A SHOOT DIEBACK IN *PINUS RADIATA* CAUSED BY *DIPLODIA PINEA*

II. INOCULATION STUDIES

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ABSTRACT

Inoculation trials confirmed the ability of **Diplodia pinea** (Desm) Kickx to invade undamaged young green shoots of **Pinus radiata** D. Don, resulting in dieback. The top 5 cm of young shoots of seedlings and rooted cuttings from 5-year-old trees, were brushed with droplets of a spore suspension and kept under misting at 25°C for 48 h. Of the plants thus inoculated, 50-80% developed shoot dieback, which always originated from necrotic stem lesions. Ripened host tissue was resistant to infection, even after wounding. There was no evidence of important differences in either pathogenicity or virulence in 18 isolates of **D. pinea** obtained from different parts of New Zealand. This suggests that the severity of dieback in certain parts of Tarawera Forest has not resulted from the emergence of a more virulent pathogenic race.

These results, together with an earlier field study, provide convincing evidence that **D. pinea** was the primary incitant of leader and shoot dieback in the Putauaki Block of Tarawera Forest.

INTRODUCTION

It has been demonstrated through field observations and isolation studies (Chou, 1976) that a severe dieback condition in certain parts of Tarawera Forest was the result of *Diplodia pinea* (Desm) Kickx infection of apparently-intact young shoots during their rapid elongation in spring. In order to understand the role of this fungus as a primary or secondary incitant of dieback, it was essential to determine by inoculation studies whether *D. pinea* could invade undamaged pine shoots and so cause dieback.

Inoculation studies by various workers and their interpretation of results are summarised in Table 1. Overall, these suggested that *D. pinea* was a weak or wound parasite, particularly with respect to the infection of *Pinus radiata* D. Don and the ability of the fungus to produce shoot dieback. The evidence for successful inoculation without wounding was somewhat inconclusive. In some trials there was no clear indication of whether or not dieback was produced (Eldridge, 1957; Brookhauser and Peterson, 1971); in others no figures were given to quantify their success (Slagg and Wright, 1943; Saravi Cisneros, 1950), or the given figures were too low to be convincing (Ludbrook and White, 1940). Many of the inoculation trials were done under field conditions (Waterman, 1943; Slagg and Wright, 1943; Saravi Cisneros, 1950), hence the possibility of wounding by natural agents could not be entirely excluded,

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TABLE 1—Summary of inoculation

Author & Country	Pine Spp. & Age Inoculated	Parts Inoculated	Source of Inoculum	Type of Inoculum
Birch (1936), New Zealand	3-year **P. ponderosa** and seedlings (3 in. high)	Uninjured leading shoots	Not stated	Mycelium
Young (1936), Australia	**P. taeda, P. caribaea, P. patula, P. insularis**	Seedlings 45 cm high, parts not stated	Isolated from **P. radiata**	Young culture presumably mycelium
White (1937), U.S.A.	3-6 year **P. mugo** and **P. sylvestris**	Growing tips at least	Isolated from **P. mugo**	Spores
Ludbrook & White (1940), Australia	2-20 year **P. radiata, P. caribaea, P. muricata, P. pinaster, P. ponderosa, P. taeda**	Inoculated at 7.6-45.7 cm below growing point	Not stated	Presumably mycelium
Hadow & Newman (1942), Canada	**P. sylvestris** — tree age not stated	Shoots	Isolate from Scots pine	Mycelium in culture and spores from naturally-infected material
Waterman (1943), U.S.A.	5-10 year **P. nigra, P. sylvestris, P. resinosa, P. ponderosa, P. strobus**	Current season shoots	Single spore isolate from **P. nigra** and an isolate from **P. resinosa**	Spores or mycelium in culture
Slagg & Wright (1943), U.S.A.	1-year **P. nigra**	Not stated	Single spore isolate from **P. nigra**	Mycelium and infected sporulating material
Saravi Cisneros (1950), Argentina	7-year **P. palustris, P. radiata, P. canariensis, P. halepensis, P. pinea, P. pinaster**	Seedling shoot to mature stem	Isolate from **P. halepensis**	Mycelium or spores from naturally-infected material
Eldridge (1957), p.53, Australia	2-3½ year **P. radiata**	Not stated	Isolate from **P. radiata**	Mycelium and spore suspension
Milliken & Anderson (1957), Australia	2-year **P. radiata**	Upper half of tree	Not stated	Mycelium from culture, plus spores from naturally-infected material
Purnell (1957), Australia	2-3 year **P. radiata**	Growing shoots	Presumably from **P. radiata**	Spores from dead needles or culture
Stahl (1968), Australia	**P. radiata**	Detached stems	Not stated	Spores or mycelium
Marks & Minko (1969; 1970), Australia	2-3 year **P. radiata**	Green or woody stem	From **P. radiata**	Spores from infected cones or culture
Brookhauser & Peterson (1971), U.S.A.	1-year and 10-year **P. nigra, P. sylvestris P. ponderosa**	Young shoots	Not clearly stated	Spores in culture or from infected pine cones

* Results of successful inoculation without wounding the host.

results by various workers

Method of Inoculation and Conditions	Results*	Views and Conclusions
Bits of mycelia placed on shoot surface, wet wool and cellophane covering, 5.0-29.4°C, over 90% RH under glass frame 24-48 h. Field inoculation also done.	*Seven out of 15 trees developed dieback in glass frame. Little success with trees in the field and with seedlings.	Infect uninjured shoots under abnormal humidity. Normally a saprophyte, occasionally parasitic.
Wound inoculations, wet cotton wool coverings under glass and in open air.	High rate (up to 100%) dieback produced.	Wound parasite implied.
(Original paper not obtained.)	Quoted by Waterman as pathogenic on young growing tips.	
Mostly wound inoculations. Wet coverings, field and greenhouse. Unwounded **P. radiata** shoots inoculated in greenhouse.	*In **P. radiata** 4 out of 9 laterals developed dieback; 15 leaders not inoculated not infected.	Capable of actively parasitising limited portions of vigorous trees, but normally such trees are resistant.
Wound inoculation, injection with hypodermic needle, or spores applied to shoots infested with spittle-bugs.	60-90% tip blight produced.	Wound parasite implied.
On injured and uninjured shoots at various positions. Field April-July. Moist cotton wrappings.	*On uninjured shoots and buds some dieback apparently produced, though not clearly indicated by the figures given.	Will infect healthy actively-growing tissue of buds and leaves but will infect more readily through wounds.
Bits of mycelia placed on uninjured growing point in moist chamber 72 h. Field conditions. Also used naturally-produced spores.	*All seedlings within a 6-in. block dead after 30 days using mycelia as inoculum. No success with spores.	Serious parasite of first-year seedlings.
With or without wounding, wet cotton wrapping for 6 days. Field in autumn and spring.	*Dieback produced but no figures given.	Attacks young shoots directly, but attacks stems through wounds.
With or without wounding, greenhouse kept moist by a water-bath (85-95% RH). No infection below 60% RH. Temp. 30°C day, 17°C night.	Whether or not dieback was produced not indicated. More infection with mycelia as inoculum than with spores.	Prevalent in moist sheltered sites. Hail storm and hot humid weather conducive to infection.
Foliage and growing tips injured by carborundum blast. Spraying of spores. In the field in summer, polythene bag covering.	No success even with wounding.	Not an active parasite, cannot attack healthy trees.
Spore suspension applied to damaged or undamaged needles, RH 28-70%, 15.6-28.9°C.	No success with uninjured shoots.	Suggest unknown factors predisposing trees to attack.
Pre-treatment of stems, heat 42°C for 4 h, or desiccation at room temperature.	No success if not pretreated.	Cannot attack healthy trees.
Wound inoculations, hypodermic needle injection, or bark removal. Field and greenhouse in winter.	Dieback or canker produced.	Emphasise wound parasite.
Spore suspension applied to needles with medicine dropper. Field at various times of year: greenhouse 24°C 100% RH for 24 h.	Only needle infection mentioned, whether or not producing dieback not indicated.	Entry through stomata demonstrated.

and the results were inconclusive as to the implication of wounding. Mycelia were used by many as inoculum (Birch, 1936; Ludbrook and White, 1940; Saravi Cisneros, 1950); some even reported little success in using spores as inoculum as compared with mycelia (Slagg and Wright, 1943; Eldridge, 1957). The use of mycelia as inoculum and the appropriateness of covering up the inoculated parts with wet cotton wool and tape wrappings is open to question, as natural infection is not likely to originate in this way.

Variability in virulence or cultural characteristics among isolates of *D. pinea* (Laughton, 1937; Purnell, 1957; New Zealand Forest Service, 1972), may also account for some of the discrepancies in inoculation results.

The wide range of host and of environmental conditions make evaluation and comparison of these inoculation studies difficult. The varying views about the role of this fungus in pine dieback therefore remain to be resolved by inoculation studies with specified host, pathogen, and environment.

This paper reports inoculation with *D. pinea* spores of *P. radiata* seedlings of different ages, as well as on rooted cuttings from 5-year-old trees, under semi-controlled glasshouse conditions. The objective was to determine whether or not this fungus can infect uninjured pine shoots. Consideration was given to previous field observations (Chou, 1976) which showed that infection was mainly restricted to the current season's growth (and that ripened tissue was apparently resistant) and to possible variation in pathogenicity among different isolates of *D. pinea.* Eighteen isolates of this fungus were obtained from various parts of New Zealand (one being from Tarawera Forest) and their pathogenicity was tested by inoculations on *P. radiata.* A limited number of *P. contorta* Dougl., *P. muricata* D. Don and *Pseudotsuga menziesii* (Mirb.) Franco (Douglas fir) seedlings were also inoculated.

MATERIALS AND METHODS

Host Plants

Plants used for inoculation trials consisted of seedlings ranging from 3 months to 2 years old, and rooted cuttings obtained originally from 5-year-old trees. Bulked seed from a number of select trees in Kaingaroa Forest was used to produce seedlings. This seedlot has been used at FRI for various research purposes over a number of years. Seedlings were raised on a 50/50 duff and soil mixture in highly fertile conditions in the glasshouse. Seedlings less than 8 months old were grown in 1- to 2-litre pots. Cuttings were from 14 randomly selected 5-year-old trees in the Northern Boundary region of Kaingaroa Forest, and were used for inoculation about 1.5 years after the cuttings were obtained. Each rooted cutting was grown in a 4.5-litre bucket in the nursery until inoculation.

Source of Inoculum

With the exception of one experiment when the pathogenicity of *D. pinea* isolates was compared, all inoculations were with one isolate obtained from a single infected shoot in the Putauaki block of Tarawera Forest, an area where dieback was most severe (Chou, 1976). The origins of the 16 other isolates used in pathogenicity tests are given under Results and Discussion.

Inoculum Production

The fungus was cultured on 3% "Oxoid" malt agar at 25°C for 5-7 days at which time the agar surface was overgrown by the fungus. The surface of the colony was overlaid with autoclaved *P. radiata* needles and the plates were irradiated in continuous near-ultraviolet light at room temperature for 4 weeks (Commonwealth Mycological Institute, 1968). Abundant *D. pinea* pycnidia were produced on the needles under these conditions. The plates were then stored at 10°C, a temperature at which spores were found to maintain high viability for at least 3 months. All inoculations used spores stored within this duration. Spore suspensions were prepared by soaking pycnidia-laden needles in 0.5% gelatin solution or distilled water at 5°C for 30 min. followed by gentle shaking to disperse the spores; after this the needles were removed and the suspension was adjusted to the required concentration, normally 12 000-15 000 spores/ml. In some experiments a concentration of up to 50 000 spores/ml was used. The gelatin solution helped spores adhere to the host and was used in most experiments.

Inoculation Methods

Three methods were used:

1. *"Brushing plus droplet"* — This was the standard inoculation method. A soft camel-hair brush was dipped into the spore suspension and stroked gently along the surface of the topmost 5 cm of shoot. Another dip was made and the opposite side of the shoot was inoculated in the same fashion. An additional droplet of spore suspension was deposited onto the shoot tip with a medicine dropper. In some experiments this droplet was 10 μl delivered with a micro-syringe.
2. *"Droplet"* — A 10-μl droplet of spore suspension was deposited on the shoot tip or, in some experiments, onto the shoot surface of horizontally laid plants. The latter procedure allowed the droplets to remain on the inoculated spot during the misting period. This method of inoculation eliminated any possibility of wounding the host and allowed examination of the effect of inoculation on a particular spot.
3. *"Injection"* — This method was applied to ripened woody stem tissue below the second branch cluster of the rooted cuttings. The spot to be inoculated was punctured to the pith and an eighteen-gauge syringe was used to inject a suspension of 15 000 spores/ml into the hole until overflow.

During inoculation the spore suspension was kept in an ice bath to prevent spores from germinating prior to deposition on the host plant. After inoculation the plants were placed in a misting chamber for 48 h at 25°C. The misting chamber was 95 cm high with an area of 54 × 105 cm, and was fitted with a de Vilbiss-type atomiser, which has a spraying capacity of 1.5 litre/hour at 40 atmospheres. The rate of misting was regulated by a humidistat of the artificial leaf type (Bean *et al., 1957*). After incubation in the misting chamber, the plants were removed to a temperature-controlled glasshouse. Night temperatures were maintained not lower than 15°C and day temperatures not higher than 22°C. On some summer days the maximum temperature in the glasshouse occasionally reached 28-32°C for short periods.

Most of the inoculations were made during November to February. Unless otherwise specified, results were recorded 4 weeks after inoculation.

RESULTS AND DISCUSSION

1. *Inoculation Methods*

The overall results of inoculating *P. radiata* seedlings and cuttings are shown in Tables 2, 3 and 4. The standard method of inoculation, i.e., "brushing plus droplet", was highly effective in producing shoot dieback in seedlings of different ages (Table 2) as well as in rooted cuttings taken from 5-year-old trees. "Droplet" alone when applied at the shoot tip was, however, only half as effective as "brushing" alone (of subapical region of shoot) in producing dieback (Table 4).

There was little difference in inoculation results whether gelatin solution (0.5%) or water was used as the spore suspension medium. In one experiment with 8-month-old seedlings, 20 were inoculated with a spore suspension (45 000 spores/ml) made with 0.5% gelatin solution and another 20 with one made with distilled water. Results were identical in each treatment, i.e., 13 out of 20 seedlings developed dieback.

As can be seen from Table 2, inoculation of rooted cuttings with a suspension of around 10 000 spores/ml was sufficient to produce a high rate of infection resulting in dieback. No advantage was apparent when a concentration higher than 50 000 spores/ml was used. In one experiment 20 seedlings were inoculated with a spore suspension of 450 000 spores/ml and another 20 with 45 000 spores/ml; 40% of the inoculated plants in the first group developed dieback in contrast to 75% in the second. The plant, apparently, must be given a minimum number of spores to ensure a high probability of infection. In one experiment when spore load per plant was 2 500, 500, 100 and 20 (10-13 plants/treatment), the incidence of plants with dieback was 100, 100, 90 and 50%, respectively.

TABLE 2—Inoculation of **P. radiata** seedlings with spores of **D. pinea**

Age of seedlings when inoculated	Number inoculated	Number infected	Number with dieback	Relative length of dieback*	Conc. spores/ml	Method of inoculation
3 months	10	5	5	—	15 000	Droplet plus brushing
6 months						
Box 1	40	38	38	57	50 000	Droplet plus brushing
Box 2†	39	18	17	29	50 000	Droplet plus brushing
8 months	50	No record	44	No record	50 000	Droplet
2 years	24	19	18	No record	16 000	Droplet

* $\frac{\text{Length of Dieback}}{\text{Plant Height}} \times 100$

† Non-infected seedlings were re-inoculated 6 weeks after the first inoculation. All those which were re-inoculated developed dieback. The difference in initial result might be due to the two boxes being inoculated by two different operators.

TABLE 3—Effect of maturity of **P. radiata** shoot on **D. pinea** infection

Age of plant	Inoculation method	Where inoculated	Percentage of plants with: No infection	Restricted lesion	Severe lesion	Dead top	Number inoculated
2-year-old seedlings	Brushing	Topmost 5-cm of shoot	54	25	3	18	28
		Suberized region	100	0	0	0	13
7-month-old seedlings	Droplet	Shoot tip	35	30	5	30	20
		Subapical 5-cm of shoot	35	5	10	50	20
		Semi-suberized region	45	40	0	15	20
Rooted cuttings from 5-year-old trees clone no. 7	Injection	Below second branch cluster	100	0	0	0	7
	Brushing plus droplet	Topmost 5-cm of shoot	29	0	0	71	7

TABLE 4—Effectiveness of two inoculation methods — "Droplet" and "Brushing" — on **D. pinea** infection of 7-month-old **P. radiata** seedlings

Inoculation method	Percentage of plants with: No infection	Restricted lesion	Dead top	Total number of plants inoculated
Droplet (10 microlitre) at shoot tip	15	37	48	59
Brushing of subapical region	2	14	84	51

2. *Infection of Seedlings and Rooted Cuttings*

Symptoms were obvious in seedlings as early as 24 hours after inoculation (Table 5). These early symptoms were most noticeable on primary leaves. Either the whole leaf or the basal half of it became necrotic, discoloured to light-brown or straw-coloured, and appeared water-soaked. Stem lesions could also be seen at this early stage when shoots were carefully examined. A lesion appeared as a small (approx. 5 mm long) necrotic, slightly sunken and water-soaked, light brown spot on the stem. Resin exudation was rarely apparent. Later the lesions were seen to enlarge, all needles on the lesion were killed, and the shoot often drooped at the affected area. Several centimetres of shoot might be killed in as little as 5-6 days after inoculation. Most often 80-90% of the inoculated plants developed dieback in 2 to 4 weeks (Table 2).

There were indications that some of the inoculated plants were genuinely resistant. Several plants survived a second inoculation, and a few even survived a third.

It was noted throughout that on susceptible plants some inoculated spots were covered with spores, yet developed no sign of infection. When epidermal strippings were taken from such spots and examined microscopically, spore germination on the host surface was significantly lower than that on slides kept under the same conditions.

All 14 clones of rooted cuttings from 5-year-old trees were readily infected; 87% of the 71 plants inoculated had leader dieback, while 82% of 321 laterals inoculated developed dieback in a month's time. On average about half the length of the current season's leader growth was killed during this 4-week period.

In rooted cuttings severe stem necrosis was seen as early as 6 days after inoculation. Occasionally a general shoot wilting in the form of needle yellowing and desiccation was evident before necrotic stem lesions were apparent (Fig. 1a).

In the infection of *P. nigra, P. ponderosa,* and *P. sylvestris,* Brookhauser and Peterson (1971) noted that the fungus entered the host through the stomata of needles. Observations in this study gave no indications that stem infection necessarily resulted from foliage infection; apparently each can occur independently of the other. Droplets of spore suspension were placed on the stem and the inoculated spots were carefully examined after 24 and 48 hours; it was evident that infection could begin in either the primary leaf, fascicle, or stem (Table 5).

TABLE 5—Early stages of symptom expression in shoots of **P. radiata** inoculated by placing droplets of **D. pinea** spore suspension (10 000 spores/ml) onto stem surface of 6- to 7-month-old seedlings (10 in Experiment 1 and 20 in Experiment 2) laid horizontally

Expt.	Time after inoculation	Number of cases showing symptoms in:				
		Primary leaf alone	Fascicle alone	Stem alone	Both primary leaf and stem	Both fascicle and stem
1	24 h	7	10	4	1	0
2	24 h	15	1	3	3	1
	48 h	17	4	7	3	2

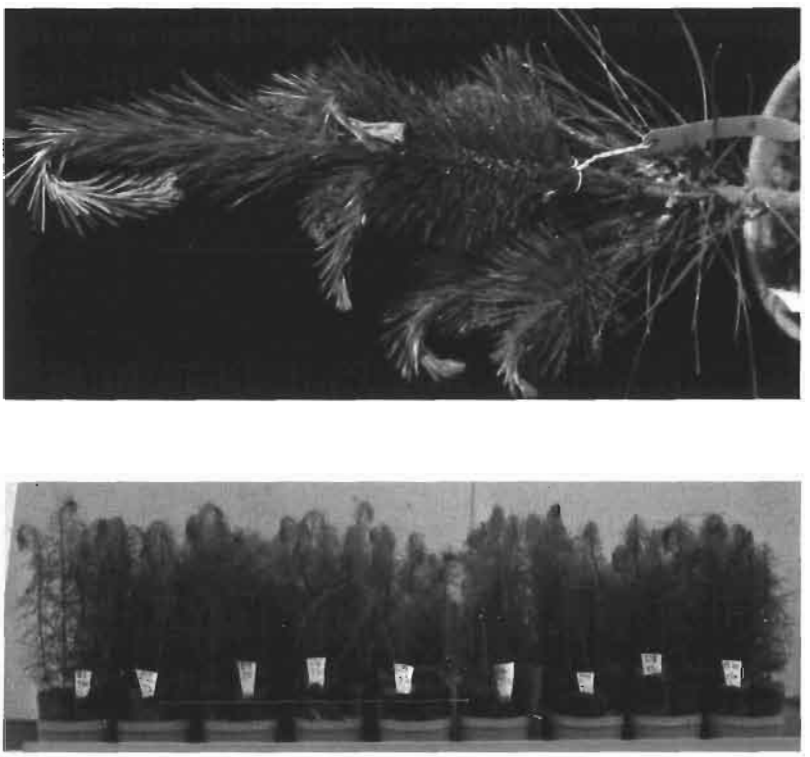

FIG. 1—(a; above, set sideways) Symptoms of shoot wilting 5 days after inoculations of rooted cutting of **Pinus radiata** with spores of **Diplodia pinea.** (b; below) Pathogenicity of different isolates of **D. pinea** on **P. radiata** seedlings, 3 weeks after inoculation. From left to right, isolates D1 to D8 and D10.

In reisolations made from infected needles and stem lesions, *D. pinea* was invariably the only organism recovered.

Pycnidia formation was sparse to nil on infected plants left in the glasshouse, presumably because pycnidia development was prevented by quick dessication of infected parts under these conditions. When a sprinkler was set up providing from 4 to 6 hours' misting, shoots freshly killed by *D. pinea* showed pycnidia by the fifth day.

3. *Infection in Relation to Maturity of Host Tissue*

When inoculum was applied to the more mature part of shoot, i.e., suberized or semi-suberized tissue, little or no infection was evident (Table 3). In the suberized region inoculation even by injection did not result in infection. In contrast, inoculations in the topmost 5 cm of shoot of the same clone resulted in a high rate of leader dieback (5 out of 7).

The tip of the shoot appeared to be slightly more resistant than the subapical region. Inoculations applied to the shoot tip produced about half as much dieback as applications

to the subapical 5 cm of shoot (Table 3, 7-month-old seedlings; Table 4) and often resulted only in the death of a few primary leaves.

4. *Infection of Douglas fir,* P. contorta *and* P. muricata

Inoculations were carried out during the mid-flush of these trees. *Pinus muricata* appeared to be highly susceptible, and Douglas fir more resistant, though no firm conclusions can be drawn from the following limited results:

Host	Age	Number inoculated	Number with dieback
P. contorta	1 year	32	16
Douglas fir	1 year	13	2
P. muricata	6 months		
Blue strain		12	12
Green strain		15	15

Diplodia pinea was re-isolated in all cases thus confirming its pathogenicity on these hosts.

5. *Isolates Variability*

The inoculation results using different isolates of *D. pinea* are shown in Table 6. All isolates produced high rates (over 90%) of dieback in seedlings within a week after inoculation. Figure 1b shows part of the inoculation results. An isolate from blue-stained timber (D 3) was just as pathogenic on *P. radiata* as the two isolates from the worst dieback area in Tarawera Forest. One isolate obtained from *P. muricata* was highly virulent on *P. radiata.*

The spore dimensions of isolates were measured by two operators at different times. Each operator measured half of the isolates assigned randomly. Length/breadth calculation has the advantage of removing obvious operator bias, and analysis of variance showed this parameter to differ significantly ($P \leq 0.001$) between some of the isolates, despite the lack of evidence of pathogenicity difference and visible difference in cultural characteristics.

CONCLUSION

The results of these inoculation tests provide conclusive evidence that *D. pinea* is capable of invading intact young green shoots of pine and causing dieback. For successful inoculations the shoots must be in their first year of growth before the epidermal tissue becomes highly resistant and infection difficult to establish even with wounding. The conflicting reports on the ability of *D. pinea* to invade intact host tissue may have arisen because of failure to distinguish between infection of young shoots and more mature woody stems.

The symptom development in these inoculation trials was very similar to that

TABLE 6—Pathogenicity of **D. pinea** isolates on **P. radiata** seedlings (7 months old). Results 1 week after inoculation

Isolate number	Host isolated	Place	Spores Dimensions Length × Breadth Mean ± S.E.	Number inoculated	Number with dead top
D1	**P. radiata**	Tarawera Forest	39.7 ± 0.2 × 15.4 ± 0.1	10	10
D2	**P. radiata**	Tarawera Forest		16	16
D3	Blue-stained timber	—	40.3 ± 0.1 × 15.4 ± 0.1	16	16
D4	**P. radiata**	Palmerston North	40.3 ± 0.1 × 15.4 ± 0.1	15	15
D5	**P. radiata**	Patunamu Forest	42.9 ± 0.2 × 15.4 ± 0.1	16	15
D6	**P. radiata**	Woodhill	42.9 ± 0.3 × 15.4 ± 0.1	16	15
D7	**P. muricata**	Woodhill	39.7 ± 0.1 × 15.4 ± 0.1	15	15
D8	**P. radiata**	Golden Downs	40.3 ± 0.2 × 15.4 ± 0.1	17	16
D9	**P. radiata**	New Plymouth	35.2 ± 0.3 × 14.1 ± 0.1	17	16
D10	**P. radiata**	Lake Taupo	39.7 ± 0.2 × 14.7 ± 0.1	19	18
D11	**P. radiata**	Hautu Prison Farm	35.2 ± 0.1 × 14.7 ± 0.1	18	16
D12	**P. radiata**	Lake Taupo Forest	37.1 ± 0.3 × 13.4 ± 0.1	15	15
D13	**P. radiata**	Matahina Forest	37.3 ± 0.2 × 12.8 ± 0.1	15	14
D14	**P. radiata**	Tokoroa	38.4 ± 0.2 × 13.4 ± 0.1	18	17
D15	**P. radiata**	Waitotara	36.5 ± 0.2 × 14.1 ± 0.1	18	18
D16	**P. radiata**	Matakana Island	—	10	10
D17	**P. radiata**	Sth Canterbury	33.3 ± 0.2 ×14.1 ± 0.1	15	15
D18	**P. radiata**	Hawke's Bay	38.4 ± 0.0 × 12.8 ± 0.1	13	13

observed in the field (Chou, 1976). This evidence lends strong support to the suggestion that *D. pinea* was the primary incitant of dieback in certain parts of Tarawera Forest.

The isolates of *D. pinea* in New Zealand did not appear to vary in pathogenicity or in virulence. It is therefore not likely that the severity of dieback in certain parts of Tarawera Forest is associated with the emergence of a highly virulent strain of this fungus.

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