

Occurrence of *Rhabdocline* Taxa in Douglas-Fir Christmas Tree Plantations in Michigan

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ABSTRACT

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A survey to determine the identity of taxa causing *Rhabdocline* needle cast in six plantations of Douglas-fir (*Pseudotsuga menziesii*) Christmas trees in northern Lower Michigan was undertaken in 1979. *Rhabdocline weirii* subsp. *oblonga* was the predominant taxon present, occurring on 83.9% of the infected needles examined. *R. pseudotsugae* subsp. *pseudotsugae*, the only other *Rhabdocline* taxon encountered, was present on 17.3% of the infected needles examined. The two taxa could be separated with 94% accuracy by characteristic methods of rupture of the host epidermis by the apothecia. The overall level of infection (percentage of needles infected) in one stand was significantly higher ($P=0.05$) in the interior of the stand than on the margins; it was also significantly lower ($P=0.05$) on the north sides of the trees.

Additional key word: epidemiology

Rhabdocline needle cast is a disease of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) that affects the needles of young trees and the lower crowns of older trees (10). It is particularly serious in Christmas tree plantations in Michigan and New England (1,5,8) and threatens the continued use of Douglas-fir for Christmas trees in these areas.

Rhabdocline needle cast is caused by

fungi in the genus *Rhabdocline* Syd. that produce sexual fruiting bodies (apothecia) exclusively on the foliage of Douglas-fir. The two species of *Rhabdocline* are differentiated by the presence (*R. weirii* Parker and Reid) or absence (*R. pseudotsugae* Syd.) of an apical annulus surrounding the pore of the ascus that gives an amyloid reaction in Melzer's reagent (1.5 g of iodine, 5 g of potassium iodide, and 100 g of chloral hydrate in 100 ml of water). These two species are further characterized by Parker and Reid (7) as consisting of a population of five distinct subspecies, three for *R. weirii* and two for *R. pseudotsugae*. Subspecies are separated by consistent differences in the location of needle lesions (epiphyllous or hypophyllous), by constant association with a conidial anamorph (*Rhabdogloenum* spp.), and by ascospore and paraphysis morphology.

MATERIALS AND METHODS

All Christmas tree plantations in the northern Lower Peninsula of Michigan known to contain Douglas-fir were examined. Five plantations infected with *Rhabdocline* spp. were sampled during a 2-wk period between 18 May and 3 June 1979, and another plantation was sampled on 1 July.

Branches with infected needles were collected along transects perpendicular to the orientation of the tree rows. Infected branches were cut so that all third-year needles were removed with the sample. In the laboratory, each branchlet was stripped of its second-year needles, and a random sample of three was chosen for microscopic examination. Infected third-year needles were examined separately. Needles were soaked for 10 min in 5% potassium hydroxide, then rinsed for 5 min in distilled water. Apothecia produced on these needles were first examined under a dissecting microscope and classified as either "*R. weirii*" or "*R. pseudotsugae*" types depending on the lateral or medial dehiscence, respectively, of the host epidermis as the apothecia enlarged. These apothecia were excised from the needles and transferred to a droplet of Melzer's reagent. They were then examined microscopically for the occurrence of an amyloid reaction of the annulus surrounding the pore of the ascus tip, and notes were taken on ascospore and paraphysis morphology.

A more intensive sampling procedure was implemented in the Cheboygan plantation for the purpose of evaluating

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patterns of occurrence of *Rhabdocline* spp. A representative block in the middle of the stand was chosen, and trees were sampled consecutively along the margin and in the interior of the block. As they were sampled, each tree was recorded as being not infected or was given subjective ratings as lightly (0–10%), moderately (11–40%), or heavily (more than 40%) infected. Sampling continued until five trees in each such “class” were collected from both the margin and the interior of the block.

Sampling consisted of removing three branchlets (equivalent to one sample) from each of the four cardinal aspects of the trees at a height of 45.7 cm above the ground. Branchlets were cut so that all second-year needles were attached. A total of 120 samples from 30 trees was collected. Needles on the left-distal portion of each branchlet were examined under a dissecting microscope. The total number of needles per sample, the number of diseased needles per sample, and the number of apothecia on each infected needle were recorded. No attempt was made to determine which *Rhabdocline* taxa were present on these needles. These data were then ordered, filed, and analyzed (one-way analysis of variance) with the help of the MIDAS statistical program (4).

RESULTS

R. weirii subsp. *oblonga* and *R. pseudotsugae* subsp. *pseudotsugae* were the only *Rhabdocline* taxa encountered in Michigan Christmas tree plantations (Table 1). *Rhabdogloeum* spp., putative

anamorphs of certain *Rhabdocline* taxa (3,7), have never been reported in Michigan and were not encountered in this survey. *R. weirii* subsp. *oblonga* was found on infected trees at all six plantations, accounting for 60.1–100% of the *Rhabdocline* apothecia examined. *R. pseudotsugae* subsp. *pseudotsugae* was found on infected trees at four of the six plantations, accounting for 21.6–39.9% of the apothecia examined from these plantations (Table 1).

Apothecia of *R. weirii* were observed on 83.9% of the infected needles examined, whereas *R. pseudotsugae* was present on 17.3%. Only four of the 330 needles examined microscopically produced apothecia of both *R. weirii* and *R. pseudotsugae*. Twelve infected third-year needles were encountered, and all were infected with *R. weirii* subsp. *oblonga*.

Attempts to identify *Rhabdocline* species solely by macroscopic apothecial characteristics were successful for 311 of the 330 apothecia examined. In all, 11 apothecia were incorrectly identified as *R. pseudotsugae* when they were actually produced by *R. weirii*, and eight apothecia of *R. pseudotsugae* were incorrectly identified as *R. weirii*. This represented an error rate of 5.8%.

Samples collected from the north sides of trees in the Cheboygan plantation had lower levels of infection (significant at $P=0.05$) than samples from the other aspects of the trees (Table 2). The number of needles produced and the number of apothecia per infected needle did not vary significantly ($P=0.05$) with the aspect

from which they were taken. Samples collected from the margin of the block were significantly ($P=0.05$) less infected than samples collected from the interior of the block (14.7 vs. 21.7%) and produced significantly ($P=0.05$) fewer apothecia per infected needle (2.2 vs. 2.7).

DISCUSSION

R. weirii subsp. *oblonga* appears to be the predominant taxon causing *Rhabdocline* needle cast in Michigan, and *R. pseudotsugae* is a minor component of the population. Given the number of needles examined and the relative abundance of *R. weirii* and *R. pseudotsugae* apothecia observed, it is statistically expected that approximately four needles would be found bearing apothecia of both species. This was exactly the case, reinforcing our estimates of the relative proportions of the *Rhabdocline* taxa present in these plantations. Both *R. weirii* subsp. *oblonga* and *R. pseudotsugae* subsp. *pseudotsugae* normally produce apothecia on second-year needles, whereas *R. weirii* subsp. *obovata* produces apothecia on third-year needles (7). All apothecia on third-year needles encountered in this study produced ascospores typical of *R. weirii* subsp. *oblonga* and not *R. weirii* subsp. *obovata*. This is consistent with reports by Parker and Reid (7) that *R. weirii* subsp. *oblonga* occasionally produces apothecia on third-year needles.

This is the first report of *R. pseudotsugae sensu* Parker and Reid in the Lake States and represents the only instance in which it has been observed outside the normal range of Douglas-fir in the United States. *R. pseudotsugae sensu* Sydow has been reported in the eastern United States (1,8), but Parker and Reid have indicated that these were probably *R. weirii* (7). *R. pseudotsugae* subsp. *pseudotsugae* is present in European plantings of Douglas-fir and appears to be the only *Rhabdocline* taxon present in Europe (7). *R. weirii* subsp. *oblonga* has been identified from collections in Rhode Island and Massachusetts (7); it has not been confirmed in the Lake States, although Morton and Miller (5) reported that *R. weirii* was present in Michigan.

Parker (6) has demonstrated that *R. pseudotsugae* has a strict requirement for a period of at least 72 hr of 100% relative humidity for ascospore release and subsequent infection of Douglas-fir. Although no similar studies have been done with *R. weirii*, our observations tend to indicate that *R. weirii* subsp. *oblonga* is somewhat less rigorous in its requirements for ascospore release than is *R. pseudotsugae* subsp. *pseudotsugae*. On the needles that we collected, *R. pseudotsugae* was observed to retain full complements of eight ascospores as late

Table 1. Number and percentage of apothecia represented by *Rhabdocline weirii* and *R. pseudotsugae* in six northern Lower Michigan plantations of Douglas-fir

Plantation	No. of needles examined	Apothecia observed			
		<i>R. weirii</i>		<i>R. pseudotsugae</i>	
		No.	%	No.	%
Cheboygan	95	187	60.1	124	39.9
Kate Hansen	33	98	100.0	0	0.0
Harbor Springs	54	182	78.4	50	21.6
Brubaker	28	125	96.9	4	3.1
Unit 12	81	267	100.0	0	0.0
Stutsmanville	39	82	73.9	29	26.1
Totals	330	941	82.0	207	18.0

Table 2. Mean numbers of needles, percentage of infection, and numbers of apothecia per infected needle for samples collected from the cardinal faces of Douglas-fir trees in the Cheboygan plantation^x

Cardinal tree face sampled	Mean number of needles per sample ^y	Mean percent infection ^z	Mean number of apothecia per needle ^y
North	103.8	11.6 a	2.4
East	101.5	19.8 b	2.6
South	95.6	21.3 b	2.3
West	100.8	20.2 b	2.5
Mean	100.4	18.2	2.4

^x Average of 30 samples per data point.

^y No significant differences were detected among means.

^z Within columns, values followed by a common letter are not significantly different ($P=0.05$) according to Duncan's new multiple range test.

as 1 July, whereas *R. weirii* was most often observed with few or no ascospores remaining in the asci at the time they were collected, as early as 18 May. Hypothetically, the release of ascospores through the apical pore of *R. weirii* asci may be more efficient than the method observed for *R. pseudotsugae*, which requires imbibition of sufficient water to cause physical rupture of the ascus wall. This difference in ascospore release method may be an important factor in determining the species composition of a population of *Rhabdocline* taxa, especially in inland areas where long periods of 100% relative humidity are uncommon. A better understanding of the nature of the mechanisms controlling ascospore release in *R. weirii* may allow better timing in the application of fungicides for control of *Rhabdocline* needle cast in Michigan and the Lake States.

Apothecial characteristics are useful in separating the species of *Rhabdocline* found in Michigan. We were able to separate the two taxa found in Michigan with 94% accuracy by using a single apothecial character. Although the

lateral or medial dehiscence of the host epidermis is the most easily recognized property of the apothecia, other differences such as the color and thickness of the hymenium were also noted but not quantified in this study.

Rhabdocline spp. are cold-tolerant fungi with optimum growth rates at about 10 C (6). The lower infection rates observed on the north sides of trees, unusual in needle-cast diseases, probably reflect the ability of *Rhabdocline* spp. to grow at low temperatures during winter months on sun-warmed foliage. Lower infection rates along the margins of the stand may be caused by rapid drying of foliage on the margins after periods of rainfall.

Extremely wide variation in the level of infection of adjacent trees was noted, indicating the well-documented variation in resistance of strains and individuals of Douglas-fir to *Rhabdocline* needle cast (1,2,9). Varieties of Douglas-fir resistant to needle cast would be eminently practical for control of *Rhabdocline* needle cast in the Lake States and should be considered as a tool for combating

this important disease of Douglas-fir Christmas trees.

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