

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

1. SYMPTOMS, DISEASE DEVELOPMENT, AND ISOLATION OF PATHOGEN

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ABSTRACT

A locally occurring dieback, similar to previously reported "red top", "shoot blight", and "tip blight", was investigated during 1971-73 in *Pinus radiata* in Tarawera Forest. Observations of symptoms and disease development, and isolation of fungi indicated that the dieback originated in necrotic stem lesions from which *Diplodia pinea* was invariably isolated. Succulent green shoots became infected in late spring and early summer while ripened shoots were apparently resistant, so dieback was restricted mainly to current season's growth. There was no evidence that infection was associated with wounds or frost damage, but there was every indication that it occurred through intact host tissue.

INTRODUCTION

Stem malformation after leader death in young (under 10 years) *Pinus radiata* D. Don has long been a matter for concern in plantations on the pumice plateau in the central North Island of New Zealand (e.g., Birch, 1936). Foresters became more aware of the problem when the scale of pruning and thinning of young stands increased in the early 1960s. Since 1967 a very high incidence of dieback has been observed in some stands three years old and older in Tarawera Forest (J. Mitchell, pers. comm.). Although the stands so affected were confined mainly to a few hundred hectares on a single valley site, the incidence was considered the worst ever seen by one observer (H. V. Hinds, pers. comm.), and concern was expressed about the cause of the dieback and the potential danger to young plantings surrounding the area.

Three factors, either singly or in combination, were suspected initially as causes of the dieback. They were nutrient deficiency, infection by the fungus *Diplodia pinea* (Desm.) Kickx, and frost injury. A deficiency of boron was suggested (Bannister and Burdon, 1968) largely because of the existence of certain tissue disorders, but also because there were known cases of dieback of *Pinus* spp. due to boron deficiency in New Zealand (Stone and Will, 1965). Sulphur and zinc deficiencies were also suggested. Intensive testing (field fertiliser trials, foliar and soil analyses, pot trials, and a soil replacement experiment) ruled out both nutrient deficiency and local soil factors as

the cause of dieback (Knight, 1970). Furthermore, vigorous trees with plentiful nitrogen appeared to be much more susceptible to dieback than those which were nitrogen deficient (New Zealand Forest Service, 1971; p. 31), and trees on valley flats were much more severely affected than similar trees on nearby hill sides (Smith, in prep.; New Zealand Forest Service, 1971, p. 31; 1973, p. 50).

Diplodia pinea appeared to be consistently associated with this dieback, and the pathogenic role of the fungus was therefore suspected.

Smith (1972) reported that shoot dieback appeared to originate in characteristic lesions, and that artificial inoculation of shoots with spores produced lesions indistinguishable from those occurring naturally. However, as the presence of *D. pinea* in those lesions was not tested by isolations, Koch's postulates were not fulfilled and the pathogenic role of *D. pinea* could not be regarded as proven. Moreover, Smith's observations did not indicate whether this fungus could invade intact shoots, or whether previous wounding was required.

Birch (1936) concluded that *D. pinea* was ubiquitous in pine forests in New Zealand, normally as a saprophyte but occasionally as a parasite on weakened trees. Subsequently most of the work on *D. pinea* has been carried out in Australia (Purnell, 1957; Milliken and Anderson, 1957; Stahl, 1968; Marks and Minko, 1969; 1970). These results and observations and preliminary inoculation trials in New Zealand (P. D. Gadgil, pers. comm.) appeared to support the view that this fungus was mainly a weak or wound parasite, unable to invade intact and healthy shoots.

As the areas worst affected by dieback were in valleys regarded as frost flats, it was surmised that frost damage followed by a fungal infection might be the primary cause (J. W. Gilmour, pers. comm.). *Sclerophoma pithyophila* (Corda) v. Höhn was identified on diseased specimens sent in from various parts of New Zealand (New Zealand Forest Service, 1968, p. 47; 1969, p. 50; 1970, p. 51), and pathogenicity studies showed that this fungus could kill *P. radiata* seedlings by spreading from frost-damaged parts to adjacent healthy tissue (New Zealand Forest Service, 1971, p. 46).

Clearly there was insufficient evidence for a firm hypothesis about the cause of the dieback; there was little information on when it was most prevalent, correlation with frost, or symptomatology. Distinctive diagnostic features may be lost when a shoot is dead so it is important to know how a healthy shoot develops dieback and what fungi, if any, are constantly associated with such development. In this way the sequence of events leading to dieback can be established and the primary incitant determined. Observations to this end are reported here.

OBSERVATIONS

Site

This was in the worst affected area—the Putauaki block in Tarawera Forest (about 8 km WSW of Mt Edgecumbe), a distinctive flat basin enclosed on three sides by hills. Soil analyses and a full description of the site have been given by Knight (1970). *Pinus radiata* was planted in 1968.

General Symptoms

Observations over a 3-year period showed that only current season's growth was affected. Varying lengths of the green leaders or later laterals died. The dead shoots appeared mummified (hardened and shrivelled) with persistent dead needles, and many of

the shoots were bent near the tip giving an inverted "hockey-stick" appearance (Fig. 1a). Recently killed shoots were reddish brown, and those dead for longer were greyish brown or dark grey. *Diplodia pinea* pycnidia were abundant on most of the specimens examined (Table 1), and were often the only fungal fructifications. On some specimens none were seen; however primordial fructifications may have formed but were not then readily discernible. Of 135 dead leaders examined at the peak of disease occurrence, 53% clearly displayed *D. pinea* and 40% showed no fungal fructifications of any kind (Table 1). However, when isolations were made from the latter group *D. pinea* was detected in 50%, making a total of 73% of the specimens demonstrably associated with this fungus.

On a few of the diseased specimens, *Sclerophoma pithyophila* was apparently the only fungus present. Of the 66 dead leaders examined by hand lens and microscope in November 1973, only 11 had *S. pithyophila* fructifications and the rest showed *D. pinea*. The *S. pithyophila*-associated dead shoots were dark purplish, spongy, soft, and sometimes water-soaked, and invariably had numerous small lenticular cracks or splits in the epidermal tissue (Fig. 1b). The origin of these cracks was not clear, but they resembled some of those described (D. R. Smith, in prep.) as epidermal splits caused by drought. Observations reported below indicate that these cracks always occurred after severe frost. *Sclerophoma pithyophila* dieback appeared to be wound-associated and to have symptoms distinct from the *D. pinea* dieback in which there was little evidence of wound association. Random sampling of 50 leaders on 14 December 1972 showed that 34 of them had dieback: 19 of the dead leaders were free of epidermal splits, and 14 of the 16 live leaders had splits. Thus, dieback was not correlated with the presence of cracks.

Initiation of Dieback

Close examination of a large number of green shoots from lower branches revealed that some had necrotic lesions of various sizes, which were brown-to-purplish-brown, slightly sunken, and sometimes water-soaked. The less obvious lesions were 1-2 mm in diameter or less, and were often found at the base of a dead stunted needle fascicle hidden by still-intact green needles. They were therefore very difficult to detect unless the green needle fascicles were removed (Fig. 1c). The more conspicuous lesions were several centimetres long, nearly girdled the shoot, and had many dead needles with *D. pinea* pycnidia often visible (Fig. 1d). Some of the shoots were bent, and close

TABLE 1—Association of *Diplodia pinea* and other fungi with dieback—based on hand-lens examination of fungal fructifications on killed shoots

No. of specimens examined	Condition	Percentage of specimens with:			Date examined
		<i>D. pinea</i>	<i>S. pithyophila</i> and other fungi	None	
25	Recently killed*	100	16	0	Oct.-Nov. 1971
25	Killed over a year	100	52	0	Oct.-Nov. 1971
135	Recently killed*	53	7	†40	25 Oct. 1972
66	Recently killed*	83	17	0	Nov. 1973

* Within about one month of date examined

† From half of these specimens *D. pinea* was isolated

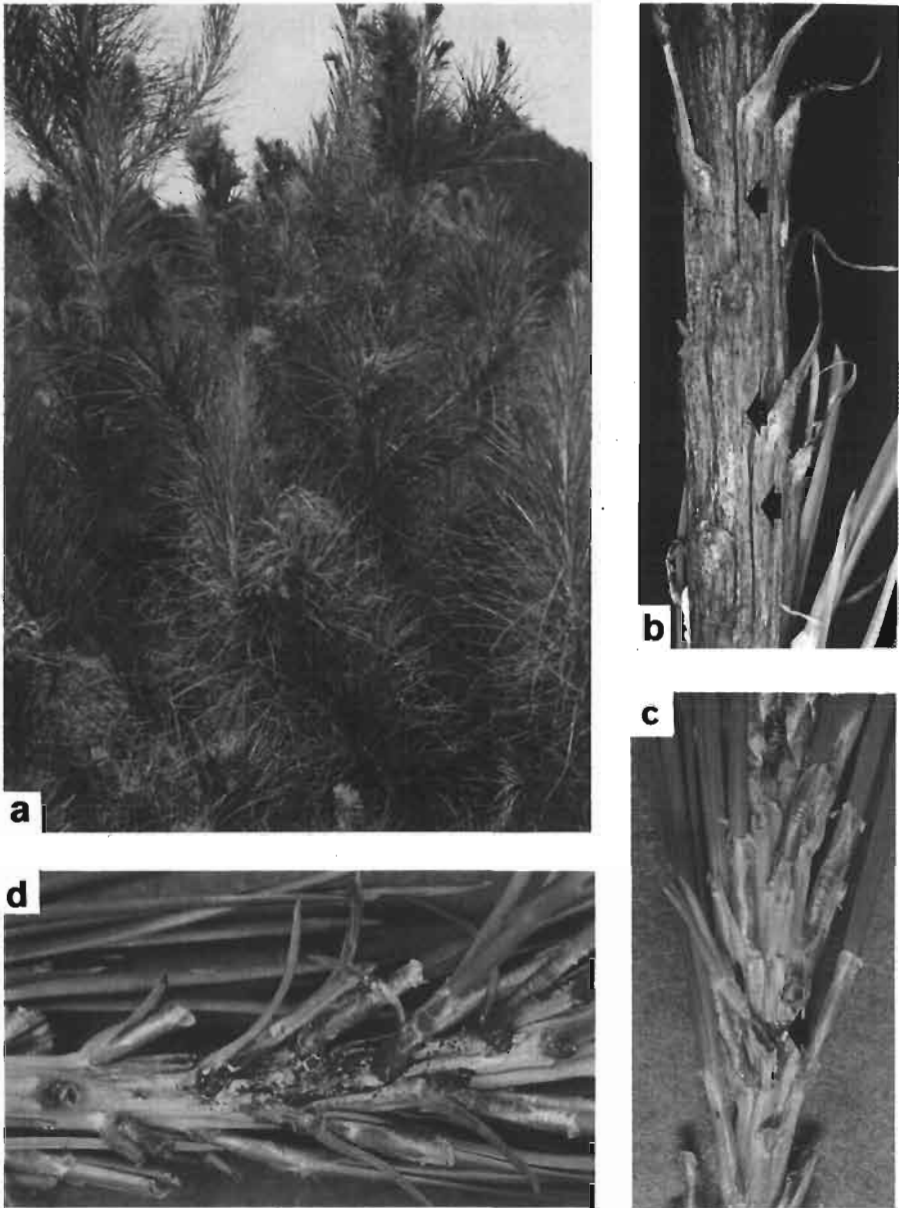


FIG. 1.—(a) Dieback of badly affected *P. radiata* (5-year-old tree) in Tarawera Forest. (b) Putative frost cracks (arrowed) and *S. pithyophila* pycnidia on dead *P. radiata* shoot, killed probably by a combination of these two. (c) Early stage of *D. pinea* infection of *P. radiata* shoot, with needles partly removed to show the semi-watersoaked necrotic lesion and dead needle fascicle. (d) Later stage of infection showing enlargement of lesion and emergence of *D. pinea* pycnidia.

examination of a kinked shoot often revealed such a concealed lesion. There was no indication that the lesions were associated with wounds.

The link between these lesions, *D. pinea*, and shoot dieback was evident from observations made November 1971-March 1972. During the periods 24 November 1971 to 5 January 1972, 5 January to 2 February, and 7 February to 3 March, three batches of approximately 20 shoots bearing small lesions (less than 1 cm long, and half the width of the stem) were marked and observed at weekly intervals. Out of a total of 64 shoots, 43 showed *D. pinea* pycnidia on the lesions from the outset or later in the period and, of these lesions, 10 were seen to enlarge and eventually girdle and kill the shoots. Pycnidia were not seen on the remaining 21 shoots so the lesions may or may not have been associated with the fungus as dieback developed in only 2 of them. On 36 of the shoots the lesions remained undeveloped, while in 14 they enlarged without killing the shoot during the observation period.

Seasonal Variation of Leader Dieback

Preliminary observations during 1971 and 1972 showed that leader dieback suddenly became rampant at the height of the spring growth flush, generally in late October-November. The dead leaders were soon replaced by laterals, and after February fresh occurrences of dieback were rarely seen. To establish how leader dieback was initiated, how it subsequently developed, and how it was affected by season and stage of shoot development, detailed examination of the leaders was carried out.

A ladder was used for tree-top examination of 30 trees selected from an area about 75 m × 50 m. One of every five trees along each row was chosen—selection determined mainly by the tree-top reached most conveniently with the ladder. When observations began eight of the selected trees had previously been badly affected, three were apparently free of leader dieback, and the rest had light-to-moderate dieback in shoots among the topmost branch clusters. At 1- or 2-week intervals each shoot in the topmost branch cluster of a tree was examined by hand lens for the occurrence and development of lesions, for dieback, for *D. pinea* pycnidia and any other readily recognisable fungal fructifications, and for wounds. In addition, the length of the leader was measured from the topmost branch cluster to the terminal bud and a note was made of the ripeness of the leader according to the appearance of the epidermis, whether green, yellowish green, or suberised. Observations started on 28 March 1973 and terminated in December 1973 when nearly 50% of the trees had leader dieback. This was as high an incidence as any observed within a comparable period. Meteorological data were provided by Tasman Pulp and Paper Co. from recorders in the Putauaki block.

The results of the examinations can be summarised briefly (Fig. 2). Development of leader dieback followed exactly the same course as previously seen in the dieback of young laterals in the lower crowns; it started with typical necrotic stem lesions, closely resembling those which had been associated with *D. pinea* infection. Lesions were seen most often on tender succulent green shoots which were elongating rapidly and had no visible wounds. On semi-mature shoot tissue (semi-suberised, yellowish green) lesions were present but their development appeared to be greatly restricted. Lesions were never seen on suberised stem tissue of the previous season's growth.

Although fresh infections, as evidenced by stem lesions and the subsequent development of dieback, could be observed throughout the year, there was a pronounced peak

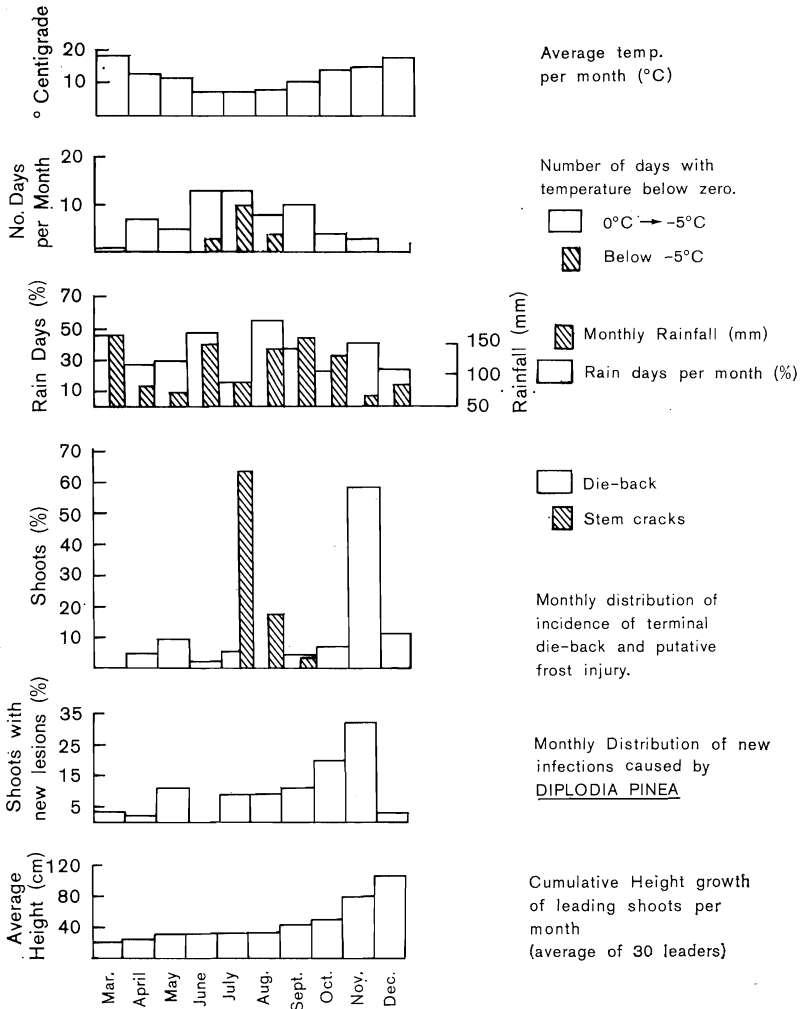


FIG. 2.—Seasonal variation in leader growth, terminal dieback, and incidence of new infections.

in new infections and shoot dieback in mid-to-late spring (October-November) when leader elongation was most rapid. The incidence of dieback in the 3-month period, October-December, accounted for two-thirds of all shoot dieback recorded in the 10-month period of observations.

Stem cracks or epidermal splits occurred mainly in July when leader growth was at a standstill (Fig. 1b). They were very noticeable after a period of severe frost. For example, heavy frosts occurred from 6-12 July, with ground frost temperatures down to -9.5°C , and the highest incidence of stem cracks was recorded on 17 July. Fresh stem cracks were also noted after a minimum temperature of -8.5°C on 8 August, and after frosty nights on 15-16 September. Most of the cracks occurred on the basal semi-

ripened region of the shoot, which had extended during the previous autumn. The cracks seemed to have little effect on subsequent shoot growth in the spring, and there was no indication that they served as entry points for *D. pinea* infection. When dissected, cracked shoots showed only superficial damage restricted to the outer cortical layer, and the wounds were evidently completely healed and suberised by mid-spring when *D. pinea*-associated dieback was most prevalent.

Isolations

Shoots showing different degrees of dieback and some with newly developed lesions were collected during each spring 1971-1973. On shoots with dieback, isolations were made at the junction of diseased and apparently healthy tissues (from the pith and, in a few cases, from the cortical tissue as well). On shoots with newly developed lesions, the affected areas were cut off the stem in 2- to 3-mm thick pieces, surface-sterilised in 3.5% sodium hypochlorite solution for 3-5 min, subdivided, rinsed thoroughly in sterile water, and plated out on 3% malt agar. The plates were incubated at 25°C and sporulation was induced by placing autoclaved pine needles on the fungus culture and exposing the plates to near-UV light (Commonwealth Mycological Institute, 1968).

Isolations were attempted from 18 shoots with newly developed lesions; *D. pinea* was recovered in every case—moreover, it was the only fungus isolated. Further isolations were attempted from 39 shoots showing symptoms of dieback; only 14 of the specimens yielded *D. pinea*, and no organisms were obtained from the remaining 25.

DISCUSSION AND CONCLUSIONS

Diplodia pinea was consistently associated with dieback during the various stages of development, and the invariable presence of the fungus in stem lesions—the earliest observable sign of dieback development—strongly suggests that *D. pinea* is the primary incitant of this dieback. The apparent absence of wounds associated with the initiation of lesions suggests that infection occurred through intact host tissue. A conspicuous feature was the infection and subsequent death of succulent green shoots of the current season's growth; yet, death of host tissue rarely extended beyond the top of the previous season's growth, and was often checked well above this point. Attempted isolation of fungi from the disease margin, generally presumed to be the margin of advance, often gave negative results and microscopic examination of this region frequently revealed dead host cells with no sign of fungal hyphae. This suggests that it may well be a margin of inhibition where the resistance mechanism of the host tissue checks the advance of the fungus. Overall, it seems that the more mature the host tissue, the more resistant it is to both infection and extended invasion by the pathogen.

The type of infection described is probably the same as that reported by Birch (1936) within the category "red-top", by Purnell (1957) as "shoot blight", and by Peterson and Wysong (1968) as "tip blight". It was also reported in detail by Waterman (1943).

The reason for the high incidence of dieback in the area studied is beyond the scope of this paper, but there was no evidence to suggest that it was connected with predisposing factors such as wounding. Dieback could apparently be associated with wounding and subsequent *S. pithyophila* infection, but only in a minority of cases.

Earlier concern that the condition might spread to adjacent young stands has proved

unfounded. Severe dieback has remained confined to the Putauaki block, and trees in the surrounding blocks are most likely out of danger now as those over 10 years old are seldom severely affected (R. B. Lawrence, pers. comm.).

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