until mailed.

What to NOT send: Needles with disease symptoms distinct from those of DNB and needles harboring obvious insects should not be submitted. If there is an unusual or high priority sample that appears to be DNB, but there are also other diseases or insects on the same branches or needles, separation of needles with putative DNB should be done. This may be done by sorting needles or selecting the portion of needles with DNB symptoms.

Other information: Photographs of diseased sites, trees, and needles on branches would be useful. Notes regarding severity of the disease, patterns of disease incidence, and hosts on which DNB was not found (where DNB is present on another host) would be valuable.

Submission of samples: Place all the needles from one collection into a plain tyvek envelope and seal that inner envelope. Place the inner envelope into the outer tyvek envelope and seal the outer envelope. Record as much of the requested information on the back of the outer envelope as possible, and keep a record of your collections. Mail the sample the same day as collected, or keep the samples in a freezer (preferred) or cooler until shipping. Multiple inner

envelopes can be placed in a single outer envelope, but the mailing permit is only for up to 3 ounces, so weigh the package if you want to include multiple inner envelopes. Also, write needed/available collection information on the inner envelopes in this case.

If you will use your own tyvek envelopes, address the envelope to: NDSU Plant Diagnostic Lab, NDSU Dept 7060, PO Box 6050, Fargo, ND 5810806050.

Symptoms and signs of Dothistroma needle blights on pines:

Symptoms: *Dothistroma* species cause a necrotic band or spot on needles, followed by death of the needle distal to the band. The band or region with fruiting bodies may have distinct, faint, or no red coloration.

Signs: The presence of a dark stroma pushing up the needle epidermis in a spot, band, or near the base of or within dead areas on a needle is indicative of either *Dothistroma* or brown spot.

Alternative causes: Brown spot is a very similar disease. Hosts tend to differ, but there is considerable overlap. Insect damage may also cause similar symptoms. Various stresses, e.g., winter damage, high salts, and drought can cause distal ends of needles to die. Those tend to result in relatively uniform dieback patterns, while location of pathogen infection on a needle tends to be more random.



Figure 1. Tree, shoots, and needles with symptoms and signs of *Dothistroma* needle blight. Infected needles tend to be on the lower portion of a tree, on a protected side of a tree, and on the oldest attached age-classes of needles.

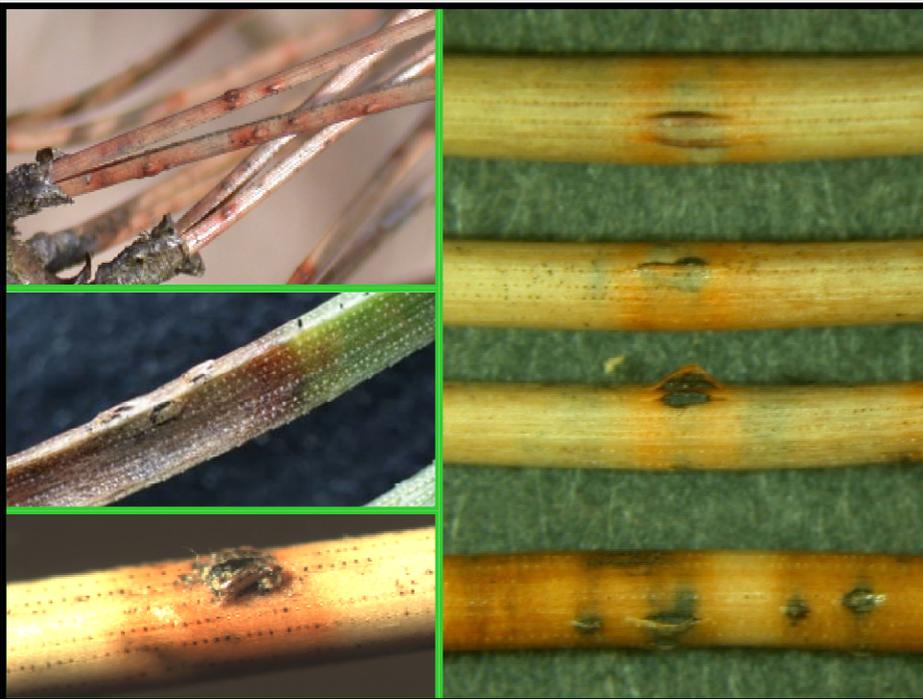


Figure 2. Needles with symptoms and signs of DNB. Fruiting bodies are black pustules that push up through the needle surface. Fruiting bodies may occur in areas with distinct, faint, or no red pigment. They may occur in bands, at the basal end of necrosis, or within dead areas of needles.