EFFECT OF TEMPERATURE AND LEAF WETNESS PERIOD ON INFECTION OF *PINUS RADIATA* BY *DOTHISTROMA PINI*

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ABSTRACT

Infection of radiata pine (**Pinus radiata** D. Don) by **Dothistroma pini** Hulbary was studied at all combinations of four different temperature regimes (day/night temperatures: 24/16°C, 20/12°C, 16/8°C, and 12/4°C) and four leaf wetness periods (8, 24, 48 hr, and continuous moisture). Germination of conidia, although favoured by higher temperatures, did not vary greatly between treatments. Successful infection occurred under all treatments and was greater on foliage more than one year old than on foliage less than a year old. Stromata appeared sooner with higher temperatures and longer leaf wetness periods. The incidence of infection, however, increased greatly under continuous moisture at 20/12°C and, to a lesser extent, at 24/16°C. No other significant differences between treatments in incidence of infection were detected.

INTRODUCTION

Dothistroma pini Hulbary (Fungi Imperfecti, Sphaeropsidales) causes needle-blight in many species of *Pinus,* including radiata pine (*Pinus radiata* D. Don) (Gilmour, 1967). Apart from the work of Parker (1972) on the effect of temperature and relative humidity on infection of radiata pine by *D. pini,* there is little information available on the effect of environmental conditions on infection by this fungus. Such information should be useful in predicting the risk of infection by *D. pini* in New Zealand forests. Work done on the effect of combinations of four different temperature regimes with four different leaf wetness periods on infection of radiata pine by *D. pini* is reported here. The temperature regimes used in this experiment were selected to approximate the range of temperatures that might be expected in the various forested areas of New Zealand. The leaf wetness periods were chosen arbitrarily.

MATERIAL AND METHODS

Four growth rooms at the Controlled Climate Laboratory, Plant Physiology Division, DSIR, Palmerston North, were used for the experiment. In all rooms a vapour pressure deficit of −4 mb was maintained, which gave the relative humidities shown below.

A photoperiod of 16 hr with a 12 hr photosynthesising period in the middle of the photoperiod of 185 W/m^2 (400-700 nm) was maintained. Other conditions were:

Room	Temperature °C (day/night)	Relative humidity % (day/night)
A	24/16	86/78
B	20/12	82/71
C	16/8	78/62
D	12/4	71/50

Each room was divided into four by screens made of heavy building paper coated with aluminium foil. The screens reflected 98% of the incident light. Each division was fitted with a wide-angle atomising spray nozzle which was operated through a solenoid valve controlled by a time switch. The time clock was set to turn the sprays on for 15 sec every 10 min; this kept the foliage moist without appreciable run-off.

One-metre-high, 2-year-old rooted cuttings of radiata pine were used for the experiment. These were taken from 5-year-old plants which originated as rooted cuttings from 7-year-old trees. The plants belonged to two clones, and four plants (two of each clone) were placed in each of the 16 divisions.

All plants were inoculated by spraying them twice with a conidial suspension (20 ml/plant/application; conidial density 5×10^6 conidia/ml). This suspension was obtained by flooding 1-week-old cultures of *D. pini* growing on 10% malt agar with sterile water. Immediately after inoculation, the plants in the respective divisions of each growth room were given leaf wetness periods lasting 8 hr, 24 hr, 48 hr, and the duration of the experiment. It was known that the foliage dried in about 20 min if the spray was turned off. Accordingly, the spray nozzle in a division was disconnected 20 min before the appropriate leaf wetness period elapsed. Sprays in the fourth division in each growth room were left on to provide continuous leaf wetness.

To study conidial density, conidal germination, and early development of the fungus on the needle surface, samples of needles (two needles/plant/collection) were collected immediately after inoculation and 2, 4, 6, 8, 10, 12, and 14 days after inoculation. The needles were killed and fixed in FAA and the fungal tissue stained by the periodic acid-Schiff technique (Preece, 1959). They were stored in methyl alcohol. All needles collected were examined under a microscope using transmitted light, with a 100 W quartz-iodine lamp as the light source. Only the abaxial surface of the needles was examined. General observations on mycelial growth were made by scanning the entire surface but no quantitative measurements were made on this aspect. On collections made immediately and 2, 4, and 6 days after inoculation, five 1 mm long sections were chosen randomly along the length of the needle. The width of each section was measured with an ocular micrometer. All ungerminated and germinated conidia within these chosen areas were counted. From counts made on needles collected immediately and 2 days after inoculation the number of conidia/mm^2 was calculated. Germination percentages were calculated from counts made on collections done 2, 4, and 6 days after inoculation.

All plants were examined twice every week and the date when the first stroma was detected on each plant was noted. Three months after inoculation, all needles which were present when the plants were inoculated were removd. The needles less than one year old and older needles were kept separate. All infected and uninfected needles were

counted and the percentages of visibly infected needles were calculated. On average, there were 2700 needles per plant.

All data were treated by analysis of variance. Clonal effects proved negligible, leaving a two-way factorial classification with two fixed effects, temperature and wetness period. Raw percentages were analysed as inspection of data indicated that there would be no advantage in using the arcsin transformation.

RESULTS

Counts of conidia on individual needle sections showed that in general the distribution of conidia was fairly even over the needle surfaces, the mean count being 43 conidia/mm^2 (S.D. = 15). Highest germination percentages were recorded on needles collected after 4 days in all treatments and these percentages are given in Fig. 1. For statistical analysis, counts from all sections of all needles collected from one plant on the same day were grouped to give a single composite sample. Germination percentages showed a significant effect of temperature ($P < 0.05$), but not of wetness period, nor of temperature/wetness period interaction. Germination showed a linear increase with temperature.

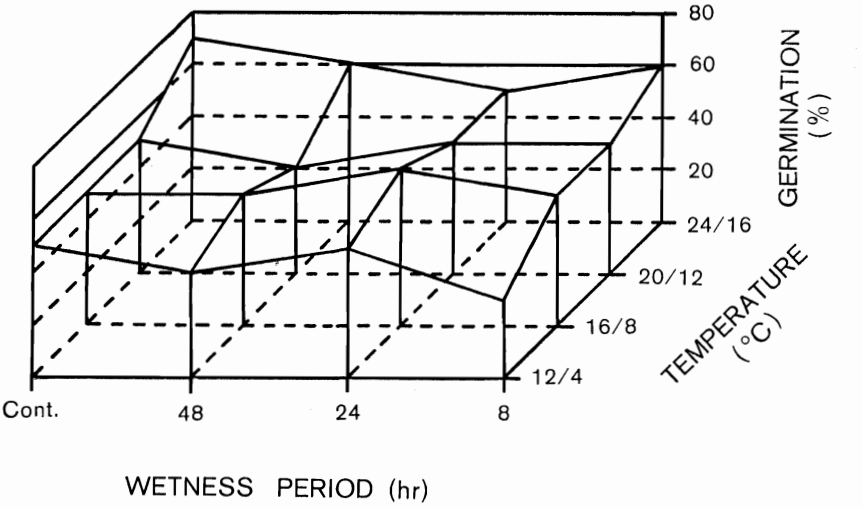

FIG. 1—Germination percentages of **D. pini** conidia 4 days after inoculation at different temperatures and leaf wetness periods

The length of the wetness period appeared to have little effect on mycelial growth on the needle surface. Temperature, however, seemed to have an appreciable effect. Mycelial growth on the needles of plants kept at 24/16°C increased for up to 8 days after inoculation. The amount of mycelium then began to decrease and samples taken after 12 days showed only a few faintly-staining hyphae. Plugs of hyphae, which may have been appressoria-like structures, were first observed over stomata on samples taken after 8 days (for a description of these plugs, see Gadgil, 1967). Increasing mycelial development was observed during the first 6 days on needles of plants kept at 20/12°C and only faintly-staining hyphae were seen on samples collected after 12 days. On needles

of plants kept at 16/8°C and 12/4°C, the fungus did not make much growth on the surface; maximum growth was seen 10 days after inoculation. Mycelial plugs over stomata were first seen 6 days after inoculation on plants kept at 20/12°C and 14 days after inoculation on plants kept at 16/8°C and 12/4°C. Anastomoses of hyphae and production of secondary conidia by the surface mycelium were observed under all treatments.

The pre-reproduction period (the time elapsed from inoculation to appearance of conidia on stromata) decreased significantly ($P < 0.01$) with increasing temperature and increasing wetness period. The temperature effect was linear, and there was no significant temperature/wetness period interaction. Conidia-bearing stromata of *D. pini*, which were taken to be the definite evidence of infection, were first seen 2 weeks after inoculation on plants kept continuously wet at 24/16°C, but took as long as 7 weeks to appear in other treatments (Fig. 2).

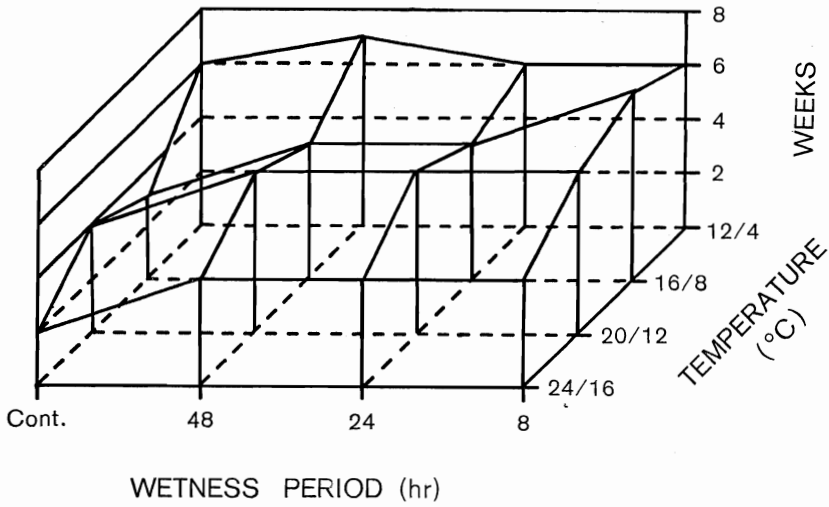

FIG. 2—Pre-reproduction period of **D. pini** at different temperatures and leaf wetness periods

Percentages of infected foliage less than one year old and older foliage under the various treatments are given in Fig. 3. Severity of infection followed the same trend regardless of age of foliage except that older needles were always more severely infected. Overall, the effects of temperature, wetness period, and the interaction were all highly significant ($P < 0.01$). The significant temperature effects, however, were confined to the continuous wetness class, 20/12°C giving significantly ($P < 0.01$) more infection than 24/16°C, which in turn gave significantly ($P < 0.01$) more infection than 16/8°C and 12/4°C. At the latter temperature levels no significant differences were noted between different wetness treatments.

DISCUSSION

The experiment showed that infection of radiata pine by *D. pini* occurred at all the temperature and wetness period combinations tested but the severity of infection remained low except at 20/12°C and 24/16°C under continuous moisture. The high

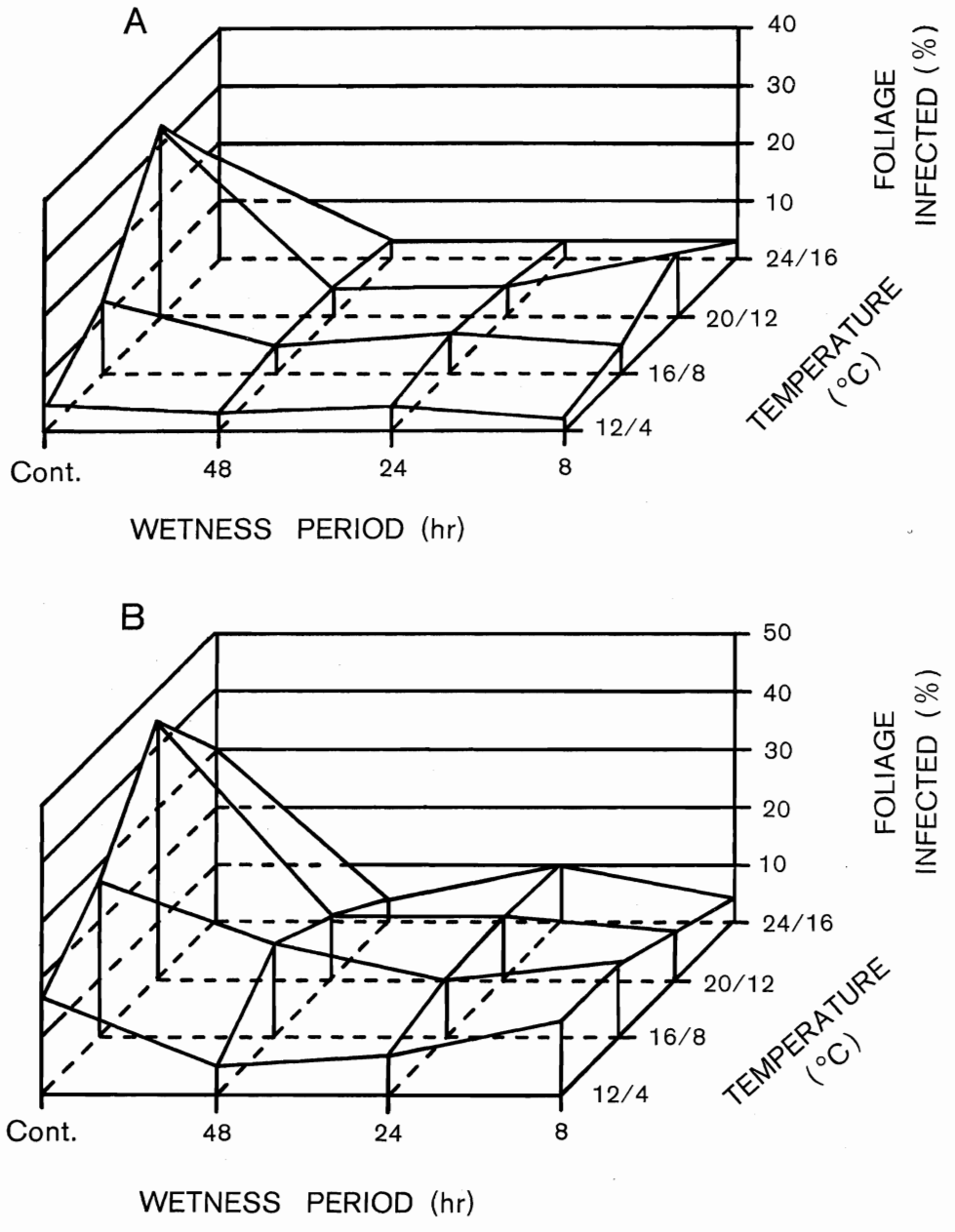

FIG. 3—Percentage of infected foliage after 3 months at different temperatures and leaf wetness periods

A: Needles less than one year old B: Older needles

level of infection on plants receiving these treatments does not appear to be due to a higher percentage of conidial germination at these temperatures (compare Figs. 1 and 3). It is possible that, as the experiment lasted 12 weeks and the pre-reproduction period with continuous moisture at 24/16°C was 2 weeks and at 20/12°C, 4 weeks, the high percentage of infected foliage found in these treatments was partly a result of infections caused by later generations of conidia. It is also possible that the secondary conidia which were produced by the *D. pini* mycelium on needle surfaces were able to germinate and cause infection under conditions of continuous moisture and relatively high temperatures, but either did not infect or caused few infections at lower temperatures or with short wetness periods. Unfortunately, no quantitative measurements were made on the numbers of secondary conidia nor were observations made on their germination.

The results reported here differ markedly from those of Parker (1972) who tested the effect of three temperature regimes (24/21°C, 21/16°C, and 16/13°C) and different humidities on infection. From his experiments, Parker concluded that "Both low temperature and high relative humidity values during incubation enhance infection levels. . . . Infection levels were greatest at the lowest temperature regime tested (16/13°C) and probably would be enhanced at still lower ones. . . ." In the present experiment, infection levels remained low at 16/8°C and 12/4°C regardless of the duration of the wetness periods. There are several factors, though, which might explain the difference in results. Parker worked with 4-month-old seedlings which had infections mainly on the primary leaves rather than on needles and, in his experiments, the light intensity was much reduced during the 100% relative humidity treatments. In the present experiment, the plants carried mature needles only and the light intensity was the same in all the treatments. In Parker's experiments, the lowest incubation temperatures had the highest relative humidity whereas, in the work reported here, relative humidities were lowest at the lowest incubation temperatures because the vapour pressure deficit was kept the same at all temperatures. Parker's results were also obtained over a much shorter period than the 3 months of this experiment. It is also possible that the isolate of *D. pini* (from ascospores in fruiting bodies on *Pinus contorta* Dougl., Vancouver Island) used by Parker had different temperature requirements from the isolate (from conidia on stromata on radiata pine, Kaingaroa Forest) used in this experiment.

This experiment was done under entirely artificial conditions—inoculation was done only once, there were no fluctuations in temperature except for the drop under the "night" regime, and the foliage was either continuously moist for 3 months or continuously dry after the initial wetness periods were over. These conditions were very different from those in the field. The results, however, do show that if sufficient inoculum is present, infection can occur over a wide range of temperatures and with relatively short leaf wetness periods. A study of meteorological data (Garnier, 1950) shows that temperatures suitable for infection occur from November to March in all radiata pine growing areas of New Zealand.

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REFERENCES

GADGIL, P. D. 1967: Infection of **Pinus radiata** needles by **Dothistroma pini**. **N.Z. J. Bot. 5:** 498-503.

GARNIER, B. J. 1950: "New Zealand Weather and Climate". N.Z. Geog. Soc., Misc. Publ. No. 1.

GILMOUR, J. W. 1967: Distribution and significance of the needle blight of pines caused by **Dothistroma pini** in New Zealand. **Plant Dis. Reptr. 51:** 727-30.

PARKER, A. K. 1972: Artificial inoculation of **Pinus radiata** with **Scirrhia** (**Dothistroma**) **pini:** Effect of relative humidity and temperature on incubation. **Phytopathology 62:** 1160-4.

PREECE, T. F. 1959: A staining method for the study of apple scab infections. **Plant Path. 8:** 127-9.