DIPLODIA PINEA INFECTION OF PINUS RADIATA SEEDLINGS: EFFECT OF TEMPERATURE AND SHOOT WETNESS DURATION

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(Received for publication 27 July 1982; revision 13 October 1982)

ABSTRACT

Artificial inoculation studies with **Diplodia pinea** (Desm.) Kickx in the glasshouse and growth cabinet established that initiation of infection (as indicated by the first appearance of stem symptoms) of **Pinus radiata** D. Don requires a minimum shoot wetness duration of 3 h but no more than 48 h within the temperature range 10°–30°C. Duration of wetness seems important to infection as interruption of the wetness period by a dry period of 12 h or longer reduced the disease level.

Temperature requirements for the initiation of infection and the subsequent development of the disease are not the same. Infection cannot be initiated at 5°C, but at 10°C the level of infection can be high if wetness is not a limiting factor. However, at this temperature lesion extension is limited and dead tops do not develop, even if the temperature is subsequently raised to the optimum. On the other hand, when infection is initiated at a high temperature, e.g., 25°–30°C, lesion extension continues until a large part of the shoot is killed even at a subsequent temperature as low as 10°C. Thus, temperature and duration of wetness during the early stages of infection largely determine the ultimate level of disease. Briefly, temperatures of 10°–12°C, 15°C, and 20°–25°C or higher during 1–2 wet or humid days would represent respectively, light, moderate, and serious dead top development.

INTRODUCTION

During the time of spring–summer flush, elongating green shoots and leaders of *Pinus radiata* are often susceptible to infection by *D. pinea* which causes leader dieback and stem malformation (Chou 1976a). The disease is apparently very sensitive to aspect and topographic changes (presumably because of microclimatic effects) and severe incidences are largely confined to a few valley sites in the central North Island (Chou 1976a; N.Z. Forest Service 1973, p. 50).

There is ample evidence that the fungus can penetrate the intact surface of a green stem (Chou 1976a, 1976b, 1978). The first sign of infection (a discoloured, water-soaked lesion on the stem) can occur within a few days of inoculation, lesion extension leading to the development of a dead top usually in 1–2 weeks but no more than

1 month (Chou 1976a, 1976b, 1977, 1978). Previous inoculation trials showed that a high level (80–90%) of dead top occurred when plants were misted for 48 h at 25°C after inoculation and then placed in a glasshouse at 15°–25°C without humidity control (Chou 1976b, 1977, 1978). In a resistance screening trial, 39% of over 2000 seedlings inoculated developed dead top when given a post-inoculation misting for 24 h at 20°C (Burdon *et al.* 1982). In both natural and artificial infections, only a proportion of infected plants develop dead top, as a lesion may stop extending soon after its first appearance (Chou 1976a, 1976b, 1978); a restricted stem lesion may thus be considered a type of host resistance response (Burdon *et al.* 1982).

Major reports on this pathogen from various parts of the world have been published by Birch (1936), Petrak (1961), Waterman (1943), Marks & Minko (1969), Brookhauser & Peterson (1971), and Chou (1976a, 1976b). However, information on the effect of climatic factors on this disease appears to be extremely meagre. This paper reports results of inoculation studies under growth cabinet and glasshouse conditions designed to determine the effects of temperature and shoot wetness duration on *D. pinea* infection of *P. radiata* seedlings.

MATERIALS AND METHODS

The Pathogen

An isolate of *D. pinea* (ATOC 34924) taken from an infected shoot in a severely affected area was used as the source of inoculum. The same isolate has been used in several previous studies (Chou 1976b, 1977, 1978), as also has the method of inoculum production and inoculation.

Spore Germination

A concentration of 15 000 spores/ml distilled water was used in slide germination tests at temperatures of 5°, 10°, 15°, 20°, 25°, 30°, 35°, and 40°C. After incubation periods of 3, 6, 12, 24, and 48 h, spore germination was halted with a drop of formalin. Two hundred spores in each treatment were then examined under a microscope to determine the number germinated (with germ tube at least half as long as the width of the spore). Germ tube lengths of 20 spores per treatment were measured at this time.

Host Plants

In all experiments, 6-month- to 1-year-old *P. radiata* seedlings were used. They were potted either singly in 8-cm-diameter polythene tubes, or in groups of 10 in 5-*l* buckets.

Inoculation Methods

Unless otherwise stated, inoculum concentration was 15 000–20 000 spores/ml. Inoculation was by stroking opposite sides of the topmost 5 cm of the stem with a soft camel-hair brush which had been dipped into the spore suspension. Inoculations were all carried out during the summer months (November to February) when shoot susceptibility is at its peak.

Misting

After inoculation, shoot wetness was maintained for various periods by misting in one of two ways:

(1) AT misting was done in specially designed misting chambers ("Aquatrons") each fitted with a de-Vilbiss-type atomiser and an artificial-leaf-type humidistat as used in previous inoculations (Chou 1976b).

(2) CAB misting was done in a growth cabinet fitted with four t-jet nozzles regulated by a time switch, with 1 min of misting every 10 min.

In temperature-effect experiments the misting duration was 48 h in all treatments.

Glasshouse and Growth Cabinet Conditions

The thermostat in the glasshouse was set for 15°C at night and 20°C during the day. Although the day limit was often exceeded during hot spells in the summer, the glasshouse temperature was fairly constant, with the mean temperature of several separate experiments close to 19°–20°C.

Photoperiod was 12 h, full light 650 $\mu E/m^2/s$ or 140 W/m^2 and twilight about 50–60 W/m^2. Relative humidity was 60–70%.

Assessment of Infection

Between 4 and 7 weeks after inoculation each plant was placed into one of the following categories: (1) no infection – that is, exhibiting no stem symptoms; (2) restricted stem lesion (RSL) – that is, having a lesion less than 3 cm long and not more than half the stem circumference in width; (3) dead top (DT) – that is, total necrosis of the shoot above the inoculated region or, infrequently, "severe lesion" affecting most of the shoot top but not completely girdling the shoot tip. Few intermediate types of host response were observed in these or in past experiments (Chou 1976b; Burdon *et al.* 1982).

The term "infection total" (I_t) refers to the sum of all infection types, i.e., DT + RSL, and measures success in infection initiation but not necessarily the degree of severity of infection represented by the development of dead top.

Temperature Effect

Experiment 1: The effect of four different temperatures on infection was studied. Inoculated plants were given a 48-h AT misting at 5°, 10°, 15°, or 23°C and then placed in growth cabinets. Misting and post-misting temperatures were the same in each treatment.

Experiment 2: A factorial of three misting temperatures and three post-misting temperatures. Inoculated plants were subjected to a 48-h AT misting at 15°, 20°, or 25°C. They were then divided into three equal lots (about 30 each) and placed in growth cabinets at 15°, 20°, or 25°C.

Experiment 3: A study of misting temperature effect – AT misting at 12° and 24°C, CAB misting at 24°/10°C or 20°/10°C day/night, for 48 h after

inoculation. The plants were then placed in a growth cabinet at 24°/10°C day/night.

Experiment 4: Further investigation of misting temperature effect – AT misting at 12°, 16°, 20°, 30°C; CAB misting at 16°/10°C day/night. After misting for 48 h, all plants were placed in a growth cabinet at 16°/10°C day/night.

Experiment 5: This experiment consisted of 10 treatments (each with 10–14 plants) in two series:

(a) A common AT misting at 24°C for 48 h after inoculation, followed by five post-misting temperatures of 28°/20°C, 28°/15°C, 28°/10°C, 15°/10°C, 20°/20°C day/night.

(b) Misting carried out in the growth cabinet, with temperatures during and after misting kept the same – 28°/20°C, 28°/15°C, 28°/10°C, 15°/10°C, and 20°/20°C.

Effect of Misting Duration

Two aspects of the effect of misting duration on infection were studied.

A. *Uninterrupted misting* (A1): The effect of misting (AT) for 6, 12, 18, 24, and 48 h at two different temperatures (15° and 25°C) on infection was studied. After each prescribed period of misting, 15 plants were taken out, dried with a warm-air blower, and placed in a glasshouse.

The experiment was repeated (A2) with misting periods of 3, 6, and 24 h at 25°C, and 20 plants per treatment.

B. *Interrupted misting:* Seedlings were subjected to AT misting at 25°C for a 24–h period or two 12-h periods separated by dry periods of 12, 24, 48, and 96 h. The treated plants were then placed in a growth cabinet at 22°/15°C day/night.

RESULTS

Effect of Temperature on Spore Germination

Figure 1 shows the percentages of spores germinated at temperatures from 5° to 40°C for a germination period ranging from 4 to 48 h. At 10°C germ tubes had emerged within 12 h of being placed in water and maximum germination had taken place after 48 h. At 20°–33°C emergence of germ tubes occurred in less than 3 h and a near-maximum percentage of spores germinated within 6 h. Germination was fastest at 30°C as shown by the 3-h curve.

Figure 2 shows the extent of germ tube elongation as affected by temperature and time. At germination times longer than 12 h, germ tube lengths in some treatments have become too extensive to be measurable. The effect of temperature on germ tube elongation became very pronounced at 12 h, with a sharp optimum apparent at 25°C.

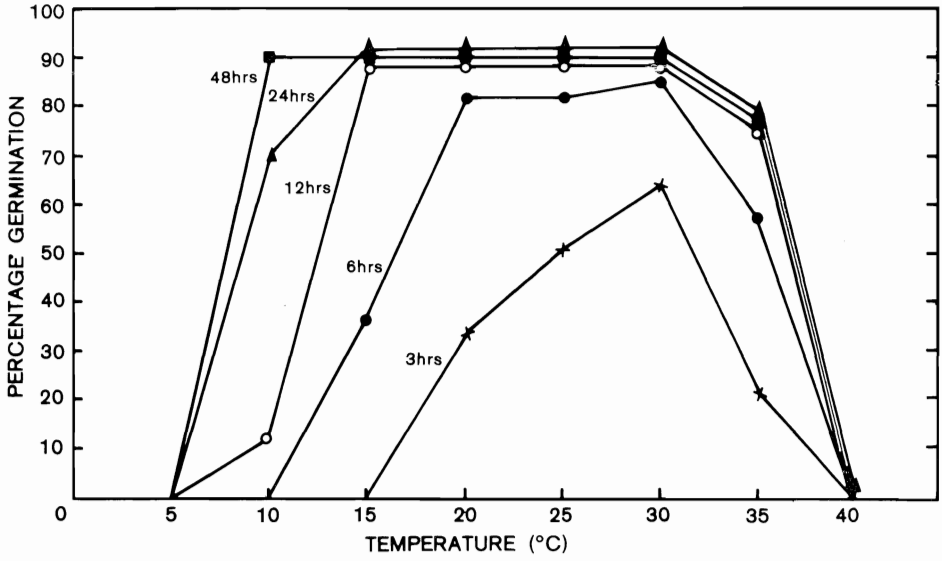

FIG. 1—Temperature response curve of **D. pinea** spore germination after various intervals of placement of spores in water.

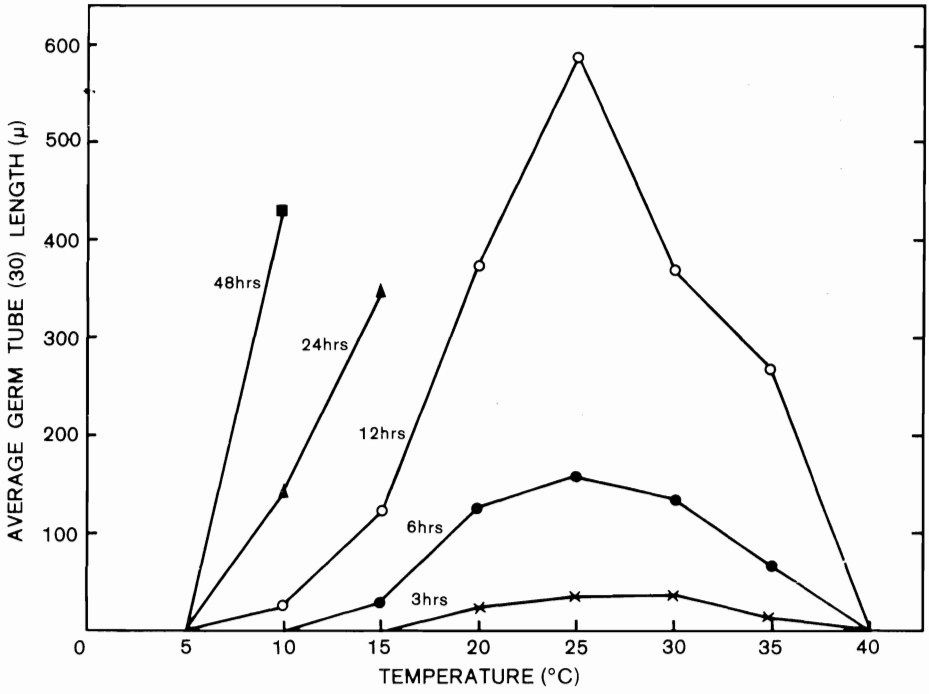

FIG. 2—Temperature response curve of **D. pinea** germ tube elongation after various intervals of placement of spores in water.

Effect of Temperature on Infection

Experiment 1: Constant temperature during and after misting. It can be seen from Table 1 that although infection occurred at a fairly wide temperature range, substantial development of dead top required a temperature 15°C or higher. At 10°C the percentage of plants infected was as high as at 23°C, but lesion extension remained limited and as few dead tops developed as at 5°C. The difference in dead top development between 15°C and 10°C, though apparently large, is not significant (chi-square = 1.01, not significant at p = 0.1). An unexpected result was that some infection occurred at 5°C although spores on slides showed no germination at this temperature.

TABLE 1—Effect of four constant temperatures on **D. pinea** infection of **P. radiata** seedlings (inoculum concentration 45 000–50 000 spores/ml; results 4 weeks after inoculation)

Temperature (°C)	Total inoculated	Number of seedlings with		
		RSL*	DT	I_t
5	10	1	1_a	2_a
10	10	8	1_a	9_b
15	10	5	4_a	9_b
23	10	0	10_b	10_b

*RSL = restricted stem lesion
DT = dead top
I_t = infection total, i.e., RSL + DT.
Figures bearing the same letter of the alphabet are not significantly different at p = 0.05 by chi-square analysis.

Experiment 2: Factorial of three misting temperatures and three post-misting temperatures. The levels of dead top at 15°, 20°, and 25°C when misting temperature equalled post-misting temperature were 9.7, 24.1, and 62.1% respectively (Table 2a); the differences between results at 25° and 20° or 15°C are highly significant, but not between 20° and 15°C (Table 2b). The levels of infection total with the same misting and post-misting temperatures were 32.3, 77.4, and 82.8% respectively; the difference was significant between 25° and 15°C, and 20° and 15°C, but not between 25° and 20°C (Table 2b).

Results from all treatments with a common misting temperature were combined for comparison of the misting temperature effect *(see* three columns at the right of Table 2a). The effect of misting temperature on dead top development is significantly different between 25° and 20° or 15°C, but not between 15° and 20°C (Table 2b). Infection total is significantly different between 15° and 20° or 25°C, but not between 20° and 25°C (Table 2b).

The effect of post-misting temperature is shown by the infection percentages in the bottom row of Table 2a. The effect on dead top

TABLE 2a—Effect of misting and post-misting temperatures on **D. pinea** infection of **P. radiata** seedlings (results 7 weeks after inoculation)

Misting temperature (°C)	Post-misting temperatures (°C) 15				20				25				Totals over all misting temperatures			
	Total inoculated	Percentage with RSL*	DT	I_t	Total inoculated	Percentage with RSL	DT	I_t	Total inoculated	Percentage with RSL	DT	I_t	Total inoculated	Percentage with RSL	DT	I_t
15	31	22.6	9.7	32.3	31	19.4	35.5	33.3	30	13.3	20	33.3	92	18.5	21.7_a	40.2_l
20	30	43.3	16.7	60.0	29	41.4	24.1	77.4	31	35.5	41.9	77.4	90	40	27.8_a	67.2_m
25	31	12.9	41.9	54.8	29	48.3	27.6	82.8	29	20.7	62.1	82.8	89	27	43.8_b	70.8_m
Totals over all post-misting temperatures	92	26.1	22.8_a	48.8_l	89	36.2	29.2_{ab}	65.4_m	90	23.3	41.1_b	64.4_m				

*RSL = restricted stem lesion.

DT = dead top.

I_t = infection total.

Figures bearing the same letter of the alphabet are not significantly different at p = 0.05 by chi-square analysis. DT comparisons are denoted by a, b, and I_t comparisons by l, m.

TABLE 2b—Comparison of effect of misting and post-misting temperatures (m.t. and p.m.t.) on **D. pinea** infection of **P. radiata** seedlings from results in Table 2a (figures indicate chi-square values)

Temperature (°C)	DT			I_t		
	m.t. effect	m.t. = p.m.t.	p.m.t. effect	m.t. effect	m.t. = p.m.t.	p.m.t. effect
25 **v.** 20	4.59***	7.03***	2.11*	0.15	1.44	0.03
25 **v.** 15	9.41***	15.85***	3.95**	16.68***	13.57***	4.78**
20 **v.** 15	0.60	1.33	0.15	12.82***	5.37**	7.2***

* = significant at p = 0.1
** = significant at p = 0.05
*** = significant at p = 0.01

development is significantly different between 25° and 15° or 20°C, but not between 20° and 25°C (Table 2b). The same trend can be seen with respect to the effect on infection total (Table 2b).

Experiment 3: The effect of various misting temperatures was examined with a common post-misting temperature of 24°/10°C day/night. Forty-eight hours of AT misting at 24°C produced far more dead tops than that at 12°C, though the infection total was identical in the two treatments (Table 3). CAB misting at 24°/10°C or 20°/10°C for 48 h produced virtually no dead tops, although 50–70% of the inoculated plants had stem lesions. Hence, infection was initially successful, but lesion extension remained limited throughout the experiment.

TABLE 3—Effect of misting temperature on **D. pinea** infection of **P. radiata** seedlings (post-misting conditions: 12-h photoperiod, 24°/10°C day/night, and R.H. = 60–75%; results 4 weeks after inoculation)

Misting condition	Total inoculated	Number with RSL	Number with DT
AT* 12°C	11	10	0
AT 24°C	10	3	7
CAB 24°/10°C day/night	10	5	0
CAB 20*/10°C day/night	10	7	1

* AT = misting in "Aquatron", a specially designed misting chamber
CAB = misting in growth cabinet

Experiment 4: The effect of various misting temperatures was further examined in this experiment at a common post-misting temperature of 16°/10°C. The levels of infection total and dead top were lower at a misting temperature of 12°C (AT) or 16°/10°C (CAB), but near maximum at a misting temperature of 20°C or above (Table 4). If infection is initiated at a favourable temperature, subsequent disease development apparently continues even at a fairly low post-misting temperature of 16°/10°C.

TABLE 4—Effect of misting temperature on **D. pinea** infection of **P. radiata** seedlings (post-misting temperature = 16°/10°C day/night)

Misting system	Misting temperature (°C)	Total inoculated	Number with	
			RSL	DT
AT	30	10	0	9
	20	10	0	10
	16	10	1	6
	12	10	2	2
CAB	16°/10°C day/night	10	2	2

Experiment 5: Dead top development was 90–100% in all treatments except when misting and post-misting temperatures were 15°/10°C day/night. In that treatment only one in 14 inoculated plants developed dead top.

Effect of Misting Duration on Infection

A. *Uninterrupted misting:* Results from Experiment A showed that, within the limits of wetness duration of 6 to 48 h, the levels of both infection total and dead top increased linearly with increasing wetness periods at the two test temperatures of 15° and 25°C (Table 5 and Fig. 3). Group comparisons showed significant slope differences between dead top at 15°C and at 25°C, but not between infection total at the two temperatures, though the levels differed significantly. At a misting temperature of 15°C slope differences between DT and I_t are highly significant (Table 5); the increase of dead top with increasing wetness periods was slower than that of infection total and the relationship between Dt/I_t and wetness periods is apparently random. At a misting temperature of 25°C the lines DT and I_t are almost parallel to each other (Fig. 3) and slope differences between the two were not significant (Table 5). Moreover, DT/I_t also regressed linearly on wetness periods. Data from both experiments (A1 and A2) showed the minimum wetness duration required for infection was around 3 h, and at a misting temperature of 25°C near-maximum infection and dead top development required no longer than 48 h wetness. It should be noted that in the two treatments in Experiment A, only temperatures during the misting periods differed; post-misting temperatures were kept the same.

B. *Interrupted misting:* A 12-h interruption of misting did not affect infection total, but dead top development was significantly affected (Table 6). Interruption of misting for longer than 24 h markedly affected both infection total and dead top development.

CONCLUSIONS AND DISCUSSION

It is evident from this study that temperature and humidity conditions during the early stages of infection (largely in the first 48 h after inoculation) have a decisive effect on the whole disease process and the final outcome of infection. Shoot wetness is required at this stage since a film of free water on the host surface is essential to spore germination and host penetration; a minimum wetness time of 3 h is required,

TABLE 5—Effect of misting duration at 15° and 25°C on infection of **P. radiata** seedlings by **D. pinea**

Experiment	Misting temperature (°C)	Response type (%)	Misting duration† (h)						Data computation						
									Linear regression					Group comparisons	
														Slopes	Levels
			3	6	12	18	24	48	a	b	R	R^2	F (VR)		
A1	15	DT	—	0	20	20	31	39	4.74	0.8	0.88	0.776	10.56*	I_t 15°C / DT 15°C	5.995*	
		I_t	—	13	27	47	44	83	8.57	1.58	0.976	0.952	59.21**			
		DT/I_t	—	0	74	42	70	47	35.36	0.52	0.28	0.08	0.267			
														I_t 25°C / DT 25°C	0.272	20.98**
	25	DT	—	6	19	20	43	86	-6.82	1.93	0.99	0.98	140**			
		I_t	—	24	50	47	79	100	22.9	1.72	0.94	0.88	22*	DT 15°C / DT 25°C	14.62**	
		DT/I_t	—	25	37	42	55	86								
A2	25	DT	0	0	—	—	16	—						I_t 15°C / I_t 25°C	0.1	9.142*
		I_t	5	0	—	—	37	—								
		DT/I_t	0	0	—	—	43	—								

* = significant at p = 0.05

** = significant at p = 0.01

† Experiment A1 did not include 3-h misting; Experiment A2 included only 3-, 6-, and 24-h misting.

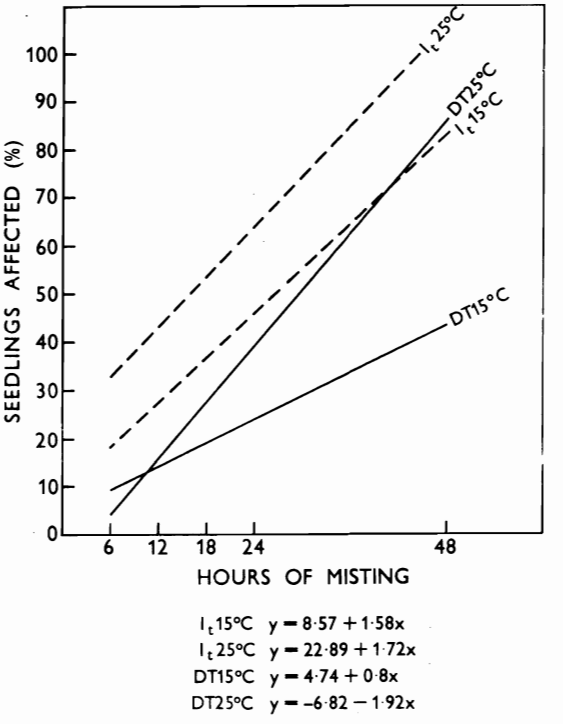

FIG. 3—Effect of misting duration at 15° and 25°C on **D. pinea** infection of **P. radiata** seedlings (from data in Table 5); DT = dead top, I_t = infection total.

TABLE 6—Effect of interrupted misting on **D. pinea** infection of **P. radiata** seedlings

Total inoculated	Treatments			Number of plants with		
	Initial misting* (h)	Interruption (h)	Final misting (h)	RSL	DT	I_t
24	24	0	0	13_a	4_a	17
25	12	12	12	18_a	1_b	19
25	12	24	12	7_b	2_b	9
23	12	48	12	4_b	2_b	6
22	12	96	12	2_b	1_b	3

* AT misting at 25°C. Seedlings were dried in the glasshouse between mistings, and all were then placed in a growth cabinet at 22°/15°C day/night. Figures bearing the same letter of the alphabet are not significantly different at p = 0.05 by chi-square analysis.

but the maximum is apparently no longer than 48 h. The level of infection total is a measure of initial infection success, and it can be seen that even at a temperature of around 10°–12°C a high level of infection total can be reached with a 48-h misting. Success in initial infection, as shown by the first appearance of a stem lesion, may also be the end of the disease process (restricted lesion); hence, a high level of infection

total is not necessarily associated with a high level of dead top. When a lesion ceases to extend at an early stage, infection becomes "aborted" or ineffective. DT/I_t is thus a measure of "abortion rate" or effectiveness of infection – the higher the value of DT/I_t the lower the abortion rate, or the more effective the infection. The increase in dead top with increased wetness duration suggests a plant/wetness interaction. The increase of DT/I_t with increase in wetness duration at 25°C but not at 15°C suggests that wetness had an effect on disease processes other than spore germination and host penetration at 25°C, and that the increase in dead top with increased wetness duration at 15°C was due entirely to an increase in the level of initial infection.

The minimum temperature for infection is around 5°C; the maximum has not been directly determined but could be somewhere between 35°C and 40°C. It is apparent that temperature is not critical in infection initiation which can occur to a high level within the range 10°–25°C or higher, given a 48-h misting period. A high level of dead top, however, can occur only when the temperature during misting is relatively high (above 20°C) despite lower post-misting temperatures. When a lower misting temperature is followed by a higher temperature during the dry period, dead top development is limited. Hence the level of dead top is more closely related to temperature level during the brief "wet" phase of infection than to the mean temperature taken over the whole period of infection development. Roughly, temperature levels of 10°–12°C, 15°C, and 20°–25°C or higher during the "wet" phase of infection can be related respectively to minimum, intermediate, and maximum levels of dead top development.

It should be cautioned that the concept of limiting or optimum levels of a factor affecting infection is relative rather than absolute (Rotem *et al.* 1971). The effects of temperature and wetness on infection are obviously interdependent and can probably be modified by other factors as well, e.g., the level of host susceptibility (Chou 1982).

It is not entirely clear why temperature during the wet or early phase of infection is crucial not only to infection initiation, including spore germination and host penetration, but also to the subsequent disease process leading finally to the development of dead top. Lesion limitation is no doubt an expression of host resistance, but the effect of temperature (or wetness) on dead top development probably relates more to the pathogen than the host. One may postulate that the effectiveness of host resistance depends on the "size" of the invading force and that there is a critical size beyond which the host is incapable of coping with the invasion. At a temperature of 10°–12°C, which is unfavourable to its growth, the pathogen can be subdued easily soon after host penetration. At 20°C or higher, faster pathogen build-up makes it easier to overcome host resistance. Apparently under such favourable conditions the pathogen can develop within 48 h to the extent that it is no longer vulnerable to host defences, even if conditions later become less favourable to its development. The data suggest that hot humid spells could be important to the natural occurrence of shoot dieback caused by *D. pinea* in nature and that the effect of topography and aspect on the disease may be related to differences in the duration and frequency of such spells during the period of shoot susceptibility.

ACKNOWLEDGMENTS

The author wishes to thank Mr Ian Andrew for advice on statistics, Mrs Hignet and Mrs Currie for assistance in the work, and Dr S. Carson for critical review of the manuscript.

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