DURATION OF LEAF WETNESS PERIODS AND INFECTION OF *PINUS RADIATA* BY *DOTHISTROMA PINI*

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ABSTRACT

Two experiments were done to study the effect of leaf wetness on infection of **Pinus radiata** D. Don by **Dothistroma pini** Hulbary. In the first experiment, inoculation with **D. pini** conidia was followed by: continuous leaf-wetness for 92 days; short leaf-wetness periods of up to 8 h interspersed with 24-h dry periods; a 24-h dry period followed by continuous wetness for 91 days; no wetness period at all. Infection, as evidenced by stromata of **D. pini**, was seen in all treatments but the percentage of infected needles was low except in those treatments in which continuous leaf wetness was provided for 91 or 92 days. In the second experiment inoculated foliage was allowed to dry for periods of 0, 2, 7, 14, 30 and 60 days before it was remoistened. The percentage of infected needles was highest in plants given the 0-, 2-, and 7-day dry periods, followed by those given the 14-day dry period treatment, and it was lowest in plants receiving the 30- and 60-day dry periods. The pre-reproduction period of the fungus was 19-21 days on plants given the 0-, 2-, 7- and 14-day dry period treatments; it was 35 and 70 days respectively on plants given 30- and 60-day dry periods. In the treatments involving 7- to 60-day dry periods, stromata were noticed within 10 days of rewetting of foliage. It appears that stromata formation, rather than germination and penetration, is inhibited by the absence of leaf surface moisture.

INTRODUCTION

In previous work (Gadgil, 1974) it was shown that visible infection by *Dothistroma pini* Hulbary (= *Dothistroma septospora* (Doroguine) Morelet) of *Pinus radiata* D. Don foliage (recognised by the appearance of stromata of *D. pini* on the needles) occurred under four different day/night temperature regimes (24°/16°, 20°/12°, 16°/8° and 12°/4°C) with the foliage kept wet for 8 h or longer after inoculation with a conidial suspension. It was also found that the length of the leaf wetness period had a marked effect on the severity of infection (as measured by the percentage of visibly infected foliage) and on the length of the pre-reproduction period of *D. pini* (the time elapsed from inoculation to appearance of conidia on stromata). At 24°/16° and 20°/12°C the severity of infection (as defined above) was much higher and the pre-reproduction period shorter when the foliage was kept moist continuously than when it was kept wet for only 8, 24 or 48 h after inoculation. Results from two experiments investigating further the effect of leaf wetness periods on severity of infection and on the length of the pre-reproduction period are given in this paper.

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MATERIAL AND METHODS

Three growth rooms at the Controlled Climate Laboratory, DSIR, Palmerston North, were used. In both experiments, the rooms were kept at a temperature of 20° ± 0.3°C and 12° ± 0.3°C, day and night respectively. The vapour pressure deficit was maintained at 4 mb which gave relative humidities of 82% day and 71% night. The photoperiod was 16 h with a 12-h photosynthesising period in the middle of the photoperiod. The light intensity during the photosynthesising period was 185 ± 10 W/m^2 (400-700 nm) at plant top height. These temperature, humidity and light conditions were known to be favourable for the infection of *P. radiata* by *D. pini* (Gadgil, 1974). Each room was fitted with 10 wide-angle atomising spray nozzles which operated through a solenoid valve controlled by a time switch. The sprays were turned on for 10 s every 10 min when the treatment required the foliage to be kept moist. The various leaf-wetness treatments are described under the individual experiments.

Three-year-old rooted cuttings of *P. radiata* (1.5 m high) were used (Clone 452 for Experiment 1 and Clone 450 for Experiment 2). These cuttings were taken from 5-year-old plants which had originated as rooted cuttings from 7-year-old trees.

All plants in both experiments were inoculated by spraying the foliage with a suspension of *D. pini* conidia taken from 10-day-old cultures on 10% malt agar (20 ml/plant; conidial density 5 × 10^6 conidia/ml). Samples of needles were taken at different intervals (see under individual experiments for details) for study of conidial density and germination. In both experiments, half of each needle sample was stained and examined under a light microscope as described before (Gadgil, 1974) and the other half was air-dried, coated with gold, and examined under a scanning electron microscope to see whether the SEM offered a less laborious way of examining the needle surface. The same procedure for calculating conidial densities and germination percentages was followed in both types of microscopy (for details of this procedure see Gadgil, 1974). Counts of conidia were made on samples collected immediately after inoculation and 2 days after inoculation, and germination percentages were calculated from counts made on samples taken 2, 4 and 6 days after inoculation in Experiment 1 and on samples taken 2 and 4 days after inoculation in Experiment 2.

All plants were examined by eye twice a week for the first 3 weeks and once a week thereafter. The week when the first stroma was detected (i.e., the first definite sign of infection) in each treatment was noted. In Experiment 2, all plants were rated visually for severity of infection on a 0-7 scale (Bartram and Kershaw, 1974) from the time the first stroma was detected to the end of the experiment. At the end of both experiments, all needles which were present when the plants were inoculated were removed. All visibly infected and uninfected needles were counted and the percentages of infected needles calculated. These percentages were transformed to arcsin values before analysis of variance was carried out. Differences between treatments or groups of treatments were tested by the method of orthogonal contrasts (Steel and Torrie, 1960).

EXPERIMENT 1

Treatments

There were eight plants per treatment. Spraying with the inoculum wetted the foliage and drying took about 20 min. The wetness-period treatments were applied after the foliage had dried. Needle samples (two needles/plant) were taken immediately

after inoculation and 2, 4, 6, 8, 10 and 14 days after inoculation. The experiment lasted 92 days. The treatments were:

1.1 Foliage kept moist continuously for 92 days.
1.2 Foliage kept moist for two 4-h periods separated by a 24-h dry period. Foliage dry for the rest of the experimental period.
1.3 Foliage kept moist for four 2-h periods separated by three 24-h dry periods. Foliage dry for the rest of the experimental period.
1.4 Foliage kept dry for 24 h after inoculation, then moist for 8 h. Foliage dry for the rest of the experimental period.
1.5 Foliage kept dry for 24 h after inoculation. Foliage moist for the rest of the experimental period.
1.6 Foliage kept dry continuously for 92 days.

Results

The mean count of conidia on the needle surface was 31/mm^2 (s.d. 11). Germination percentages in treatments 1.1, 1.4 and 1.5 were significantly greater ($P < 0.05$) than those in treatments 1.2, 1.3 and 1.6 (Table 1). The pre-reproduction period was shortest (21 days) in the two treatments which had the longest wetness periods (1.1 and 1.5); in the other treatments 42 to 77 days elapsed before stromata were first seen.

There were, on average, 8300 needles per plant. The percentages of infected needles in the various treatments are given in Table 1. Infection was observed in all treatments, including treatment 1.6 where the needles were moist only when they were sprayed with the inoculum. Plants given treatments 1.1 and 1.5 were significantly more severely infected ($P < 0.01$) than plants in treatments 1.2, 1.3, 1.4 and 1.6. The difference in infection percentage between treatments 1.1 and 1.5 was not statistically significant nor were there significant differences in infection percentage between treatments 1.2, 1.3, 1.4 and 1.6.

TABLE 1—Data on germination percentage, pre-reproduction period, and percentage of infected foliage from Experiment 1

Treatment	Germination percentage*	Pre-reproduction period (days)	Infected foliage percentage**
1.1 Continuously moist	34 a†	21	6.1 a†
1.2 Moist for two 4-h periods separated by a 24-h dry period	14 b	42	0.21 b
1.3 Moist for four 2-h periods separated by three 24-h dry period	19 b	63	0.35 b
1.4 Dry for 24 h after inoculation, wet for 8 h, dry thereafter	34 a	42	0.39 b
1.5 Dry for 24 h after inoculation, wet thereafter	28 a	21	7.7 a
1.6 Continuously dry	20 b	77	0.02 b

* Six days after inoculation ** Mean of 8 plants
† Treatments followed by different letters are significantly different ($P < 0.01$)

EXPERIMENT 2

Treatments

There were six plants per treatment. Needle samples (two needles/plant) were collected immediately after inoculation, 4 days after inoculation, and 2 and 4 days after the beginning of the wetness period. The experiment lasted 96 days. The treatments were:

2.1 Foliage kept moist continuously for 96 days.
2.2 Foliage kept dry for 2 days after inoculation and then wet for 94 days.
2.3 Foliage kept dry for 7 days after inoculation and then wet for 89 days.
2.4 Foliage kept dry for 14 days after inoculation and then wet for 82 days.
2.5 Foliage kept dry for 30 days after inoculation and then wet for 66 days.
2.6 Foliage kept dry for 60 days after inoculation and then wet for 36 days.

Results

The mean count of conidia on the needle surface was 46/mm^2 (s.d. 18). Germination percentages of conidia 4 days after inoculation are given in Table 2. The length of the dry period after inoculation appears to have had no direct effect on the germination percentages. Samples of needles were taken 2 and 4 days after the beginning of the wetness periods to see if conidia remained dormant over the dry period and germinated when the foliage was moistened. Germinated conidia were present on these samples but their numbers did not increase with time, indicating that these conidia had probably germinated some time ago and then dried out. Ungerminated conidia were present on these needles but they were not seen to germinate over the short period the samples were taken. The pre-reproduction periods in treatments 2.1, 2.2, 2.3 and 2.4 were very similar (19 to 21 days) but they were much longer in treatments with long dry periods (treatment 2.5: 35 days; 2.6: 70 days. See Table 2).

The gradual increase in disease severity over the experimental period in the various treatments is shown in Fig. 1. At the end of the experiment, the percentage of infected needles on plants given treatments 2.1, 2.2 and 2.3 was significantly higher ($P < 0.01$) than that on plants in treatment 2.4 which in turn were significantly more infected ($P < 0.01$) than plants in treatments 2.5 and 2.6 (Table 2). There was an almost exponential increase in the severity of infection with decreasing number of dry days (Fig. 2).

TABLE 2—Data on germination percentage, pre-reproduction period, and percentage of infected foliage from Experiment 2

Treatment	Germination percentage*	Pre-reproduction period (days)	Infected foliage percentage**
2.1 Continuously moist	19	19	21.5 a†
2.2 Dry for 2 days, wet for 94 days	21	19	21.0 a
2.3 Dry for 7 days, wet for 89 days	17	21	19.6 a
2.4 Dry for 14 days, wet for 82 days	20	21	10.3 b
2.5 Dry for 30 days, wet for 66 days	31	35	4.7 c
2.6 Dry for 60 days, wet for 36 days	23	70	2.7 c

* Four days after inoculation ** Mean of 6 plants

† Treatments followed by different letters are significantly different ($P < 0.01$)

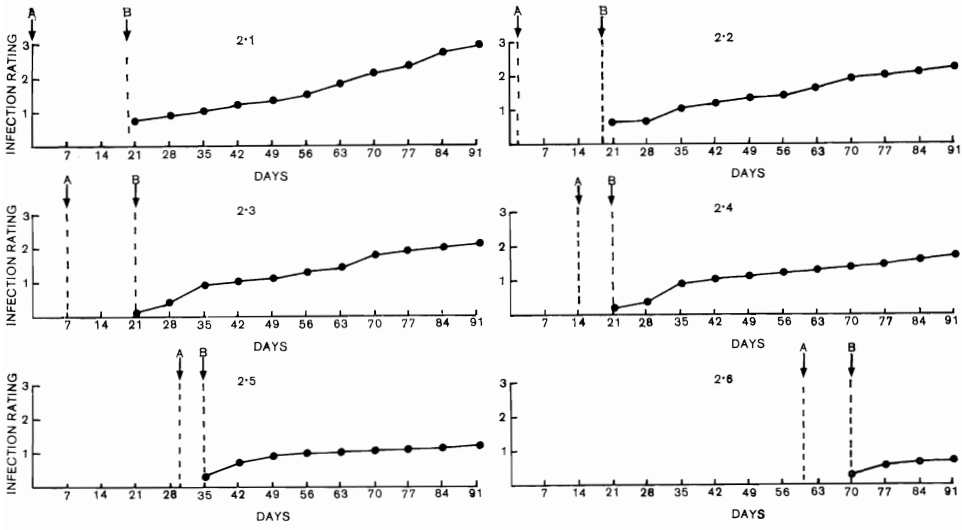

FIG. 1—Progress of infection severity (assessed on a 0-7 scale) under the various treatments of Experiment 2.
A denotes the time when the wetness period began
B denotes the time when the first stroma of **D. pini** was detected

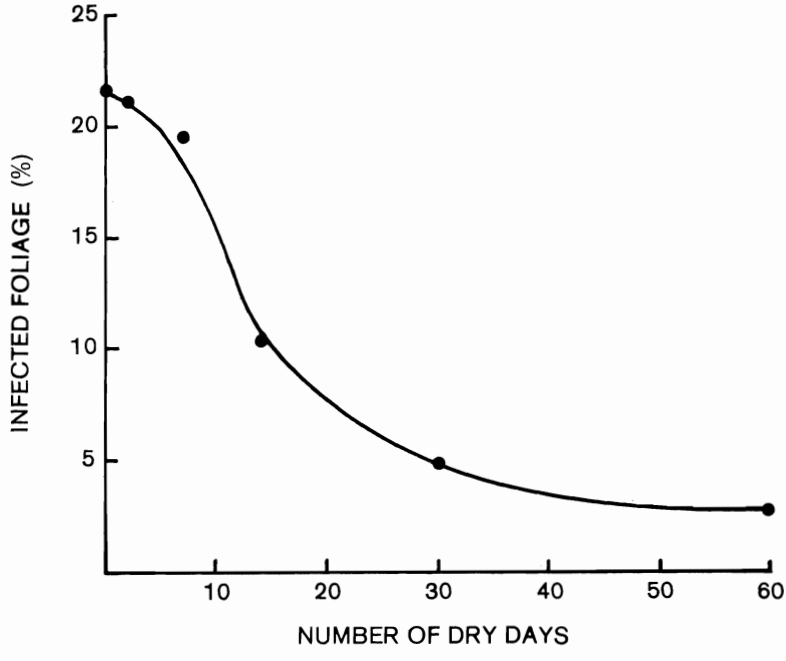

FIG. 2—Relationship between infection severity (measured by calculating the percentage of infected needles) and the length of the dry period after inoculation. Note the marked drop in infection severity between the 7 days dry period treatment and the 14 days dry period treatment. Data from Experiment 2.

DISCUSSION

The first experiment showed that when short wetness-periods were interspersed with long (24-h) dry-periods, the severity of visible infection was low and that it remained at about the same level regardless of whether there were one 8-h, two 4-h, or four 2-h wetness periods. Initial drying of the inoculum for 24 h did not cause any reduction in the severity of infection. When no wetness period was provided after inoculation, infection did occur although the number of visibly infected needles was very low. In the second experiment, the severity of infection was similar in treatments 2.1, 2.2 and 2.3 (0, 2 and 7 days dry), treatment 2.4 (14 days dry) showed a marked reduction in infection severity, and treatments 2.5 and 2.6 (30 and 60 days dry) showed the least infection. It appears that up to 7 days dryness after inoculation reduced infection very little while longer periods of dryness tended to retard visible infection.

In the first experiment, conidial germination percentages were higher in treatments with longer wetness periods; in the second experiment, no such effect of leaf wetness on germination was apparent. In both experiments germination percentages appeared to bear little relationship to the percentage of visibly infected needles at the end of the experiment. For example, treatments 1.1 and 1.4 had the same germination but the percentage of infected needles on plants given treatment 1.1 was 15 times greater than that on plants given treatment 1.4. It would seem that the length of the leaf wetness period did not affect germination but that development of stromata was suppressed in treatments with short (up to 8-h) wetness-periods. Results of the second experiment support this suggestion. Here, stromata were first observed 19 days after inoculation when the plants were kept in temperature, moisture, and light conditions considered favourable for disease development. This period of 19 days may be taken to be the minimum required for germination, penetration, growth of hyphae within the needle tissues, and formation of stromata under the conditions of the experiment. A 2-day dry period after inoculation did not limit development of the fungus since the same pre-reproduction period (19 days) was observed in this treatment. A 7- or 14-day period after inoculation increased the pre-reproduction period by only 2 days. In the 30- and 60-day dry period treatments, the first stromata were observed 5 and 10 days respectively after wetting commenced — a period unlikely to be long enough for completion of the whole process from germination to stromata formation. Besides, no germinating conidia were seen on needle surfaces on samples taken 4 days after wetting began. It appears that penetration occurred when the foliage was dry but that stromatal development was much reduced until the foliage was moistened. It is possible that the presence of free water on the needle surface led to an increase in the water content of the needle tissues or to saturation of intercellular spaces, and that this stimulated the growth of the fungus: the rate of growth of *Phytophthora infestans* (Mont.) de Bary has been shown to be directly proportional to the water content of the host leaves (Butler and Jones, 1949), and in fire-blight of apples and pears the growth of the bacterium that causes the disease is greatly increased when the intercellular spaces are saturated with water (Shaw, 1935; Gäumann, 1950).

The finding that visible infection occurred when the foliage was wet for only 20 min showed that stromatal development was not entirely suppressed through lack of leaf surface moisture and that free water may not be essential for germination of *D. pini*

conidia. In these experiments, the conidia used for inoculation were suspended in water and had probably imbibed enough water to permit germination. It is possible that if dry conidia had been used for inoculation, germination and penetration would not have taken place with such a short wetness period. Inoculation with dry conidia would, however, be impractical because *D. pini* conidia are produced in a slimy mass and cannot be picked off unless they are suspended in water. There is also some evidence that, in nature, conidia are liberated only when the foliage is moistened and that they are dispersed by a splash mechanism. Gibson *et al.* (1964) showed that the great majority of conidia on *D. pini* were released during periods of rain or heavy mist when the infected foliage was dripping wet. They also found that no conidia were released when a strong current of air was directed at dry or moist foliage. The report of Cobb *et al.* (1968) appears to contradict these findings. They found that using a rotorod sampler "conidia of *D. pini* were collected during all sampling periods and dissemination was not dependent upon rain or wind." Our unpublished work with a Hirst spore trap which was operated continuously in a heavily infected *P. radiata* stand from August 1974 to February 1975, and which caught very few *D. pini* conidia during this period, supports the findings of Gibson *et al.* (1964). The Hirst trap has a high collection-efficiency for dry spores but it is unsuitable for trapping splash-dispersed spores (Gregory, 1961, p. 101) and its failure to trap *D. pini* conidia indicates that they were not present as dry conidia in the air. There is therefore a strong possibility that naturally dispersed conidia are likely to be hydrated when they come in contact with the needle surface of the host plant. The conidial suspension used for inoculation of plants in these experiments differed in one noteworthy aspect from conidia released from infected needles. The conidial suspension was prepared by washing the conidia from cultures growing on 10% malt agar and it is likely that some nutrients from the culture medium were carried over in to the suspension. These nutrients may have enhanced germination of the conidia.

Results of these experiments suggest (1) that if hydrated conidia are deposited on the surface of a susceptible host, germination and penetration will occur regardless of the length of the wetness period that follows deposition, provided that suitable temperatures prevail, and (2) that the severity of infection depends on the length of the dry period which follows deposition of conidia — the longer the dry period, the lower the infection severity.

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