Effect of Relative Humidity and Temperature on Needle Cast Disease of Douglas Fir

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ABSTRACT

High levels of infection by *Rhabdocline pseudotsugae* subsp. *pseudotsugae* occurred on Douglas fir in growth chamber experiments when a treatment of 100% relative humidity and 13 C was applied for 3 days immediately following inoculation. Infection levels were lower when the treatment was applied at higher temp or for less than 3 days. After the 3-day treatment period, temp did not affect significantly the level of infection. Phytopathology 60:1270-1273.

Additional key words: needle blight, northwestern United States, British Columbia.

Many of the natural stands of Douglas fir (*Pseudotsugae menziesii* [Mirb.] Franco) in the dry-belt regions of British Columbia and the northwestern United States are now under intensive management for the production of Christmas trees. Formerly, stands undergoing periodic needle cast outbreaks caused by members of the genus *Rhabdocline* could be left to recover, but present intensive management practices require all areas to be fully stocked with high-quality trees. Weir (12) described the needle cast disease in 1917 from observations made in the northwestern United States, while Sydow (10) described the causal agent in 1922, erecting the genus *Rhabdocline* with one member, *R. pseudotsugae*. Recently, Parker & Reid (6) revised the generic description and added four new members: *R. pseudotsugae* subsp. *epiphylla, R. weirii* subsp. *weirii, R. weirii* subsp. *obovata,* and *R. weirii* subsp. *oblonga.* All members are common throughout the natural range of Douglas fir with the exception of *R. weirii* subsp. *oblonga,* which is more prevalent in plantations in the northeastern United States (2). *Rhabdocline pseudotsugae* subsp. *pseudotsugae* is the only member of the genus reported from plantations in Britain (3, 7, 13) and Europe (1, 9, 11), and is the most prevalent and serious cause of needle casting in the Christmas tree-growing areas of British Columbia.

The present report is concerned with the environmental conditions required for infection of Douglas fir by *R. pseudotsugae* subsp. *pseudotsugae,* and is one of a group of studies undertaken to determine the cause of periodic outbreaks of the disease.

MATERIALS AND METHODS.—A total of 290 plants was used in the experiment, 208 grown from seed and 82 obtained from the field. Seeds for 68 plants were obtained from one locality on Vancouver Island (C) and for 140 plants from one locality near Cranbrook in southeastern British Columbia (I). Ten weeks prior to inoculation, and after a 3-week period of stratification, the seeds were sown in pots in a growth room having a 16-hr photoperiod (800 ft-c) at 21 C and 80% relative humidity (RH), and 8 hr of dark at 18 C and 71% RH. Field plants, 15-60 cm high and 10-30 years of age, obtained from the Cranbrook area, were potted 2 months prior to inoculation and transported to Victoria. The needles of the previous year, severely infected with *R. pseudotsugae* subsp. *pseudotsugae,* were removed before the bud-opening period, 20 May-4 June.

Inoculum, consisting of spores in mature apothecia on severely infected 1-year-old needles, was obtained 2 days prior to inoculation of the plants on June 8-9 from an area near Invermere in eastern British Columbia. Before the plants were placed in the inoculation chamber, three or four needles on each of two plants selected for a given temp and humidity treatment were marked, and a needle bearing a mature apothecium was attached to the upper or lower surface of each. The needles bearing apothecia were held in position for 3 days with a drop of nail polish at one end. All plants were then placed in the inoculation chamber on a greenhouse bench and exposed to a spore discharge period lasting 12 hr at approximately 13 C. The chamber consisted of a rectangular wooden framework covered with burlap kept moist by water from mist sprayers. The inoculum was suspended on a wire mesh shelf above the plants. Spore discharge was checked by placing glass slides on the soil surface beneath the plants.

Ten plants, selected at random from each of the three sources, served as checks. They were placed in the inoculation chamber after removal of the inoculum, but otherwise received treatment identical to that given the inoculated plants.

Immediately after inoculation, the plants were subjected to three postinoculation periods of 0, 24, or 72 hr at 100% RH, and three temp regimes in growth rooms having 16-hr photoperiods (800 ft-c supplied by incandescent and Gro-Lux fluorescent lamps). Temperature regimes were 13-13 C, 24-13 C, and 24-18 C, light and dark periods, respectively. A RH of 100% was obtained by placing the potted plants under plastic and spraying the inside with water. After the required period of time at 100% RH the plastic was removed and the plants were incubated at the above temp regimes for 9-14 months. Relative humidity was 60% in the 13-13 C incubation room and within the range of 70-80% in the other rooms.

One or two of the marked needles, inoculated using individual needles bearing apothecia, were removed for sectioning 3 weeks after inoculation, and two were

removed after 6 weeks. Sections were stained using the periodic acid-Schiff technique developed by Farris (4).

The results were subjected to tests of chi-square (8). The chi-square value of each factor was determined by summing-over the data of the other factors in the experiment (e.g., in determining the chi-square values for temp, the RH data were summed-over). Comparisons also were made among individual plant origins.

RESULTS.—The first symptoms, pale-yellow lesions 1-2 mm in diam, appeared on needles 7 weeks after inoculation. By the end of the 3rd month, the pale-yellow lesions on several plants had turned red-brown, characteristic of the disease in the field. After 9 months, 66 of the 103 plants developing symptoms had both red-brown and pale-yellow lesions. Pale-yellow lesions were the only symptom on the other 37 infected plants. Apothecia failed to develop on the 24 heavily infected plants which were retained for 14 months at the three temp regimes. Field plants receiving a 72-hr postinoculation treatment at 100% RH and 13-13 C developed symptoms 2-3 weeks earlier than plants from other sources or plants which received other treatments. The 30 check plants were free of symptoms.

Histological sections, prepared from the needles individually inoculated, revealed that infection had taken place within 3 weeks in plants incubated at 13-13 C following a 72-hr postinoculation period of 100% RH at 13-13 C. At 6 weeks, the fungus was well established in the mesophyll on plants held at all three incubation temp regimes following the 72-hr postinoculation period at 13-13 C, but not at 24-13 C or 24-18 C. At 6 months, sectioning confirmed the presence of the fungus in pale-yellow lesions on eight lightly infected plants held at the three incubation temp regimes following the 72-hr postinoculation period at 100% RH and 24-18 C. At all times of sectioning, the hyphae and pattern of infection appeared identical to that observed in previous studies (4, 11).

Infection, recorded as the percentage of plants becoming infected, increased with an increase in the length of the 100% RH period following inoculation, and was highest at the 13-13-C temp regime (Table 1). The highly significant chi-square values for individual factors indicated that temp ($\chi^2 = 67.9$) had a greater effect than length of 100% RH periods ($\chi^2 = 32.2$). Differences in geographical origins did not attain significance. Some infection (24%) took place at a temp regime of 13-13 C when the plants were in 100% RH only during the 12-hr inoculation period. No infection occurred, however, at 24-13 C or 24-18 C.

Percentage infection was greater at the lower temp regimes during the postinoculation period and during incubation (Table 2). The highly significant chi-square values for individual factors indicated that temp during the postinoculation period ($\chi^2 = 64.1$) had a greater effect than temp during incubation ($\chi^2 = 24.9$). The greatest number of plants was infected, and the heaviest level of infection on individual plants occurred, when they were given a postinoculation treatment of 100% RH for 72 hr at 13-13 C and then transferred to the 3 incubation temp regimes. Forty-five of the 47 plants inoculated under these conditions developed numerous lesions on current-year needles. The two symptom-free plants were raised from seed of trees grown on Vancouver Island.

Under conditions outlined in Table 2, when the temp regimes during the postinoculation period were varied, infection levels were significantly higher on plants obtained from the field than on those from seed originating on Vancouver Island ($\chi^2 = 6.8$, $P < .01$) and eastern British Columbia ($\chi^2 = 5.2$, $P < .05$). There were no significant differences between infection levels on plants grown from the two seed sources under any of the conditions tested.

DISCUSSION.—Symptom development in growth rooms closely paralleled that of highly susceptible trees in the field, which develop pale-yellow lesions on current-year needles during August-December following spore dispersal in June. Van Vloten (11) suggested that the change from pale-yellow to red-brown lesions was brought about in plantations by frost. In the present study, the change occurred at temp well above freezing. Infected plants in growth chambers failed to develop mature apothecia 11-12 months after inoculation, as occurs in the field, but this is not surprising since 16-hr photoperiods and the temp regimes tested differ greatly from those in nature during the winter and spring months.

A period of high RH is necessary for spore discharge by *Rhabdocline pseudotsugae*, and studies with artificial media indicated that a temp in the range

TABLE 1. The effect of length of 100% relative humidity periods following inoculation on infection by *Rhabdocline pseudotsugae* subsp. *pseudotsugae* of Douglas fir from three sources at three incubation temp regimes

Length of 100% relative humidity (RH) periods, hr	% Plants infected at three incubation temp regimes											
	13-13 C[a]				24-13 C				24-18 C			
	F	C	I	All sources[b]	F	C	I	All sources	F	C	I	All sources
0[c]	20	0	33	**24** (17)[d]	0	0	0	**0** (21)	0	0	0	**0** (17)
24	100	40	91	**81** (21)	0	0	0	**0** (21)	20	0	0	**6** (16)
72	100	67	100	**94** (16)	100	67	0	**50** (12)	20	0	14	**14** (14)

[a] Temperatures during light (16 hr) and dark (8 hr) periods, respectively.
[b] Source of plants: field-grown in southeastern British Columbia (F); grown from seed collected in the coast region of British Columbia on Vancouver Island (C) and near Cranbrook in southeastern British Columbia (I).
[c] All plants were held in the inoculation chamber at 100% RH for 12 hr overnight.
[d] Number of plants inoculated.

TABLE 2. The effect of postinoculation and incubation temp on infection by *Rhabdocline pseudotsugae* subsp. *pseudotsugae* of Douglas fir from three sources

Postinoculation period[a] Temp regimes	% Plants infected at three incubation temp regimes											
	13-13 C[b]				24-13 C				24-18 C			
	F	C	I	All sources[c]	F	C	I	All sources	F	C	I	All sources
13-13 C	100	67	100	**94** (16)[d]	100	100	100	**100** (17)	100	67	100	**93** (14)
24-13 C	100	17	46	**50** (24)	100	67	0	**50** (12)	40	0	40	**33** (12)
24-18 C	60	0	33	**33** (24)	100	0	0	**29** (14)	20	0	14	**14** (14)

[a] 72 Hours at 100% relative humidity.
[b] Temperatures during light (16 hr) and dark (8 hr) periods, respectively.
[c] Source of plants: field-grown in southeastern British Columbia (F); grown from seed collected in the coast region of British Columbia on Vancouver Island (C) and near Cranbrook in southeastern British Columbia (I).
[d] Number of plants inoculated.

of 1-15 C was necessary for germination and growth, 10 C being optimal (5). June weather records for southeastern British Columbia show that temp below 13 C prevail during rain periods. From the present study, the level of infection was related to both the duration of the 100% RH postinoculation period and the temp during the same period. Given a 100% RH period for 72 hr at the lowest temp tested (13-13 C), a very high level of infection resulted, no matter which of the three temp regimes followed. Reducing the duration of the 100% RH period or raising the temp during the period reduced levels of infection. Some infection always occurred at the temp regimes tested, provided there was a 72-hr postinoculation period at 100% RH, and even when the 100% RH period was reduced to the period of inoculation (12 hr), a small amount of infection occurred at the continuing temp regime of 13-13 C. However, long periods at 13-13 C or rain periods lasting 72-84 hr in the temp range of 13-24 C are unlikely to occur within the natural range of Douglas fir following spore dispersal in June.

In this study, the ascospores germinated and penetrated very young needles a few days after being discharged from the asci. Still to be determined is whether the spores may lie dormant on the surface of needles for several weeks or months until a suitable temp-humidity period occurs, and whether needle tissue remains susceptible for that period. Because the spores are held tightly to needles by a water-resistant mucilaginous coating, and one cell of each spore forms a thick dark wall, they may be able to survive lengthy periods of low humidity and high temp. This information would help to relate weather data to fluctuating infection levels in the field.

The number of plants from all sources becoming infected was considerably greater with the postinoculation treatment of 72 hr at 100% RH and 13-13 C than with any other treatment. A reduction in the level of infection on any one plant source may be explained by variability in the rate of spore germination at the temp tested. Apparently 72 hr at 100% RH was sufficient time for most spores to germinate and penetrate all susceptible plants at a temp regime of 13-13 C, but only a few spores were able to penetrate the host in less time or at higher temp. Under optimal conditions for infection, only the plants originating from seed obtained on Vancouver Island showed signs of resistance while, under suboptimal conditions, there were significant differences between the infection levels on field plants and plants grown from the two seed sources. The differences may have been caused by the deliberate selection of field plants on the basis of susceptibility in previous years and/or by the difference in age of field plants (10-30 years) and the plants grown from seed (2-3 months). In natural stands and in provenance experiments conducted in Europe, the intermountain variety of Douglas fir appears more susceptible to the disease than the coast variety, although highly susceptible individuals are found in both varieties (7). In the present study, the level of infection on the intermountain variety of Douglas fir grown from seed was slightly higher than on the coast variety, but the differences were not statistically significant. Since susceptibility may vary with age of the host and since the genetic basis of resistance is unknown, the results obtained from a relatively small number of plants from only one seed source of each variety can neither support nor contradict the observations made in natural stands and provenance experiments.

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