Artificial Inoculation of Pinus radiata with Scirrhia (Dothistroma) pini: Effect of Relative Humidity and Temperature on Incubation

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ABSTRACT

In the temperature range 13-24 C and relative humidity range 70-100%, infection of *Pinus radiata* by conidia of *Scirrhia pini* was greater at the lower temperatures when accompanied by high humidity values. With a 768-hr incubation period containing an average of 580 hr of 100% relative humidity, 97, 65, and 0% of the pine became infected in temperature ranges 16-13, 21-16, and 24-21 C, respectively. With relative humidity values in the range 91-81%, only a few leaves on 47% of the pine were infected in the temperature range 16-13 C. The results suggest that leaf infection would be greater at even lower temperatures and longer incubation periods than those tested at high relative humidities.

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In 1961, needle blight of pines caused by *Dothistroma pini* Hulbary was considered to be a minor disease of several species of pine planted in the midwestern United States. The disease is now a major concern in several countries (8, 9), and occurs in at least 12 countries on 5 continents (17). Hosts now include Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco), larch (*Larix decidua* Mill.) and many species of pine (1, 3, 13, 16). Monterey pine (*Pinus radiata* D. Don) is generally considered the most susceptible

host. Variation in size of conidia and conidial locules from one part of North America to another (18) and from one continent to another (11) has led to the description of several varieties. The perfect state of the fungus has been reported only from western North America (2, 4), France (14), and Rumania (10). Since the perfect state occurs throughout British Columbia on all pine species affected, the fungus is referred to in this paper as *Scirrhia pini* Funk & Parker.

Little is known of the environmental conditions required for infection by the fungus, although a number of pine species, including Monterey pine, were inoculated successfully in greenhouse and field experiments (2, 5, 6, 15). More precise information on the temperature and humidity combinations which both limit and favor infection will be of value for determining the relative susceptibility of hosts and for locating low-risk sites where each species may be grown successfully. This paper reports on the artificial inoculation of Monterey pine with conidia of *Scirrhia pini* in growth chambers at combinations of three controlled temperature and humidity regimes.

MATERIALS AND METHODS.—Seeds for the 720 plants used in the experiment originated in Chile and were sown in greenhouse flats at Victoria, B.C., after a 3-week period of stratification. One to 2 weeks later, the young seedlings were transplanted, four to a 15-cm pot, in sterile soil (equal proportions of peat and sand) and kept in a growth room where they received a 16-hr photoperiod (1,800 ft-c) at 24 C and 70% relative humidity (RH), and an 8-hr dark period at 21 C and 77% RH. Fertilizer, 2 g Plant Prod (28-14-14)/liter of tap water, was added once a week at the rate of 100 ml/pot until the plants were inoculated at the age of 4 months.

Inoculum consisted of conidia suspended in sterile distilled water. Approximately half of the spores were obtained from mycelia of 3-week-old malt agar cultures and half from conidial locules in 10-week-old malt agar cultures. The cultures originated from ascospores in fruiting bodies on lodgepole pine (*Pinus contorta* Dougl.) growing on southern Vancouver Island.

I inoculated all plants by inverting each pot and briefly dipping the foliage in the inoculum (8,000 spores/cm^3), and by spraying each plant with ca. 1 ml of inoculum (18,000 spores/cm^3) from an atomizer. Immediately after inoculation, 240 plants were placed in each of three growth rooms having 16-hr photoperiods adjusted to 1,800 ft-c at bench height and supplied by incandescent and Gro-Lux fluorescent lamps. Temperature and humidity regimes in the growth rooms were 24-21 C with 70-77% RH, 21-16 C with 73-74% RH, and 16-13 C with 91-81% RH, light and dark periods, respectively. In each growth room, 72 plants were subjected to 54 consecutive hr of 100% RH (early-incubation treatment), as were 72 to 144 hr and 72 to 288 hr. The remaining 24 plants in each growth room were not given an early incubation treatment. I obtained 100% RH by bending a 5-cm-wide strip of wire mesh over the top of the plants in each pot and anchoring the ends in the soil, placing a thick strip of moist gauze on the wire mesh, and covering the entire unit with a plastic bag moistened on the inside with water. Sheets of aluminum foil were then placed on the top surface of the bags to prevent a temperature rise inside during the photoperiod. The plants received only 50-125 ft-c during photoperiods as a result of the methods employed to control relative humidity and temperature.

Following each early-incubation treatment, the plastic bags, wire mesh, and moistened gauze were removed; the plants were divided into three groups of 24 plants each, and each group was placed in one of the three growth rooms. Of the 240 plants in each growth room, the 24 that had not received an early-incubation treatment and 108, or half of the remainder, were given 100% RH treatments for 17 hr each day for the duration of the experiment (late-incubation treatments). Each afternoon, the relative humidity was held near saturation by the covering of the pots with plastic bags moistened on the inside after the plants had been sprayed with tap water. A single strand of light wire was bent over the plants in each pot to support the bags and prevent contact with the foliage. Sheets of aluminum foil covered all plants in the growth rooms during the daily 17-hr treatment periods, and as a result the plants were subjected to 1,800 ft-c of light for 7 hr and 50-125 ft-c for 9 hr each day.

I terminated the experiment 32 days after inoculation, and determined infection levels by counting the number of leaves on each plant with asexual fruiting bodies of the fungus. Data were evaluated by analysis of variance with orthogonal comparisons.

RESULTS.—The first symptoms, small, irregularly shaped chlorotic or necrotic areas on leaves, were usually followed 1-3 days later by the appearance of fruiting bodies in the form of conidial locules. Fruiting bodies were first noted 28 days after inoculation. Necrotic areas frequently expanded to include the distal portion of leaves, but occasionally they remained as distinct areas less than 1 cm in length in the central portion. The brick-red bands, characteristic of the disease, usually appeared several days after the locules had developed, but occasionally they appeared at the same time. Most infections occurred on the primary leaves on the main stems of plants and, except for the most heavily infected plants, only a small percentage of leaves in fascicles became infected.

A summary of infections occurring at all combinations of treatments during incubation is presented in Table 1. Infection increased very significantly ($P < .01$) when temperatures were reduced in the late-incubation treatments, and at the two lowest temperature regimes, 21-16 C and 16-13 C, infection was significantly greater at the highest relative humidity regime. Infection was not significantly affected by the temperature regimes of the early-incubation treatments.

At the late-incubation temperature regime of 16-13 C, infection levels were significantly greater

TABLE 1. The effect of different temperature and humidity regimes in early and late incubation periods on infection of *Pinus radiata* by *Scirrhia pini*

Early-incubation treatment	Late-incubation treatments											
	No. plants (P) and avg no. leaves infected per plant (L/P)											
	24-21 C[b]				21-16 C				16-13 C			
	70-100%	RH[c]	70-77%	RH[d]	73-100%	RH[c]	73-74%	RH[d]	91-100%	RH[c]	91-81%	RH[d]
Temperature regimes[a]	P	L/P	P	L/P	P	L/P	P	L/P	P	L/P	P	L/P
24-21 C	0(60)[e]	0	0(36)	0	26(36)	6	0(36)	0	34(36)	38	11(36)	1
21-16 C	0(36)	0	0(36)	0	39(60)	4	0(36)	0	35(36)	52	13(36)	3
16-13 C	0(36)	0	0(36)	0	25(36)	3	0(36)	0	58(60)	26	17(36)	2

[a]At each temperature regime, an equal number of the 240 plants were given 100% relative humidity treatments for 0, 54, 144, or 288 consecutive hr immediately after inoculation. Groups of plants were then transferred to the three growth rooms for late incubation.
[b]Growth room temperatures during light (16-hr) and dark (8-hr) periods, respectively.
[c]Plants subjected to 17 hr of 100% relative humidity (RH) daily.
[d]Growth-room relative humidites during light and dark periods, respectively.
[e]Number of plants inoculated.

TABLE 2. The influence of temperature and humidity during early-incubation treatments on infection of *Pinus radiata* by *Scirrhia pini* when followed by late-incubation treatments at 16-13 C

Early-incubation treatment		Late-incubation treatments at 16-13 C[a]				
		No. plants (P) and avg no. and range of infected leaves per plant (L/P) at two humidity regimes				
			91-100% RH[b]		91-81% RH[c]	
Temp regimes	100% RH consecutive	100% RH	P	L/P	P	L/P
C	*hr*	*hr*[d]				
24-21	54	510	12(12)[e]	85(1-278)	3(12)	<1(0-6)
	144	422	11(12)	15(0-59)	2(12)	<1(0-2)
	288	340	11(12)	13(0-29)	6(12)	2(0-12)
21-16	54	510	12(12)	96(1-286)	0(12)	0
	144	442	11(12)	31(0-124)	4(12)	<1(0-5)
	288	340	12(12)	29(6-84)	9(12)	6(0-22)
16-13	54	544	24(24)	16(1-68)		
		510	12(12)	67(1-227)	1(12)	<1(0-2)
	144	442	11(12)	20(0-47)	7(12)	3(0-15)
	288	340	11(12)	13(0-60)	9(12)	4(0-11)

[a]Growth room temperatures during light (16-hr) and dark (8-hr) periods, respectively.
[b]Plants subjected to 17 hr of 100% relative humidity (RH) daily.
[c]Growth room RH during light and dark periods, respectively.
[d]Total accumulated hours, at the rate of 17 hr/day, of 100% RH, the plants received during the late-incubation treatment.
[e]Number of plants inoculated.

when the plants were subjected to 100% RH treatment of 54 hr during the early-incubation treatments and 510 hr during late incubation, than at any of the other humidities tested (Table 2). Infection levels were approximately the same in the absence of 100% RH early-incubation treatment as at 288 hr at 100% RH. At the 91-81% RH regime during late incubation, there was a small increase in infection levels with an

increase in length of the 100% RH treatment during early-incubation.

Two months after the results reported in Tables 1 and 2 were recorded, 24 plants which appeared healthy were selected from each of the three growth rooms and, over the next 45 days, were subjected to 981 hr of 100% RH in a growth room having a temperature and humidity regime of 16-13 C and 76-79% RH. The method and daily rate of application of the 100% RH treatments were those used during late-incubation treatments. Of the 24 plants selected from a growth room, each group of eight had been held at one of the three temperature regimes while receiving either 54 or 144 of 100% RH during the early-incubation treatments. None of the plants had received a 100% RH treatment during late incubation. At the end of the extended incubation period, 56 of the 72 plants had become infected, each having 1-57 leaves with conidial locules. Infection levels did not appear to have been influenced by earlier incubation treatments.

DISCUSSION.—The wide range in levels of infection on plants receiving the same treatment (Table 2) suggests that a control program based on the selection of plants with high levels of inherited resistance holds considerable promise. However, in this paper, treatments are probably best described as the minimum conditions for infection and the differences noted may disappear under environmental conditions which give rise to maximum levels of infections. For example, the differences may disappear under longer periods of incubation and/or more favorable temperatures. In addition, only an estimated 10% of infected leaves were those in fascicles, the remainder being primary leaves, and the resistance level expressed by one type of leaf on a plant may not be the same in the other type.

The relatively low levels of infection obtained in the 16- to 13-C growth room with continuous 100% RH early-incubation periods of 144 and 288 hr, as compared to the high values obtained with only 54 hr, is probably more a reflection of the method used to achieve the high humidity than of its duration. The reduced infection levels may have been related to the lack of air circulation around the plants caused by the use of plastic bags to maintain the atmosphere near saturation. There was probably sufficient change in the composition of the air in 144 hr to interfere with the infection process. A second possibility, suggested by the observations of Gibson et al. (7) on the effects of shade on disease development, is that the low light intensity maintained during the rather extensive early-incubation treatments may have been responsible for the reduction in infection levels. The light intensity was reduced by the aluminum foil covers used to assist in controlling the temperature inside the plastic bags.

The results clearly indicate that both low temperatures and high relative humidity values during incubation enhance infection levels, but the average value of 88% RH maintained in the 16- to 13-C growth room compared to 73 and 72% in the 21- to 16- and 24- to 21-C growth rooms, respectively, makes it difficult to determine which of the two factors exerted the greater influence. Infection levels were greatest at the lowest temperature regime tested (16-13 C), and probably would be enhanced at still lower ones, since the fungus is able to germinate at temperatures below 13 C (4, 12).

The minimum incubation period in the temperature range 13-21 C may be expressed either as 4 weeks at the 73-100% and 91-100% RH regimes, or as ca. 550 hr at 100% RH. Since the period at 16-13 C was only several days shorter than that at 21-16 C, and since the fungus in vitro grows more slowly at temperatures below 13 C, it is unlikely that still lower incubation temperatures will shorten the minimum period. The appearance of conidial locules on plants subjected to a second period of incubation, at 16-13 C and 91-100% RH, after failing to appear under other treatments, indicates that infection had been arrested during the early-incubation treatments, and had not proceeded until the more favorable conditions of the extended incubation period were applied. Further experiments are needed to clarify this point; but in either case, the effect of temperature and humidity combinations favoring infection is a cumulative one, and suggests that incubation periods in the field could vary widely. In addition, the results indicate that the potential of disease in a forest stand may be determined by the measurement of the average number of hours of 100% RH which occur after spore dispersal.

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