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Field performance of progenies of *Pinus radiata* selected for resistance to Diplodia-associated shoot dieback

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Abstract

Background: A severe outbreak of Diplodia-associated dieback of *Pinus radiata* D.Don shoots in 1967 prompted a pilot programme of selection for resistance to dieback. Plus trees were selected in 1970 for absence of dieback, growth and tree form, and seed collected.

Methods: Twenty open-pollinated progenies plus two control lots were field-tested on two sites, and assessed around 6.5 years after planting. Alternative measures of shoot dieback were recorded, as were dbhob and scores for growth and form variables. Data were analysed for seedlot differences, heritability estimates and various genetic correlations.

Results: Dieback at one site allowed good resolution of progeny differences, whatever the dieback measure. Remarkably, no response to field selection for resistance was evident, unlike in a glasshouse inoculation trial. Nor did progeny rankings correlate with those in the inoculation trial. Between the trial sites no clear seedlot rank changes were evident for any trait.

Conclusions: The disparity with results from the inoculation trial is unexplained, although a role of endophyte status is postulated. Together with non-recurrence of past dieback outbreaks, the disparity means that selection for field resistance is not promising.

Keywords: *Diplodia pinea*; disease resistance; genetic correlation; heritability; radiata pine; selection response; shoot dieback.

Introduction

Epidemics of shoot dieback in *Pinus radiata* D.Don have been recorded in the North Island of New Zealand (Burdon 2011). One such epidemic, noted in 1967, involving hollows in Tarawera Forest near Kawerau, was locally spectacular, although lower incidences of leader dieback had been common in young *P. radiata* plantations nearby. *Diplodia pinea* (Desm.) Kickx (*Sphaeropsis sapinea* (Fr.) Dyko & B.Sutton) ('Diplodia') was consistently implicated as a causative pathogen (Chou 1976a, b). Concern was heightened by the fact that it involved the potentially most valuable part of the tree, namely the butt log. This prompted a pilot programme to study the prospects of breeding for resistance to Diplodia-associated dieback. It began with 'plus-tree' selection for resistance (Burdon et al. 1982), choosing trees of good vigour and form in addition to minimal signs of dieback.

Two studies were undertaken of response to selection, using open-pollinated progenies of the select trees: a glasshouse inoculation trial (Burdon et al. 1982), and a field progeny trial which is the topic of this paper.

In the inoculation trial, the open-pollinated progenies of the select trees showed better resistance overall to Diplodia spore suspensions compared with controls. Individual progenies ranged from being substantially more resistant than controls to being marginally and non-significantly worse. As a group, however, the progenies were markedly superior to the controls for resistance. These results met classical expectations for response to intensive selection for a continuously varying trait of modest but worthwhile heritability. The overall rates of infection and dieback were 72% and 39% respectively, which the results of Sohn and Goddard (1979) suggest would have given near-optimal resolution of progeny differences.

The field progeny trial was replicated on two sites where considerable dieback was expected. Both were subject to combinations of high temperatures and high humidity. One was close to Fenton's Mill Flat, on a river flat. The other was in a locality where shoot dieback was often prevalent although not dramatic. The trial served not only as a test of selection response under field conditions but also as a methodological study for scoring shoot dieback incidence and analysis of the data.

The results of this trial, which are reported in this paper, were strongly counter to expectations. Unlike in the glasshouse trial, they were effectively negative in that progenies overall were no more resistant than controls. And, while they were positive in respect of progenies showing differences in resistance, they were negative insofar as progeny resistance was negligibly correlated between the field and the glasshouse trials. Because of these results, and other factors (Burdon 2011), the programme was abandoned, but the results are reported as a record of research done. Although the work was done and reported internally in 1980 (Supplementary document 1), and the primary data are no longer available, this paper meets the general obligation to publish any substantial study whether the results are positive or negative. This obligation is deemed to be accentuated by the apparently substantial conflict with the already published results of the greenhouse trial (Burdon et al. 1982).

Methods

Material

The seed parents were in a *P. radiata* stand at Fenton's Mill Flat in Tarawera Forest near Kawerau, New Zealand (Lat. 38°08' S, Long. 176°38' E; ca 65 m a.s.l.; mean annual precipitation ca 1750 mm). The site was an enclosed flat, often isolated from sea breezes, and often experiencing both high temperatures and high humidity. In 1967, after five years from planting, the stand had started to suffer a very high incidence of shoot dieback of a type associated with Diplodia (Chou 1976a; Burdon et al. 1982). Almost every tree had multiple shoots affected, with very few having leaders unaffected. Twenty-eight parents were intensively selected (ca. 1 : 5,000) in 1970, for vigour and tree form, in addition to minimal signs of dieback. Open-pollinated seed was then collected from each parent and extracted, with 20 parents producing enough seed for the field progeny trial. The progenies were deemed to be approximately half-sib families.

In 1975, a fresh seed collection was made from the select parents for the glasshouse inoculation trial (Burdon et al. 1982; Supplementary document 2), together with collection of cortical oleoresin samples from ramets of the selected parents (cf Burdon et al. 1992).

Two *P. radiata* control lots were included in the field trial. One (SO) was from the Gwava Seed Orchard, with a Growth and Form Improvement Rating (Vincent 1987) of 14. The other (Bulk) was from a 'bulk' seed collection from Kaingaroa Forest with a Rating of 1, and was one of the controls used by Burdon et al. (1982). Included as

an outgroup was a seedlot of 'blue' *Pinus muricata* D.Don (Mendocino County, CA, USA origin).

Seedlings of the 23 lots were raised in the New Zealand Forest Research Institute nursery at Rotorua as bare-rooted stock, and planted out on two nearby high-index sites of similar rainfall where a fairly high incidence of Diplodia-associated shoot dieback was expected.

Trial sites and field layouts

One of the sites was a river flat in Tarawera Forest (Lat. 38°07' S, Long. 176°40' E; ca 50 m elevation), with a site index (van der Colff & Kimberley 2013) of nearly 40 m. As a river flat, it belonged to a site category that was widely associated in the region with high incidence of stem malformation in *P. radiata*, which presumably resulted from prevalent leader dieback (Burdon 2011). The other site was in northern Kaingaroa Forest (Lat. 38°16' S, 176°42' E; ca 400 m elevation), on undulating terrain with a site index of nearly 35 m. In that locality shoot dieback was prevalent in the hollows and otherwise at nuisance levels. Of the 20 *P. radiata* progenies, five were not represented in Tarawera Forest.

Field layouts were randomised complete block, with 12 replicates of eight-tree row plots at each site. Some minor imbalance of classification arose at Kaingaroa from shortages of seedlings of some progenies. All progeny plots had the full eight seedlings, the shortages leading to a few missing progeny plots that were made good with supplementary plots of controls.

Assessment

The trial was assessed at around 6.5 years from planting, in March 1978. Assessment was primarily for shoot dieback incidence, and was done while the crowns were still sufficiently visible for shoot dieback to be mostly recognised as dead shoots in the outer crown. On each tree, counts were made of visible dieback occurrences, definite and doubtful, on the leader and laterals, respectively. Other traits assessed were: stem diameter at breast height (1.4 m) over bark ("dbhob" in mm); stem straightness score ("Str", 1 = very crooked to 9 - very straight); branch habit quality ("Bran", 1 = heavy, rough and irregular to 9 - light, regular and with short intervals between clusters); and malformation ("Malf." 1 = multileadered to 9 = no malformation).

Data analysis

Preliminary analyses converted the dieback counts into alternative individual-tree scores (Table 1), partly as a study of alternative assessment methods. For each tree, occurrences of dieback were recorded on both leader and laterals, distinguishing between definite and doubtful cases. These records were used with various weightings to derive alternative composite ("Dbk") scores (Table 1) for individual trees.

Because malformation scores and composite dieback scores showed strongly non-normal distributions (Supplementary document 1, Table 3), alternative values were used for subsequent data analysis: (i) the raw values; and (ii) normalising transformations. The transformations were aimed at converting the raw

TABLE 1: Derivation of dieback variables, with boldfacing denoting variables for which detailed results are presented in this paper. Results for the remaining variables are in Supplementary document 1.

Dieback variable	Weighting (w) for each occurrence				Algorithm/conditions	
	Leader		Laterals			
	Definite	Doubtful	Definite	Doubtful		
Dbk1	1	0	0	0	w = 0 or 1	
Dbk3	2	1	0	0	Σw	
Dbk4	2	1	0	0	$(\Sigma w)^{0.65}$ Whole-plot basis	
Dbk5	0	0	2	1	Σw	
Dbk6	0	0	3	1	$(\Sigma w)^{0.65}$	
Dbk7	10	5	2	1	Σw	
Dbk8	10	5	2	1	$(\Sigma w)^{0.65}$	
Dbk9	5	0	1	0	$(\Sigma w)^{0.65}$	

composite scores, across both sites, into class intervals corresponding to the percentile intervals in a normal distribution (cf Gianola 1982). For each malformation score and multinomial dieback score, this was achieved by deriving the value $(a + x)^b$, where x is the raw value, a and b being empirically chosen values to give roughly the desired class intervals ($a = 0, b \sim 0.65$) (Table 1). Success in thus normalising distributions, however, was limited (Supplementary document 1, Table 3).

All variables for the *P. radiata* material were subjected to analysis of variance (ANOVA) for individual sites. Effects in the classification were: replicates (deemed fixed), seedlots (random), replicates \times seedlots (random, deemed to be plot-environment effects), and the within-plots residual (random). Separate analyses were conducted for each site. At Tarawera, the Method of Unweighted means was used, using mean squares based on plot means in combination with within-plot mean squares. Thereby, tests for significance of effects and variance component estimates were made. The approximations entailed in that method were very minor, what with 91% effective survival (assessable trees) overall. For Kaingaroa, with 92% effective survival but some additional imbalance in the classification, Henderson's Method 1 (Searle 1968) was used, treating all effects as random. Thereby, expectations of mean squares and variance component estimates were obtained, and only minor bias in *F*-tests was indicated. Since replicates were inherently a fixed effect, creating reservations concerning Method 1, results were cross-checked with a least-squares analysis, showing low sensitivity of results to whether replicates was deemed fixed or random.

At both sites, the random-effect variances were estimated: seedlots (σ^2_{β}), replicates \times seedlots interaction (σ^2_{rf}) and the within-plots residual (σ^2_w). Within-site narrow-sense heritabilities (h^2_s) for individual variables were estimated according to the relationship:

$$h^2_s = 4\sigma^2_f / (\sigma^2_f + \sigma^2_{rf} + \sigma^2_w) \quad (1)$$

the statistical significance being that of the seedlots (progenies)¹ effect. For the heritability estimate of the binary (0 or 1) variable, leader dieback, a correction to that for a continuous underlying scale of susceptibility was used (van Vleck 1972).

The heritability (or repeatability) of progeny means (h^2_{fs}) for a variable was calculated according to the relationship:

$$h^2_{fs} = \sigma^2_f / (\sigma^2_f + \sigma^2_{rf} / 12 + \sigma^2_w / 96) \quad (2)$$

if there were no imbalance in the classification resulting from missing subclasses or trees. In practice, this is given by $(F - 1)/F$, F being the *F*-ratio in testing for progeny differences, which entailed slight approximation with the minor imbalance.

ANOVA and estimation of heritability across sites are not addressed here because of data properties resulting from very different rates of dieback at the two sites. Instead, Type B genetic correlations, between expression of the same variables at the two sites (Burdon 1977), were estimated as a complement to within-site results, such correlation analysis being very robust with respect to the data properties.

Genetic correlations (r_{gxy}) between variables x and y were estimated according to the relationship

$$r_{gxy} = \text{Cov}_{gxy} / (\sigma_{fx} \sigma_{fy}) \quad (3)$$

where Cov_{gxy} denotes the progenies covariance between the variables, and σ^2_{fx} and σ^2_{fy} denote the corresponding variances for the two variables. Covariances were estimated from cross-products analogously to estimation of variances from mean squares. The following genetic correlations were estimated: between variables (Type A genetic correlations – Burdon 1977) within each site: between the expression of the same variable between the

¹ For this, and in connection with the following equations, controls were grouped with progenies, but that could not have caused appreciable bias, except with variables for which the progenies as a group differed materially from controls.

sites (Type B genetic correlations – *op. cit.*); and between variables expressed in the field and in the glasshouse inoculation trial (Burdon et al. 1982) (also Type B genetic correlations). The Type B genetic correlation estimates ($r_{g_{tk}}$) were actually calculated according to the equivalent relationship

$$r_{g_{tk}} = r_{ptk} / (h_{ft}^2 h_{fk}^2) \quad (4)$$

where $r_{g_{xy}}$ denotes the correlation between progeny means for the variables in the respective trials (denoted t and k), the statistical significance of $r_{g_{tk}}$ being that of r_{ptk} and h_{ft}^2 and h_{fk}^2 denote the repeatabilities of means for a variable at the respective sites. Deriving the denominator factors was complicated by some items in the classification for the inoculation trial being arguably either fixed or random effects, but the observed values of r_{ptk} meant that choice between the alternative assumptions could not materially affect the answers.

Results

Incidence of dieback (Tables 2 and 3) was less than expected, especially in Kaingaroa Forest (Table 3), where also growth was clearly slower. However, it was enough at Tarawera to allow good resolution of family differences (Table 2). At Tarawera, all derived dieback variables showed progeny differences and heritabilities that were statistically highly significant ($p < 0.01$), unlike at Kaingaroa where dieback incidence was low. The transformations of the dieback variables did not materially affect heritability estimates, so results for transformed variables are not shown, except those for malformation. Nor did the alternative dieback variables

differ appreciably in estimated heritability, except for a lower raw value for leader dieback. However, adjusting its narrow-sense heritability estimate to a value for continuously varying underlying susceptibility raised the estimate from 0.08 to 0.30.

At Kaingaroa, however, there were no statistically significant differences among progenies for any dieback variable (with a marginal exception for Dbk1, leader dieback), the estimated heritabilities being very low (<0.025). Adjusting the narrow-sense heritability estimate for leader dieback for continuously varying susceptibility raised the value from 0.024 to 0.09.

For the growth and form variables at both sites differences among the progenies, and therefore the heritabilities, were statistically significant throughout ($p < 0.001$), meaning good resolution of progeny differences even though the narrow-sense heritability estimates were low to moderate.

With the low incidence of dieback at Kaingaroa, across-sites heritability estimates were of less interest than whether there were any indications ($r_{g_{xy}} < 1$) of rank changes between the sites. Accordingly, results from across-sites analysis of variance were not pursued. The Type B genetic correlation estimates, between sites for each trait, although mostly imprecise, were generally around +1 (Table 4), none approaching any statistically significant departure from +1. This indicates a complete lack of rank-change genotype-environment interaction for any of the variables studied.

Comparing the control lots with the select progenies as groups for the dieback variables, the latter showed no overall superiority, the differences all being trivial. Comparing the two control lots (SO and bulk), while the bulk showed less dieback at both sites than the seed

TABLE 2: Ranges of means of progenies and means of the two control lots and overall, with heritability estimates, for individual variables at Tarawera.

Category/statistic	Variable						
	Dbhob (mm)	Str (1-9)	Bran (1-9)	Malf [†] (transf.)	Dbk1 (0-1)	Dbk5 (score)	Dbk7 (score)
Progenies							
Max.	173	6.28	5.73	0.80	0.50	9.76	13.40
Mid.	162	5.71	5.05	-0.05	0.34	6.10	9.46
Min.	151	5.14	4.37	-0.90	0.19	2.45	5.51
SO	166	5.58	5.09	0.38	0.34	2.96	7.99
Bulk	160	5.28	4.95	-0.07	0.43	4.53	10.42
All lots	161.9	5.79	5.08	0.17	0.36	3.82	8.77
LSD [§]	8.29	0.58	0.51	0.68	0.14	1.74	2.93
\hat{h}^2	0.15	0.20	0.15	0.11	0.08	0.14	0.13
\hat{h}_f^2	0.77	0.78	0.78	0.67	0.64	0.67	0.72
p	***	***	***	***	**	***	***

[†]denotes normalising transformation.

[§]Least significant difference, $p < 0.05$.

** denotes highly significant $p < 0.01$; *** very highly significant, $p < 0.001$, for h^2 and h_f^2 .

NOTE: \hat{h}^2 confidence limits will be strongly asymmetric (skew positive) with small numbers of progenies and low point estimates.

TABLE 3: Ranges of means of progenies and means of the two control lots and overall, with heritability estimates, for individual variables at Kaingaroa

Category/statistic	Variable						
	Dbhob (mm)	Str (1-9)	Bran (1-9)	Malf [†] (transf.)	Dbk1 (0-1)	Dbk5 (score)	Dbk7 (score)
Progenies							
Max.	159	6.29	5.54	1.37	0.175	2.26	3.49
Mid.	150	5.40	4.72	0.76	0.098	1.21	2.21
Min.	141	4.51	3.90	0.16	0.022	0.16	0.93
SO	158	5.62	4.88	1.19	0.127	0.79	2.21
Bulk	149	4.84	4.69	0.60	0.098	0.77	2.06
All lots	151	5.40	4.72	0.80	0.097	1.00	2.25
LSD [§]	8.69	0.50	0.48	0.089	-	-	-
\hat{h}^2	0.11	0.25	0.23	0.09	0.025	0.024	0.015
\hat{h}^2_f	0.63	0.82	0.83	0.60	0.35	0.22	0.17
<i>p</i>	***	***	***	***	≥ 0.05	NS	NS

[†] denotes normalising transformation.

[§] Least significant difference, *p* < 0.05.

NS denotes not significant, *p* > 0.05; *** very highly significant, *p* < 0.001.

NOTE: \hat{h}^2 confidence limits will be strongly asymmetric (skew positive) with small numbers of progenies and low point estimates.

orchard lot (Tables 2 and 3), no statistically significant difference was evident. For growth and form variables, however, the seed orchard (SO) was consistently superior to the bulk. Most of these differences were statistically significant (LSD, *p* < 0.05), although the differences were marginal in respect of branching score.

Estimation of Type B genetic correlations between progeny dieback in the field and the glasshouse inoculation trial (Burdon et al. 1982) is complicated by some experimental design effects in the glasshouse being arguably either fixed or random, creating uncertainty as to exact values of \hat{h}^2_{fx} and \hat{h}^2_{fy} in Eq 4. Irrespective of the assumption used, the correlations of progeny means (r_{pxy}) for dieback variables between the field and the inoculation trial were consistently weak. Indeed, none of these correlations (phenotypic or genetic) even approached statistical significance, a few more actually being weakly negative than were positive (Supplementary document 1, Table 15). At Tarawera, where progeny differences were quite well expressed, the progenies showing extremes of infection and dieback in the glasshouse were well represented. This argues against any statistical artifact of correlations being depressed by missing progenies there. Type A genetic correlation estimates, between traits within the same site(s) (details in Supplementary document), are of limited interest in such a small study

population. However, consistently negative correlations were observed at Tarawera for level of leader dieback with dbhob (-0.23), straightness (-0.54), branching habit (-0.62) and malformation (-0.75). Type A genetic correlation estimates among different dieback variables there were all very close to +1.

The *P. muricata* seedlot, which was excluded from the main data analysis (Supplementary document 1), was much slower growing. For dbhob, it averaged 60% and 23% less than for *P. radiata* at Tarawera and Kaingaroa respectively. Based on Least Significant Differences between individual *P. radiata* lots (Tables 2 and 3) these species differences point to *p* → 0. It also had somewhat more dieback than *P. radiata* at both sites (details not shown), pointing on a similar basis to *p* < 0.05 to < 0.001 at Tarawera depending on the dieback variable, but *p* > 0.05 throughout at Kaingaroa. However, its incidence of needle cast, while not formally scored, was clearly much less than in the *P. radiata*.

Discussion

It was assumed that the observed dieback was essentially all associated with Diplodia infection, the basis for this being necessarily circumstantial (cf. Chou 1976a).

The absence of some progenies at the site (Tarawera) that gave better resolution of progeny differences was unfortunate, but did not obscure a definite but

TABLE 4: Estimates of Type B genetic correlations, between sites, for selected variables

Dbhob	Str	Bran	Malf	Dbk1	Dbk5	Dbk7
0.98	1.22	1.24	0.96	0.65	1.04	1.09

unexpected picture. The number of progenies was inadequate, especially at Tarawera, for reasonably precise estimates of heritabilities and between-trait (Type A) genetic correlations. To a lesser extent, the select nature of the progenies' seed parents could bias genetic parameter estimates; however, such an effect is unlikely to have been important given that much the most intensive selection was for dieback resistance which showed no selection response. Anyway, there were enough progenies to give a realistic test of response in field conditions to selection for dieback resistance, especially in the light of the inoculation trial results (Burdon et al. 1982).

Regarding the alternative measures of dieback incidence, as a methodological study, there was generally little to choose between the alternatives tried. The binary scoring of leader dieback gave the lowest crude heritability estimate, but the available correction of binary-data heritability estimates, to values for continuously varying underlying susceptibility, gave higher estimated heritability than for the other dieback variables. The data transformations tried had no material effect on heritability estimates compared with using untransformed data, despite far from ideal distributions.

The results of this field progeny trial were highly counterintuitive. While agreement between laboratory-based and field trials is routinely in question, the default expectation is that field-trial results, if at all precise, would show the clearer response to field selection. Yet, despite good resolution of progeny differences in dieback at Tarawera, quite the opposite was the case.

Reasons for this disparity are unclear. However, problems with execution of the trials seem unlikely. Substantial misidentification of lots in the nursery, while they cannot be totally excluded, seems very unlikely. If misidentification did occur it evidently affected both sites co-equally, judging from the estimated Type B genetic correlations ($\sim +1$) between the sites. Even if some misidentification did occur, it appears unlikely to have affected more than a few specific replicates, judging from the resolution of progeny differences and typical values for estimated heritabilities of DBH and scores for straightness, branch habit and malformation (cf. Wu et al. 2008). Moreover, the performance of the two control lots relative to each other for these traits generally fits expectations. Admittedly, some of the statistical contrasts are weak, but it was early days for selection response for these growth and form traits to become clear. Indeed, whatever selection was done for growth rate in the parents was likely to have been too early to be optimal. Whatever the situation, the lack of superior field dieback resistance in the select-parent progenies overall was clear. Bias in heritability estimates resulting from grouping controls with progenies must therefore be negligible for the dieback traits, and could not be major for the growth and form traits.

Attempting to explain the results biologically is complicated by the fact that Diplodia can attack *P. radiata* in various situations, although damp heat has been consistently implicated as a strongly predisposing factor (e.g. Scott 1960; Eldridge 1961; Chou 1978; Burdon et

al. 2017). Beyond that, the recognised circumstances for Diplodia attack generally fall into three categories that may define main shoot dieback types for *P. radiata*:

1. New, unripened shoot growth being attacked in the absence of wounds (Chou et al. 1976b; 1978), which appears to have been generally the case in this study;
2. Fresh wounds being attacked, most notoriously following severe hailstorms that often occur in summer-rainfall areas (Scott 1960). It has also been observed that pruning off green branches of *P. radiata* in summer incurs a greatly increased risk of Diplodia-associated stem cankers (Chou & McKenzie 1978); and
3. Paradoxically, as in "autumn brown top" with Diplodia attack following severe drought stress (Wright & Marks 1970; Chou 1987), shoot dieback occurring only after some rain at the end of a highly stressing drought (Wright & Marks 1970).

Also, as trees get older and taller they undergo maturation, with changes in shoot morphology and anatomy, and with shoot phenology becoming more strongly seasonal (Burdon 1994), while they grow upwards from the ground-level microclimate. Moreover, there are both year-to-year fluctuations in severe climatic events and general climatic change. Furthermore, there are possibly important differences in pathogen strains.

Even if genetic variation in resistance/susceptibility is genuine, there is no guarantee that genotypic rankings for it are consistent across all circumstances, viz general dieback types (as above), more specific effects of climatic events, different pathogen strains, influences of tree ages. The question becomes one of whether, and to what extent, the overall incidence and genotypic rankings for resistance or susceptibility are influenced by the above factors, or some additional ones.

There cannot be certainty that types of dieback were the same either between the two studies or between Fenton's Mill Flat (FMF) and the field test sites. Yet, even if there were differences, they would only generate conflicting results if genotypic rankings were type-dependent. The most likely case of type specificity involving such rankings would be Type 3, in which differential drought tolerance would affect susceptibility rankings. However, in our study drought stress is an unlikely factor, given the high, evenly distributed rainfall in the region, and the high moisture-holding capacity of pumice soils observed by Will and Stone (1967).

Despite differences in maturation state of material existing between the glasshouse and both FMF and the test sites, the glasshouse results which involved the youngest material generally accorded with the field selection in older material in FMF.

Differences in pathotype strains affecting genotypic rankings for resistance cannot be tested on the material studied. The inoculum for the glasshouse trial and in the field would have been different, but not necessarily in pathogenicity.

Inferring impacts of climate change on dieback incidence, between past rotations and in future, is very problematic. Rising temperatures, and more extreme climatic events, would appear to favour increasing *Diplodia* attack, which does not fit with the evident decreasing trend in time (Burdon 2011). However, more extreme climatic events could dominate the incidence of attack — and even the types — depending on tree ages or heights.

A tentative explanation of the apparent conflict in results has been offered in terms of a suggested role of endophyte status (Burdon 2011). Indeed, the potential significance of trees' microbiome is being increasingly acknowledged (e.g. Wakelin 2018). Burdon suggested for *P. radiata* that acquisition of bioprotective endophyte strains occurring over time in the field could account for the non-recurrence of severe dieback outbreaks after the first rotation in the field. At least implicit in the suggestion was the postulate that nursery stock tended to be depauperate of bioprotective endophytes (or even phylloplane microorganisms), meaning increased susceptibility to certain pathogens. Also, while not actually stated, this could reflect poor transmission in seed and/or ill-effects of some poor temperature control in past seed extraction from cones. In this last connection, a recognised treatment of seed to eliminate unwanted endophytes in grasses is heating, but not enough to kill the seeds (e.g. Siegel et al. 1984; Pedersen & Sleper 1998). Burdon (2011) also postulated that with the passage of time in the field, endophyte populations would somehow spontaneously recover. Indeed, pollen grains are now a suspected pathway for endophyte transmission (S.A. Wakelin, pers. comm. 2022)².

However, given that the glasshouse trial seed was actually collected three years after that for the field trials, and yet the glasshouse trial showed a much better response to field selection, the hypothesis of progressive accretion of protective endophytes does not account for our particular results.

Differences in pollen clouds between the two seed collections seem an unlikely explanation. Even if genuine, they would not automatically explain rank changes among progenies and controls between the two studies. Moreover, pollen cloud differences seem unlikely, given that in *P. radiata* onset age of pollen production is earlier and more uniform among individuals than onset of female flowering.

If the field behaviour of *Diplodia* largely reflects climatic events in particular years, the dieback incidence within the effective lifetime of a field trial would depend on year of establishment. Robust characterisation of genetic resistance could then depend on replication in planting year — as well as in place — of field tests. That, however, could require prohibitive effort. And even if achieved it would still not assure robust protection of deployed material against effects of year-to-year fluctuations in infection conditions.

² A review by Frank et al. (2017) cites five studies, using surface-sterilised pollen grains, pointing to vertical transmission of beneficial bacterial endophytes in *Pinus* spp.

The largely negative results of the field trial, whatever the reasons, argued strongly against continuing the selection programme — and provide a cautionary tale. But there were additional, and probably more decisive, reasons for abandoning the programme. As mentioned earlier, the high incidence has not been repeated in the second rotation on the same sites (Burdon 2011). This was especially notable in 'Death Valley' (Chou et al 1976a), an enclosed hollow at the foot of Mt Edgecumbe (Putauaki) in Tarawera Forest, where *Diplodia*-associated dieback was even more extreme and persistent than in Fenton's Mill Flat. There an area was felled very early to make way for tests of additional selections for dieback resistance. However, the dieback incidence in those tests was too low to produce promising data. Also, the elapse of time was too little to point to progressive climatic change as causing the difference. Moreover, provided stands are well stocked, the lasting impact of such dieback on tree form and growth can be surprisingly limited. The Fenton's Mill Flat stand later showed little outward sign of the alarming level of dieback. Even in Death Valley, tree form could recover remarkably (Fig. 1 in Burdon 2011), although loss of height growth and increment would have been considerable.

Regarding alternative bases for possible *Diplodia* resistance, past reports (D.R. Smith pers. comm. 1967; Burdon & Bannister 1973) had suggested an association between carene content in cortical oleoresin and resistance to *Diplodia* infection. Chemical and statistical analyses conducted in connection with this study (Supplementary document 1, Tables 16, 17) produced no evidence for such an association. The occasional statistically significant correlation between a monoterpene fraction in parent trees and progeny dieback incidence could easily be attributed to random chance (Bonferroni correction). However, the evident unreliability of field progeny performance as a measure of general resistance prevents firm conclusions.

The comparative behaviour of *P. muricata*, with slower growth but much less needle cast, was typical of when it was included as an outgroup in *P. radiata* trials (e.g. Ades et al. 1992).

Conclusions

Reasons for the negative results in the field progeny trial are unknown, although an explanation is suggested. On the basis of these results, and subsequent low incidence in the field, breeding for resistance to *Diplodia*-associated shoot dieback has proved very unpromising.

Competing interests

None

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Authors' contributions

RDB designed the study and did the write-up. CBL participated in the field assessment and did the data analysis. The authors are agreed on the content.

Additional files

Supplementary document 1 – Detailed archival report on field trial:

Burdon, R.D., & Low, C.B. (1980). First assessment of field progeny trial of selections of *Pinus radiata* for resistance to *Diplodia* infection. *Internal Report No. 187*. Rotorua, New Zealand: Forest Research Institute. 45 p. <https://nzjforestryscience.nz/index.php/nzjfs/article/view/217/80>

Supplementary document 2 – Detailed archival report on glasshouse inoculation trial:

Burdon, R.D., Chou, S. & Currie, D. (1976). Response to inoculation with *Diplodia pinea* in progenies of apparently resistant trees of *Pinus radiata*. *Internal Report No. 122*. Rotorua, New Zealand: Forest Research Institute. 32 p. <https://nzjforestryscience.nz/index.php/nzjfs/article/view/217/81>

References

Ades, P.K., Simpson, J.A., Eldridge, K.G., & Eldridge, R.H. (1992). Genetic variation in susceptibility to *Dothistroma* needle blight among provenances and families of *Pinus muricata*. *Canadian Journal of Forest Research*, 22, 1111–1117. <https://doi.org/10.1139/x92-147>

Burdon, R.D. (1977). Genetic correlation as a concept for studying genotype-environment interactions in forest tree breeding. *Silvae Genetica*, 39, 168–175.

Burdon, R.D. (1994). Annual growth stages for height and diameter in *Pinus radiata*. *New Zealand Journal of Forestry Science*, 24, 11–17. https://www.scionresearch.com/_data/assets/pdf_file/0008/59687/NZJFS2411994BURDON11_17.pdf

Burdon, R.D. (2011). Shoot dieback epidemics in *Pinus radiata* in New Zealand: is there an endophyte story? *New Zealand Journal of Forestry Science*, 41, 131–139. https://www.scionresearch.com/_data/assets/pdf_file/0003/58773/NZJFS412011131_139-BURDON.pdf

Burdon, R.D., & Bannister, M.H. (1973). Provenances of *Pinus radiata*: Their early performance and silvicultural potential. *New Zealand Journal of Forestry*, 18, 217–232.

Burdon, R.D., Currie, D., & Chou, C.K.S. (1982). Responses to inoculation with *Diplodia pinea* in progenies of apparently resistant trees of *Pinus radiata*. *Australasian Plant Pathology*, 11(4), 37–39. <https://doi.org/10.1071/APP9820037>

Burdon, R.D., Gaskin, R.E., Low, C.B., & Zabkiewicz, J.A. (1992). Clonal repeatability of monoterpene composition of cortical oleoresin of *Pinus radiata*. *New Zealand Journal of Forestry Science*, 22(2-3), 299–305. https://www.scionresearch.com/_data/assets/pdf_file/0007/59821/NZJFS222_31992BURDON299_305.pdf

Burdon, R.D., Libby, W.J., & Brown, A.G. (2017). *Domestication of Radiata Pine*. Springer International, Forestry Sciences Volume 83. <https://doi.org/10.1007/978-3-319-65018-0>

Chou, C.K.S. (1976a). A shoot dieback in *Pinus radiata* caused by *Diplodia pinea*. I. Symptoms, disease development, and isolation of pathogen. *New Zealand Journal of Forestry Science*, 6, 72–79. https://www.scionresearch.com/_data/assets/pdf_file/0011/58934/NZJFS611976CHOU72-79.pdf

Chou, C.K.S. (1976b). A shoot dieback in *Pinus radiata* caused by *Diplodia pinea*. II. Inoculation studies. *New Zealand Journal of Forestry Science*, 6, 409–420. https://www.scionresearch.com/_data/assets/pdf_file/0008/58895/NZJFS631976CHOU409-420.pdf

Chou, C.K.S. (1978). Penetration of young stems of *Pinus radiata* by *Diplodia pinea*. *Physiological Plant Pathology*, 13(2), 189–192. [https://doi.org/10.1016/0048-4059\(78\)90033-4](https://doi.org/10.1016/0048-4059(78)90033-4)

Chou, C.K.S. (1987). Crown wilt of *Pinus radiata* associated with *Diplodia* infection of woody stems. *European Journal of Forest Pathology*, 17, 398–411.

Chou, C.K.S., & McKenzie, M. (1988). Effect of pruning intensity and season on *Diplodia pinea* infection of *Pinus radiata* stems through pruning wounds. *European Journal of Forest Pathology*, 18, 437–444. <https://doi.org/10.1111/j.1439-0329.1988.tb00233.x>

Eldridge, K.G. (1961). Significance of *Diplodia pinea* in plantations. Victoria Forestry Commission, *Forestry Technical Papers*, 7, 16–22.

Frank, C.A., Saldierna Guzmán, J.P., & Shay, J.S. (2017). Transmission of bacterial endophytes. *Microorganisms*, 5(4), 70 (21 p.). <https://doi.org/10.3390/microorganisms5040070>

Gianola, D. (1982). Theory and analysis of threshold characters. *Journal of Animal Science*, 44(5), 1079–1096. <https://doi.org/10.2527/jas1982.5451079x>

Pedersen, J.F., & Sleper, J.T. (1988). Considerations for breeding endophyte-free tall fescue forage cultivars. *Journal of Production Agriculture*, 1(3), 127–132. <https://doi.org/10.2134/jpa1988.0127>

Scott, C.W. (1960). *Pinus radiata*. FAO, Rome.

Searle, S.R. (1968). Another look at Henderson's methods of estimating variance components. *Biometrics*, 24, 749–787. <https://doi.org/10.2307/2528870>

Siegel, M.R., Varney, D.R., Johnson, M.C., Nasmith, W.C., Buckner, R.C., Bush, L.P., Burrus II, P.B., & Hardison, J.R. (1984). A fungal endophyte for tall fescue: evaluation of control methods. *Phytopathology*, 74, 937–941. <https://doi.org/10.1094/Phyto-74-937>

Sohn, K.-I., & Goddard, R.E. (1979). Influence of infection percent on improvement of fusiform rust resistance in slash pine. *Silvae Genetica* 28, 173–180.

van der Colff, M., & Kimberley, M.O. (2013). A national height-age model for *Pinus radiata* in New Zealand. *New Zealand Journal of Forestry Science*, 43: 4. <https://doi.org/10.1186/1179-5395-43-4>

Van Vleck, L.D. (1972). Estimation of heritability of threshold characters. *Journal of Dairy Science*, 55, 218–228. [https://doi.org/10.3168/jds.S0022-0302\(72\)85463-8](https://doi.org/10.3168/jds.S0022-0302(72)85463-8)

Vincent, T.G. (1987). What radiata pine seed should you use? *What's New in Forest Research* No 157. Rotorua, New Zealand: Forest Research Institute. 4 p. <https://cdm20044.contentdm.oclc.org/digital/api/collection/p20044coll15/id/143/download>

Wakelin, S.A. (2018). Managing soil microbiology: realising opportunities for the productive land-based sectors. *New Zealand Journal of Agricultural Research*, 61(3), 358–376. <https://doi.org/10.1080/00288233.2018.1428203>

Will, G.M., & Stone, E.L. (1967). Pumice soils as medium for tree growth 1. Moisture storage capacity. *New Zealand Journal of Forestry*, 12(2), 189–199.

Wright, J.P., & Marks, G.C. (1970). Loss of merchantable wood in radiata pine associated with infection by *Diplodia pinea*. *Australian Forestry*, 41, 107–119. <https://doi.org/10.1080/00049158.1970.10675516>

Wu, H. X., Ivković, M., Gapare, W.J., Matheson, A.C., Baltunis, B.S., Powell, M.B., & McRae, T.A. (2008). Breeding for wood quality and profit in *Pinus radiata*: a review of genetic parameter estimates and implications for breeding and deployment. *New Zealand Journal of Forestry Science*, 38, 56–87. https://www.scionresearch.com/_data/assets/pdf_file/0003/59043/NZJFS_38_12008_Wu_et_al_56-87.pdf