F.R.I. Rotorua
PRODUCTION FORESTRY DIVISION

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Title: Response to inoculation with *Diplodia pinea* in progenies of apparently resistant trees of *Pinus radiata*

Project: GTI 24,22 **Work Plan:** GTI 142 **Date:** December 1976 **By:** R.D. Burdon, C.K.S. Chou, D. Currie

SUMMARY

Wind-pollinated seed was collected from 20 Pinus radiata trees which had been selected for apparent resistance to Diplodia pinea infection in an area of very high disease hazard. Nearly 100 seedlings of each of these 20 progenies and 300 seedlings of each of two control seedlots were raised in a randomised block layout in a glasshouse. At the age of 5½ months these plants were inoculated in the topmost 2 cm with a spore suspension of D.pinea (15 000/ml). The material was kept for 24 hours at 20°C in misting chambers. Plants which did not become infected after this inoculation were re-inoculated later.

After the first inoculation 72% of the plants developed infection, and 39% of the total eventually showed dead tops. After the second inoculation 76% of the re-inoculated plants showed some infection, and 14% developed dieback.

The general degree of infection from the first inoculation was appreciably less in the progenies of the apparently resistant trees (69.7% infection, 36.8% dieback) than in the controls (79.4% infection, 44.4% dieback). Significant differences ($P < 0.05$) between progenies and controls were established, the exact levels of significance depending greatly on whether fixed or random block effects were assumed. However, the progenies were quite variable among themselves; the best progeny showed 53% infection and 21% dieback, and the worst 85% infection and 59% dieback. Although four of the 20 progenies showed more dieback than the controls none was found to be significantly worse.

Several alternative scoring systems were used for recording infection response, these systems giving a range of weights to infection in itself and to dieback respectively. The most significant difference between the controls and the progenies appeared to be in respect of infection rate. In respect of dieback the difference was much less clear-cut, although the incidence of dieback among infected trees tended to be higher in the lots with higher percentages of infection. The repeat inoculation led to only marginally better resolution of lot differences.

From analysis of results from the progenies alone it appears that both inoculation response and underlying resistance have shown a low narrow-sense heritability ($h^2 \leq 0.2$) at the level of individual seedlings. Repeatabilities of progeny means, however, were high (> 0.55).

No aspect of inoculation response showed any significant correlation with the level of any monoterpene in cortical oleoresin of the parent trees.

The results indicate that the phenotypic selection has been effective in some degree, but that subsequent progeny testing in the form of glasshouse inoculation trials would be beneficial. They also suggest that resistance to initial infection in the glasshouse was a better index of field resistance of individual genotypes than was the progress of established infection.

INTRODUCTION

The fungus *Diplodia pinea* is a notorious cause of stem malformation in *Pinus radiata* because it often infects and causes death in the leading shoots of trees. Affecting mainly young trees, typically five to eight years old, the malformation involves the bottom two logs which are potentially the most valuable part of the tree. One possible means of combatting the fungus is breeding for resistance (Burdon, 1974). Genetic variation in resistance is a prerequisite to successful selection, and there have been some indications that such variation exists. The natural populations of *P.radiata* have been found to vary in incidence of leader dieback (Burdon and Bannister, 1973). Moreover, two New Zealand populations which have presumably been exposed to greater pressures of *Diplodia* infection during their genetic history have shown a lower incidence of leader dieback than any of the natural populations. Added to that, where shoot dieback is prevalent, adjacent trees can differ strikingly in the amount of dieback. All this evidence, however, falls far short of proof of the existence of a worthwhile level of heritable variation in resistance within our local stands of *P.radiata*.

Even if heritable variation in resistance is present, selection for resistance will be complicated by the fact that both infection and dieback are all-or-nothing phenomena, which means that chance escapes are likely to occur. In many environments infection is obviously far too sporadic to provide reliable screening for susceptibility. On the other hand, selection in environments where infection is prevalent must restrict the pool of trees from which to select.

Preliminary selection for little or no shoot dieback has been carried out (Shelbourne, G.T.I. Work Plan 96) during 1970 in Fenton's Mill Flat, Tarawera Forest, an area of very high disease hazard. The trees were nine years old from planting, and selection was also made for general tree form and dominant crown status. Twenty-six individuals were selected thus, wind-pollinated seed was collected, and the resulting progenies were planted in 1971 on two test sites, one in Tarawera Forest and one in Kaingaroa. These progenies should now be reaching the age at which sufficient shoot dieback should occur to provide worthwhile resolution of genetic differences in resistance, both between progenies and between the progenies and control material.

Cuttings from many of these trees have been taken and are growing as hedges at FRI Headquarters. These have been repropagated, and resulting cuttings are due to be planted out on test sites.

Monoterpene composition of cortical oleoresin has shown considerable promise as an indicator of resistance to infection and consequent dieback (Smith *et al.*, 1976). More recent data (Zabkiewicz, unpubl.), however, suggested that the initial results were fortuitous, so it was important to resolve the conflict between the different sets of results.

Since the initial selection a reliable technique for inoculating seedlings with *Diplodia* has been developed (Chou, in press). It has also been possible to collect further wind-pollinated seed and oleoresin samples from the select parents. This made it possible to carry out an inoculation trial in the glasshouse with progeny of the parents. In conjunction with the field progeny trial the inoculation trial stood to achieve several objectives:

1. To establish whether resistance of *P.radiata* to infection by *D.pinea* is heritable in the narrow-sense.

2. To confirm whether there is a satisfactory juvenile/semi-adult genetic correlation for resistance.

3. To assess the feasibility of using glasshouse inoculation trials as a short-cut method of screening candidate parents for resistance.

4. To indicate which feature or features of inoculation response in the glasshouse represent the best index of resistance under field conditions.

5. To give preliminary indications as to the mechanism of field resistance.

6. To provide further information on the use of monoterpene composition of oleoresin as an indicator of resistance.

NOT POSSIBLE WITHOUT SAMPLING SEEDLINGS.

If the progenies overall were less affected than the controls, it would indicate that the field selection had been effective in some degree, and therefore that there is an appreciable degree of narrow-sense heritability for resistance. It would at the same time indicate that there is a good genetic correlation between resistance during the early seedling phase and the semi-adult phase respectively. It would also indicate that such inoculation trials are of value as a screening procedure. All such evidence would be greatly strengthened if a parallel result is obtained in the field trials of the progenies of the same parents. [The relationship between parental monoterpene composition and progeny resistance provides a test of the practical value of monoterpene composition as an indicator in selecting for resistance.] ONLY if progeny in term old enough to develop some resistance metabolism.

MATERIALS AND METHODS

Source of Seed

Twenty-one of the select parents in Fenton's Mill Flat could be relocated and were bearing a worthwhile number of cones. From each such tree all ripe unopened cones were collected, and an oleoresin sample collected from the cortex of a first year shoot in the free growing crown, on 17.4.1975. The procedure for the sampling and analysis of resin will be described separately (Burdon and Zabkiewicz, in prep.), the sampling point being chosen for its good sampling repeatability. In addition, a note was made of the amount of shoot dieback believed to have occurred on the tree during the past three years, and each tree was thence rated on a 1-6 scale (1 denoting no shoot dieback, 6 denoting multiple occurrences).

The seed from each tree was extracted as a separate lot, and was cleaned by severe blowing so as to ensure the removal of all empty seeds. Twenty progenies only were used for the experiment. Two control seedlots were used:

1. R69/854, representing a bulk collection from Kaingaroa Forest and which has been used as a control in a number of genetic experiments.

2. AK74/1048, from Waiuku Forest, where shoot dieback is not a problem and where perforce there could have been little natural or silvicultural selection for dieback resistance in the previous generation.

Experimental Design and Layout

Seedlings were grown in polythene tubes held in standard boxes which held 35 tubes each. The three compartments of the misting chamber could each hold three boxes. With these constraints the experiment was split into four inoculation runs, with a randomised complete block design of the following structure:

{20 progenies x 4 runs x 3 compartments x 4 tubes x 2 seedlings
{2 controls x 4 runs x 3 compartments x 12 tubes x 2 seedlings

In effect there was a two-factor block classification with tubes fully randomised within run/compartment sub-classes. With the particular design it was not possible to use boxes as any sort of experimental unit. The two control lots were each coded as three notional progenies in order to conceal their identity from the experimenter.

Sowing of Seed and Raising of Stock

The polythene tubes were 6.35 cm in diameter x 20 cm long. They were filled with standard nursery mix (50:50 soil + duff). Three seeds were sown in each tube, equally spaced, roughly 8 mm below the soil surface. Sowing was carried out during 3-7 June, 1975.

In addition, eight spare tubes per progeny and 25 spare tubes per control were sown as a source of replacements of experimental tubes if there were losses from non-germination or mortality.

The boxes were kept in No.3 glasshouse which was heated and ventilated to keep temperatures between 15°C and 25°C, although 25°C tended to be exceeded in hot sunny weather. Individual boxes were fully randomised within the glasshouse. The boxes were stood on metal trays to allow watering from beneath.

After the seedlings were old enough to be safe from damping-off, on 30 August, they were thinned with scissors to two per tube.

Although germination was slightly lower than expected in some progenies, the growth of the seedlings was very satisfactory, and there were no indications of nutrient deficiencies. By the prescribed date for inoculation, however, the seedlings were becoming fairly crowded, being about 25-30 cm tall.

INOCULATION PROCEDURE AND CONDITIONS

Inoculation was carried out on 17, 18, 19, 20 November, 1975.

For inoculation an isolate obtained from "Death Valley", Tarawera Forest was used throughout. The fungus was grown on 3% Oxoid malt extract agar at 25°C. When the plate was overgrown with the fungus, autoclaved *P.radiata* needles were laid over the culture and sporulation was induced by near UV light within three weeks. Mature spores were used, these being proven to have at least 75% germination rate.

Spores were extracted by soaking the pynidia-bearing needles in 0.5% gelatin solution at 5°C for 20 minutes, after which a gentle shaking was applied and the needles removed. The volume of the suspension was adjusted to give about 15 000 spores/ml.

During inoculation, an ice water bath was used to prevent premature germination in this spore suspension. Spores were applied with a soft camel hair brush to the topmost 2 cm of the shoot. Care was taken to avoid injury to the foliage, while all reasonable efforts were made to get droplets of spore suspension into the leaf axils. In addition to the brush inoculation 10 µl of spore suspension was applied to the apical tuft of each seedling with a microsyringe. Throughout inoculation the spore suspension was shaken to prevent spores from settling on the bottom of the vessel.

Only one person worked on a run/compartment sub-replicate at a time, and as soon as the inoculation of a sub-replicate was completed the three boxes were placed in the misting chamber.

All inoculated plants were kept for 24 hours, beginning at 10.30 am, at 20°C. Misting was regulated by an artificial leaf type humidistat. After misting all plants were returned to the same glasshouse, but the boxes from each run/compartment sub-replicate and from each run were kept in contiguous units.

Reinoculation

All seedlings which did not show any infection when first inoculated were subjected to a repeat inoculation on 12 and 13 January, 1974. For this the tubes containing such seedlings were segregated and regrouped at random into 16 boxes. The re-inoculation was completed in 2 runs, but all tubes involved in the re-inoculation represented a single randomised experimental unit without distinction between runs, compartments or boxes. Otherwise, inoculation procedure was as described normally.

Assessment and Response Criteria

A preliminary assessment was made on 3 December, but the main assessment which provided the basis for subsequent analysis was carried out on 18-22 December. Assessment of the re-inoculated seedlings was carried out on 9.2.76. Response was initially recorded for each seedling according to the categories shown in Table 1, Column 1. The responses were then coded according to seven alternative scoring systems (Table 1) with a view to comparing both the rankings and resolution of lot differences under alternative systems. Systems 1 and 2 recorded all-or-nothing responses with respect to dieback and infection respectively. Systems 3-5 were alternatives which appeared to be intuitively reasonable. They involved some arbitrary decisions as to what represented a meaningful ranking of certain responses, but this factor was clearly unimportant because response categories 4 and 3a, 3c and 3d were all uncommon. Systems 6 and 7 represent an *ad hoc* transformation (P.M. Burrows, per W.J. Libby, pers. comm.), each response category being coded as the approximate mean (in hundreds of standard deviations) for its percentile class (on basis of preliminary counts) if the overall frequency distribution was normal.

Analysis

The final classification was slightly unbalanced. Four tubes were missing, 42 contained only one seedling and 22 contained three seedlings, but the imbalance was spread evenly among block/chamber sub-classes. It was decided to adopt an approximate solution based on treating the data as if it were balanced, because the imbalance was not great, while any of the more rigorous solutions would have been very cumbersome because of the complexity of the classification.

Sums of squares for the various effects were obtained as shown in Table 2.

The interpretation of the mean squares was complicated by the question of whether the various main effects (lots, runs and compartments) should be regarded as fixed or random. The expectations of mean squares are shown in Table 3 under the alternative assumptions of all main effects being fixed and random respectively.

Both runs and compartments were set up as fixed effects, but it is debatable as to how closely they conform to this model. Insofar as they conform to random effects some evaluation is possible of the generality of the results. The progenies of the select trees could certainly be regarded as a random sample of potential populations resulting from intensive phenotypic selection, but against that the contrast between progenies and controls clearly conforms to a fixed effect.

In the event the results were analysed under both the extreme assumptions - that all main effects were fixed and that all effects were random. The latter assumption was employed to give the best available indication of the generality of the results with respect to other possible inoculation trials.

If all effects were assumed to be random it is clear from Table 1 that there are no direct tests for the main effects. Accordingly approximate F tests were synthesised using Satterthwaite's method (e.g. Gaylor and Hopper, 1969). For example, in testing for differences between lots the following combination of mean squares was used as the approximate F ratio

$$\frac{\text{M.S.(Lots)} + \text{M.S.(Lots x Runs x Compartments)}}{\text{M.S.(Lots x Runs)} + \text{M.S.(Lots x Compartments)}}$$

and the effective degrees of freedom for lots were estimated by

$$\frac{(\text{M.S.(Lots)} + \text{M.S.(Lots x Runs x Compartments)})^2}{\frac{(\text{M.S.(Lots x Runs)})^2}{\text{d.f.(Lots x Runs)}} + \frac{(\text{M.S.(Lots x Compartments)})^2}{\text{d.f.(Lots x Compartments)}}}$$

while the effective degrees of freedom for its denominator was estimated by

$$\frac{(\text{M.S.(Lots x Runs)} + \text{M.S.(Lots x Compartments)})^2}{\frac{(\text{M.S.(Lots x Runs)})^2}{\text{d.f.(Lots x Runs)}} + \frac{(\text{M.S.(Lots x Compartment)})^2}{\text{d.f.(Lots x Compartments)}}}$$

No sum of squares was pooled with its effective error term unless it had an F ratio of less than one. However, this generally arose with the L x R x C interaction, which was accordingly pooled with the tubes within L R C sub-classes sum of squares. For constructing Satterthwaite F ratios and for testing of the first-order interactions under the random effects model this composite mean squares was used in place of the L x R x C mean square but only assigned the original L x R x C degrees of freedom.

The sums of squares for lots and lot interactions could be partitioned according to which genotypic comparisons were of interest. Hence it was possible to obtain mean squares for the contrast between progenies versus controls and for differences among the progenies. Assuming random effects for runs and chambers each component of the lots effect was tested against a combination of the corresponding interactions. Hence the Progenies vs Control (P vs Co) effect would be tested as follows:

$$F = \frac{\text{M.S.(P vs Co)} + \text{M.S.((P vs Co) x R x C)}}{\text{M.S.((P vs Co) x R)} + \text{M.S. ((P vs Co) x C))}}$$

Where first-order interactions were non-significant the appropriate pooling procedures were not always clear and the power of alternative tests could vary substantially. Procedures finally adopted are indicated in Results, but the rule was to use the most conservative test possible.

As a preliminary measure the three "lots" within each of the two controls were pooled for subsequent analysis.

Variance components were estimated from data, for the progenies only, by solving for the expected mean squares shown in Table 3, subject to the specified procedures for pooling sums of squares which precluded negative estimates for variance. Narrow-sense heritabilities (h^2) and repeatabilities of progeny means (H^2) were estimated as follows:

$$\hat{h^2} = \frac{4\hat{\sigma_p^2}}{\hat{\sigma_p^2} + \hat{\sigma_t^2} + \hat{\sigma_w^2}}$$

$$\hat{H^2} = \frac{\hat{\sigma_p^2}}{\hat{\sigma_p^2} + \hat{\sigma_t^2}/48 + \hat{\sigma_w^2}/96}$$

and assuming random effects

$$\hat{h^2} = \frac{4\hat{\sigma_p^2}}{\hat{\sigma_p^2} + \hat{\sigma_{pr}^2} + \hat{\sigma_{pc}^2} + \hat{\sigma_{prc}^2} + \hat{\sigma_t^2} + \hat{\sigma_w^2}}$$

$$\hat{H^2} = \frac{4\hat{\sigma_p^2}}{\hat{\sigma_p^2} + \hat{\sigma_{pr}^2}/4 + \hat{\sigma_{pc}^2}/3 + \hat{\sigma_{prc}^2}/12 + \hat{\sigma_t^2}/48 + \hat{\sigma_w^2}/96}$$

where $\hat{\sigma_p^2}$ = between-progenies variance

and $\hat{\sigma_{pr}^2}$ = progenies x runs interaction variance

etc.

Owing to the approximate nature of the analysis of variance, and to a lesser extent the non-normality of data, estimates of variance components and derived parameters must be accepted with caution.

Estimates of narrow-sense heritability for infection and dieback were adjusted for binomial variation (all-or-nothing expression) to give heritability estimates for the underlying susceptibilities (Appendix II).

RESULTS

General Pattern of Response

The overall incidence of individual response categories can be seen in Table 1, Column 3. The first inoculation led to 72% of the seedlings becoming infected. The corresponding incidence of dieback was 39%, the extent of dieback (length of dead top) in seedlings showing a roughly normal distribution. Of the seedlings which got infected but did not die back roughly one-third had just a few needles infected and almost all the remainder showed stem lesions which girdled the stem by less than 50% and were 1-3 cm long. Hence irrespective of the scoring system adopted almost all seedlings fell into a limited number of more or less discrete response categories, a situation which undoubtedly reflects threshold effects.

The general levels of infection and dieback were within the desired range for purposes of obtaining good resolution of genetic differences between lots.

In the repeat inoculation involving seedlings which were initially uninfected the infection rate was marginally higher than in the first

inoculation, being 76% compared with 72%. The dieback rate, however, was considerably lower, being 14% compared with 39%. Because of the difference in size and age of the seedlings, however, no weight is attached to the differences in response between the two inoculations.

Comparisons Among Lots

Mean responses for the individual lots are shown in Table 4.

Preliminary analysis showed that the two control lots differed negligibly in their responses ($F \ll 1$), irrespective of response criterion. Moreover, the two controls showed no significant interactions with runs or compartments. These two lots were therefore pooled as a single control for all subsequent analysis.

The control material showed more infection and dieback overall than the progenies. Of the individual progenies 16 out of 20 averaged better than the controls by all criteria. This ratio which provided a simple if crude statistical comparison differs significantly from 50% (χ^2 test, $P \leq 0.015$).

Using analysis of variance (Tables 5-11) the progenies were very highly significantly better than the controls ($P > 0.001$) if fixed effects were assumed. Under the very conservative assumption of random run and compartment effects this difference was significant ($P < 0.05$) for infection, score d and score e, but not for scores a, b and c ($0.1 > P > 0.05$) and dieback ($P \cong 0.2$). Lot interactions, although generally non-significant statistically, greatly reduced the significance of lot effects when they were taken into account for testing. Progenies differed significantly among each other, even by the most stringent tests.

No progeny was significantly worse than the controls. Only for score e, assuming fixed effects, was a progeny worse than the controls by more than the least significant difference, and among the multiplicity of comparisons this isolated result can be discounted.

Interestingly, the progenies differed among each other slightly more in respect of dieback than of infection, even though they differed from the controls more in respect of infection. Considering the other response criteria, scores e and d gave the most sensitive discrimination between lots. Score e, which incorporates results from the repeat inoculation gave slightly better resolution of progeny differences, but did not give clearly better resolution of the difference between progenies and controls. Of scores a, b and c, score a gave the most sensitive overall resolution of lot differences, despite the crudeness of the scale which allowed values of only 1, 2 or 3. In the case of infection even a binomial scale gave very similar results in analysis of variance to those from the more elaborate scales.

Among the progenies inoculation response did not relate clearly to the amount of shoot dieback on the respective parents at the time of seed collection (Table 4, Col. 2).

Experimental Block Effects and Interactions

Inoculation response, irrespective of criterion, differed very highly significantly ($P < 0.001$) between runs and compartments under a fixed effects model (Tables 5-11). Because of pronounced runs x compartments interaction the two main effects were generally not significant under the random model. Tube effects, despite the smallness of the F ratios, were consistently significant ($P < 0.05$).

Infection showed much more definite effects of runs, compartments and runs x compartments interaction than did dieback, but less definite tube effects (Tables 5 and 6). Infection showed a stronger effect of chambers than of runs, in contrast to dieback.

Interactions shown by lots, even though not generally significant are worth noting. For scores d and e the progenies vs controls contrast showed significant second-order interactions. Dieback showed significant lots x runs interaction. With the threshold effects which were occurring scalar effects are an obvious potential source of interactions, even with the transformations used. What does appears to be an interaction, although not statistically significant, is the (Progenies vs Controls) x compartment effect for dieback (Table 12). In Compartment 1 the progeny seedlings actually showed marginally more dieback than the controls.

Estimates of Heritability

Estimates of variance components and of heritability (subject to reservations mentioned earlier) are shown in Table 13. The predominance of seedling-to-seedling variation is obvious.

By any of the four response criteria considered estimates for narrow-sense heritability at the level of individual seedlings were low (<0.2). Values were lower for the all-or-nothing scores with dieback and infection than for scores d and e which were designed to integrate all response phenomena and to normalise the data. Adjusting heritability estimates for all-or-nothing expression, so as to estimate heritability for underlying predisposition, almost doubled the original estimates, but still gave rather low values.

Whether fixed or random effects were assumed had a relatively minor bearing on point estimates of heritability but would clearly have a large bearing on confidence limits. With fixed effects the confidence intervals would be quite narrow, but with random effects the interval would clearly be quite wide, particularly for adjusted heritablities of infection and dieback. For this reason, together with the specialised conditions of the experiment, the approximate nature of the analysis and the non-random sample of progenies the confidence limits are of rather academic interest and so were not pursued.

Repeatabilities of progeny means were apparently high (>0.55) for all four response criteria and were not greatly dependent on whether fixed or random effects were assumed.

Interrelationships Between Response Criteria

Progeny means for different response criteria were strongly interrelated (Table 14), although the transformation error in score e must have slightly inflated the correlations between this score and other response criteria. The weakest simple correlation among primary response criteria was between infection rate and dieback rate (Fig. 1), and this was associated with a relatively weak correlation between infection and the derived criterion, dieback %/infection % (Fig. 2). The imperfect correlation between infection and dieback parallels the rather different results for these response criteria in analyses of variance.

With respect to the relationship between dieback rate and infection rate the controls were not appreciably out of line with the progenies (Figs 1 and 2). Differences between lots overall in the amount of dieback in relation to infection were tested by analysis of covariance, adjusting dieback sums

of squares for individual covariances on infection (cf. Snedecor, 1956, 13.5); although for two binomial variates this analysis (Table 15) may be less than satisfactory. For what it is worth, the analysis indicated that lots and runs differ significantly in dieback rate in relation to infection (see also Fig. 3), but only if main effects are assumed to be fixed. First-order interactions approached statistical significance.

Relationship Between Inoculation Response and Parental Monoterpene Levels

Correlations between the level of any monoterpene in cortical oleoresin of the parents and infection response (Table 16) were not statistically significant. The one exception, involving dieback/infection and myrcene ($P \leq 0.05$) is readily explicable as being fortuitous. Transformation of values for monoterpene levels could not be expected to materially affect the result.

From an examination of simple correlations between levels of individual monoterpenes it is clear that partial correlations (Snedecor, 1956, 14.6) between inoculation response and any monoterpene for constant level of any one other monoterpene, were non-significant. Partial correlations between any one monoterpene and dieback rate for constant infection rate were also non-significant.

Accordingly, it was deemed to be not worth pursuing multiple regression analysis of inoculation response in relation to linear functions of the levels of more than one monoterpene.

DISCUSSION

Genetic Implications

Results of the inoculation trial were positive by even the most stringent of statistical criteria, although they were not dramatic. They therefore give a qualifed endorsement of both the initial selection procedure and the use of inoculation trials for rapid progeny test screening.

It must still be assumed, albeit reasonably, that the controls were genetically comparable to the base population represented by the parental seedlot. Even assuming this, however, it is impossible to quantify the response to selection at this stage, partly because the absolute differences between lots must relate to the level of infection obtained, and partly because there is no means yet of projecting the observed lot effects to differences in field performance. Nevertheless, it is likely that the observed response in the glasshouse was only about half that could be expected from inter-crossing between apparently resistant parents, because the progenies were only wind-pollinated. Insofar as the pollen parents may have already undergone effective natural and/or silvicultural selection for dieback resistance, the progenies would reflect more than half the gain obtainable from initial phenotypic selection. The results, however, make it unlikely that this last factor was important.

The lack of detectable differences between the two controls is noteworthy, because these controls were chosen to represent seed collections from sites of contrasting disease hazard. Assuming that genetically comparable seedlots provided the parent stands for the two control lots the inference is that the natural selection represented in bulk collections has been of negligible significance during the preceding generation. This, in turn, suggests that the previously observed superiority of New Zealand material over any natural population must be either the cumulative result of several generations of natural and/or silvicultural selection, or an

expression of heterotic gene effects resulting from the increased outcrossing which is promoted by plantation culture. The similarity of the two controls, however, does suggest that both were appropriate yardsticks for comparing the selections.

Progeny performance under inoculation did not relate to current dieback status among the parents. There are several possible reasons for this. Firstly, an offspring-parent regression (which this situation represents) tends not to be manifested clearly when the parents represent an intensively selected sample, which was certainly the case here. Secondly, the condition of individual parent trees could reflect local variations in the amount of damage from hail at the beginning of 1975, this damage having been extremely patchy. Thirdly, and more disturbingly, resistance to *Diplodia* infection and dieback following hail damage could be under rather different genetic control from resistance to infection of uninjured shoots which was apparently the basis of the original selection (c.f. Chou, 1976).

There are some indications that there are two effectively distinct components to dieback resistance, namely, resistance to initial infection, and resistance to further progress of established infection. The pattern of lot differences was rather different for infection rate and dieback rate respectively, although it was not possible to establish clear differences between lots in the amount of dieback in relation to infection. The correlation among lots between dieback rate among infected trees and infection rate may be somewhat misleading; it could be a consequence of more multiple infections among lots with higher infection rates rather than higher rates of dieback per infection event. Most interestingly, the pronounced difference between progenies and controls in infection rate suggests that resistance to initial infection as manifested in the glasshouse may have been the important determinant of field resistance to dieback. It must be stressed though, that in the particular field situation almost all the dieback which provided the basis for selection had apparently followed selection of uninjured shoots.

The apparently low narrow-sense heritability for inoculation response, irrespective of criterion, was not unexpected. Intensive selection of parents can, however, reduce the additive genetic variation among progenies and thus reduce the apparent heritability. This could well have happened with respect to infection, and the marginally higher heritability estimates for dieback are therefore also consistent with field selection having effectively involved resistance to initial infection.

Repeat inoculation did not accentuate the difference between progenies and controls. Nevertheless, this need not indicate a poor repeatability of inoculation results. The very high infection rate in this second inoculation meant that cumulative infection rates were contributing little to the resolution of lot differences in score e, and yet it appears that infection rate was a major component of the difference between progenies and controls.

NO!

The trial has provided further evidence that monoterpene composition, or at least Δ^3-carene level, is of no value as an indicator of resistance to infection and thence to shoot dieback. Although some reservations attach to the chemical analysis (Burdon & Zabkiewicz, in prep.), this factor is unlikely to have been important. Why the preliminary studies all pointed to such an association is beyond the scope of this report.

Implications for Further Inoculation Trials

The effects of runs and chambers and their first-order interaction emphasis the importance of subtle variations in inoculation conditions on response. Hence a blocked experimental design is essential for valid comparisons between lots. It would probably have been preferable to have retained the runs and run/compartment sub-classes as discrete block units

throughout the experiment, because final response to infection is presumably influenced by not only the inoculation conditions, but by growing conditions both before and after inoculation.

The differences between the compartments of the inoculation chamber and the smallness of the compartments certainly create some problems with experimental design, in that it becomes difficult to accommodate large numbers of seedlings of large numbers of lots in a run/compartment block unit. Imbalance in sub-class numbers can become awkward in this situation. The need to handle seedlings in boxes creates another problem of layout, because although boxes are an obvious source of variation it is very difficult to create block units which correspond to single boxes without being left with numerous missing sub-classes.

The use of two seedlings per tube was adopted largely to achieve efficient use of greenhouse space with the standard sized tubes. For purposes of analysis, however, this created several difficulties which were related to the fact that the tube-to-tube effect (which was partly confounded with box effects) could not be ignored. With the number of sub-classes arising from the use of tubes in this way the only practical course was ignore the inevitable imbalance in the classification and hope that this caused no serious bias. Use of single seedlings in smaller tubes would not in itself overcome the difficulties of making boxes the ultimate block subunits.

The eventual solution may be to work with even younger and therefore smaller seedlings and to use a lattice (incomplete block) design. Smaller seedlings, either in small tubes or from seeds sown individually in grid positions in soil boxes, would save space. This, in conjunction with a lattice design, could mean that a box could serve as a block sub-unit and yet allow enough individually randomised seedlings per block/lot sub-class to prevent imbalance in sub-class numbers from becoming troublesome. The validity of inoculation results with very young seedlings would, however, need to be established.

There is the question of what is the appropriate criterion of response to record. The indications are that simply the occurrence of infection relates satisfactorily to field performance. If this is so, the duration of inoculation trials can be minimised. On present knowledge, however, a composite score integrating infection, lesion development and dieback, used instead of or in addition to infection records, would appear a safer course. Lot means for several such scores all correlated almost perfectly with infection percentages. In retrospect, it might have been better to have applied separate normalising transformations within each block sub-unit, although this would have been a cumbersome procedure. The results with score a, which did not recognise infection as such unless it involved more than the occasional needle and yet gave good resolution of the differences between progenies and controls, suggest that the criterion of infection is optional. It may therefore be better where infection is very severe to apply the more conservative criterion to obtain good resolution of lot differences. In any event there appears to be little advantage in recognising more than four response categories (no infection, occasional needles infected, stem lesions and dieback) because many categories were so uncommon.

Implications for Other Work

The obvious need is to compare the inoculation trial rankings with rankings of the corresponding progenies in the field. As prescribed, individuals which have remained uninfected through both inoculations have been lined out in the nursery, and can be clonally propagated for further screening in the glasshouse and later in the field.

It appears worthwhile to conduct further field selection along the lines of what was done in Fenton's Mill Flat, so as to give an adequate genetic base (after reselection) to selections made for *Diplodia* resistance. Such material could in turn be subjected to progeny tests (in the glasshouse and possibly in the field too), and to direct tests of the original parent clones.

Results to date have indicated a promising level of additive genetic variation in resistance to *Diplodia*. Eventually it will be desirable to test for non-additive gene effects (specific combining ability). For this further refinement of the experimental technique would be desirable, but may not be essential.

CONCLUSIONS

1. The initial intensive phenotypic selection for resistance to dieback caused by *Diplodia* infection in an environment of very high disease hazard, appears to have had some success.

2. Hence there appears to be a promising degree of additive genetic variation for resistance.

3. Resistance in the early seedling phase appears to show a satisfactory genetic correlation with resistance in the crucial semi-adult phase.

4. Present indications are that the glasshouse inoculation trial is a valid method of progeny test screening, if not yet very precise.

5. Heritability of resistance appears to be low, if only because of the threshold nature of infection and the importance of environmental conditions in infection.

6. Hence progeny test screening is probably a desirable adjunct to initial phenotypic selection.

7. The results suggest that in the glasshouse the incidence of initial infection has been a better index of field resistance than the incidence of actual dieback.

8. The trial provided no convincing evidence that the level of any monoterpene component in cortical oleoresin is a worthwhile indicator of resistance.

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TABLE 1: Alternative systems for scoring responses to inoculations, and percentage occurrence overall of individual response categories

Response to first inoculation	Response to second inoculation	% total individuals*	Criterion of response (scoring system)						
			Dieback	Infection	Score a	b	c	d	e
1. No infection	a. No infection	7	0	0	0	0	0	122	136**
	b. Lesion without dieback	17							104**
	c. Dieback	4							54**
2. Only a few needles infected	N.A.	11	0	1	0	1	4	46	46
3. Restricted lesion, ≦50% girdling	N.A.								
a. ≦1 cm long		≧0	0	1	1	2	6	1	1
b. 1-3 " "		19							
c. 3-5 " "		2				3	7		
d. 5-7 " "									
4. Severe lesion, >50% girdling	N.A.								
a. ≦1 cm long		1.5	0	1	1	3	8	-19	-19
b. 1-3 " "						4	9		
c. 3-5 " "						5			
d. 5-7 " "									
5. Top dieback	N.A.								
a. ≦6 cm		7	1	1	2	6	14	-74	-74
b. 6-10 cm		20						-113	-113
c. >10 cm		12						-147	-147

* Do not sum to 100 because of rounding errors.

** Subject to a computional error which should not have materially affected the results of analysis.

TABLE 2: Calculation of sums of squares, assuming fully balanced classification

Effect	Degrees of freedom	Solution for sum of squares
Lots (L)	N-1	SS_L
Runs (R)	3	SS_R
Compartments (C)	2	SS_C
L x R	3 (N-1)	$SS_{LR}-SS_L-SS_R$
L x C	2(N-1)	$SS_{LC}-SS_L-SS_C$
R x C	6	$SS_{RC}-SS_R-SS_C$
L x R x C	6(N-1)	$SS_{LRC}-SS_R-SS_C-SS_{LxR}-SS_{LxC}-SS_{RxC}$
Tubes (T):LRC	36N	SS_T-SS_{LRC}
Trees:Tubes	48N	Total SS-SS_T
Total	96N-1	Total SS

Where all sums of squares are corrected,

SS_T = sum of squares for tubes overall

SS_{LRC} = sum of squares for lots/runs/chambers sub-classes

etc

T:LRC = tubes within lots/runs/chambers sub-classes

etc

N = number of lots being considered

TABLE 3: Expectations of mean squares, assuming balanced classification with two seedlings x 4 tubes per lot/run/chamber sub-class. Terms in square brackets are included if lots, runs and chambers are assumed to be random effects, and omitted if lots, runs and chambers are assumed to be fixed effects

Source	Expected mean square
Lots (L)	$\sigma^2_w + 2\sigma^2_t \left[+ 8\sigma^2_{lrc} + 32\sigma^2_{lc} + 24\sigma^2_{lr} \right] + 96\sigma^2_f$
Runs (R)	$\sigma^2_w + 2\sigma^2_t \left[+ 8\sigma^2_{lrc} + 8N\sigma^2_{rc} + 24\sigma^2_{lr} \right] + 24N\sigma^2_r$
Compartments (C)	$\sigma^2_w + 2\sigma^2_t \left[+ 8\sigma^2_{lrc} + 8N\sigma^2_{rc} + 32\sigma^2_{lc} \right] + 32N\sigma^2_c$
L x R	$\sigma^2_w + 2\sigma^2_t \left[+ 8\sigma^2_{lrc} \right] + 24\sigma^2_{lr}$
L x C	$\sigma^2_w + 2\sigma^2_t \left[+ 8\sigma^2_{lrc} \right] + 32\sigma^2_{lc}$
R x C	$\sigma^2_w + 2\sigma^2_t \left[+ 8\sigma^2_{lrc} \right] + 8N\sigma^2_{rc}$
L x R x C	$\sigma^2_w + 2\sigma^2_t + 8\sigma^2_{lrc}$
Tubes within LRC	$\sigma^2_w + 2\sigma^2_t$
Seedlings within tubes	σ^2_w

σ^2_w = variance of seedlings within tubes

σ^2_t = variance of tubes within lots/runs/compartments sub-classes

σ^2_{lrc} = variance of lots x runs x chambers interaction

N = no. of lots

NOTE: When certain groups of lots are pooled, the form of analysis remains essentially the same, but the coefficients will be changed for variance components with 1 in their subscripts.

TABLE 4: Responses of individual lots to inoculation

Lot (parental no.)	Criterion of inoculation response								
	Parental dieback	Infection (%)	Dieback (%)	Dieback/ infection (%)	Score a	b	c	d	e
85	2	53	21	39	.64	1.90	4.8	40.7	39.6
73	6	60	27	46	.72	2.17	5.5	31.3	30.8
77	4	61	27	44	.70	2.18	5.5	27.3	27.2
83	5	63	26	41	.77	2.24	5.7	26.4	22.3
84	5	69	27	39	.78	2.33	6.0	20.6	19.3
68	3	63	33	53	.84	2.59	6.4	19.0	16.7
81	1	64	33	52	.92	2.59	6.4	14.5	14.1
66	1	65	36	55	.89	2.63	6.6	13.1	13.8
63	2	62	34	55	.92	2.61	6.5	14.6	12.6
86	2	65	35	53	.92	2.66	6.6	10.3	9.7
69	2	72	35	48	.90	2.71	6.8	6.5	6.2
79	1	74	32	43	.90	2.63	6.7	6.4	5.9
78	1	74	38	52	.99	2.91	7.3	-0.2	-1.0
64	5	74	39	53	1.05	3.01	7.5	-3.2	-1.1
82	4	77	38	49	1.05	3.04	7.6	-3.3	-2.4
76	3	74	42	57	1.03	3.08	7.6	-6.6	-4.9
67	1	80	49	61	1.17	3.51	8.6	-22.3	-20.7
61	3	74	53	72	1.20	3.62	8.7	-18.2	-21.7
70	1	85	59	69	1.37	4.03	9.7	-38.0	-35.6
65	4	84	54	64	1.29	3.86	9.3	-35.9	-36.0
Progenies overall		69.69	36.85	52.9	.949	2.81	6.98	5.38	4.96
AK74/1048		80	44	55	1.16	3.35	8.3	-18.37	-16.41
R69/85		78	45	57	1.15	3.35	8.2	-17.15	-16.34
Controls pooled		79.44	44.43	55.9	1.55	3.35	8.22	-17.76	-16.38
Overall mean		71.96	38.61	53.6	.997	2.94	7.27	0†	0†
Superority, progenies vs controls		9.75	7.58	3.0	.206	.540	1.237	23.13	21.34
*LSD (P=0.05) among progenies		12.4	13.9	-	.25	.73	1.63	26.4	25.2
*HSD (P=0.05) among progenies		22.5	25.1	-	.45	1.31	2.95	47.7	45.6
*LSD (P=0.05) controls vs any progeny		9.6	10.6	-	.18	.56	1.25	20.4	19.4

*Assuming fixed effects
†All means expressed as departures from this value

TABLE 5: Analysis of variance for infection (scored 0 or 1)

Effect	Degrees of freedom	Mean square	Assuming fixed effects		Assuming random effects	
			F	P	F	P
Lots overall (L)	20	0.8475	4.41	***	2.20	*†
Progenies vs Control (P vs Co)	1	4.1864	21.80	***	16.35	*‡
Progenies (P)	19	0.6718	3.50	***	1.83	*†
Runs (R)	3	3.1985	16.66	***	1.99	N.S.
Compartments (C)	2	7.8598	40.94	***	4.63	*
L x R	60	0.2215	1.15	N.S.	1.12	N.S.
(P vs Co) x R	3	0.2560	1.33	N.S.	<4	N.S.
P x R	57	0.2169	1.13	N.S.	1.12	N.S.
L x C	40	0.2548	1.33	N.S.	1.29	N.S.
[(P vs Co) x C	2	0.1007	<1	N.S.	<1	N.S.]
[P x C	38	0.2629	1.37	N.S.	1.36	N.S.]
R x C	6	1.4860	7.74	***	7.50	***
L x R x C	120	0.1981			1.03	N.S.
[(P vs Co) x R x C	6	0.2815			1.47	N.S.]
P x R x C	114	0.1937			1.01	N.S.
(P vs Co) x C (P vs Co) x R x C	8	0.2363			1.25	N.S.
Tubes:LRC	994	0.1920			1.12	*††
Within tubes	1222	0.1717				

N.S. denotes not significant (P >0.05)

* denotes significant (P <0.05)

*** denotes very highly significant (P <0.001)

† Approximate ratio using Satterthwaite's method

‡ Tested against (P vs Co) x R interaction with 1, 3 d.f.

†† Level of significance roughly interpolated

TABLE 6: Analysis of variance for dieback (scored 0 or 1)
(For meaning of asterisks, etc., see Table 6 footnotes)

Effect	Degrees of freedom	Mean square	Assuming fixed effects		Assuming random effects	
			F	P	F	P
L	20	1.0366	4.29	***	1.99	*†
P vs Co	1	2.5255	10.45	**	2.38	N.S.†
P	19	0.9582	3.97	***	1.96	*†
R	2	2.0375	8.42	***	2.55	N.S.
C	3	1.8358	7.60	***	2.34	N.S.
L x R	60	0.3238	1.34	*	1.34	N.S.
(P vs Co) x R	3	0.4562	1.89	N.S.	1.89	N.S.
P x R	57	0.3168	1.31	N.S.	1.31	N.S.
L x C	40	0.3175	1.31	N.S.	1.31	N.S.
(P vs Co) x C	2	0.7211	2.98	N.S.	2.64	N.S.
P x C	38	0.2962	1.23	N.S.		
R x C	6	0.5711	2.36	*		
L x R x C	120	0.1998	<1	N.S.		
(P vs Co) x R x C	6	0.2734	1.13	N.S.		
[P x R x C	114	0.1959	<1	N.S.]		
Tubes:LRC + P x R x C	1108	0.2414	1.18	***††		
Tubes:LRC + L x R x C	1114	0.2416	1.18	***††		
Tubes:LRC	994	0.2466	1.21	***††		
Within tubes	1222	0.2044				

TABLE 7: Analysis of variance for Score a
(For meaning of asterisks, etc., see Table 6 footnotes)

Effect	Degrees of freedom	Mean square	Assuming fixed effects		Assuming random effects	
			F	P	F	p
L	20	4.3896	5.76	***	2.76	**†
P vs Co	1	18.6457	24.45	***	11.41	*‡
P	19	3.6392	4.80	***	2.36	**†
R	3	8.7048	11.42	***	2.15	N.S.
C	2	12.8168	16.81	***	3.03	N.S.
L x R	60	0.8946	1.17	N.S.	1.17	N.S.
(P vs Co) x R	3	1.6343	2.14	N.S.	1.17	N.S.
P x R	57	0.8570	1.13	N.S.	1.13	N.S.
L x C	40	0.9690	1.27	N.S.	1.27	N.S.
(P vs Co) x C	2	1.2168	1.60	N.S.	[<1	N.S.]
P x C	38	0.9559	1.26	N.S.		
R x C	6	3.5099	4.60	***		
L x R x C	120	0.6913	<1	N.S.		
(P vs Co) x R x C	6	1.3980	1.83	N.S.		
[P x R x C	114	0.6541	<1	N.S.]		
Tubes:LRC + P x R x C	1108	0.7589	1.14	*††		
Tubes:LRC + L x R x C	1114	0.7624	1.14	*††		
Tubes:LRC	994	0.7709	1.14	*††		
Within tubes	1222	0.6666				

TABLE 8: Analysis of variance for Score b
(For meaning of asterisks, etc., see Table 6 footnotes)

Effect	Degrees of freedom	Mean square	Assuming fixed effects		Assuming random effects	
			F	P	F	P
L	20	36.049	5.45	***	2.54	**†
P vs Co	1	128.317	19.39	***	5.09	<0.1†
P	19	31.193	4.73	***	2.25	*†
R	3	77.467	11.71	***	2.37	N.S.
C	2	105.731	15.98	***	3.17	N.S.
L x R	60	8.396	1.27	N.S.	1.27	N.S.
(P vs Co) x R	3	12.874	1.95	N.S.	1.25	N.S.
P x R	57	8.161	1.24	N.S.	1.24	N.S.
L x C	40	8.4125	1.27	N.S.	1.27	N.S.
(P vs Co) x C	2	14.343	2.17	N.S.	1.39	N.S.
P x C	38	8.100	1.23	N.S.		
R x C	6	27.020	4.08	***		
[L x R x C	120	5.688	<1	N.S.]		
(P vs Co) x R x C	6	10.322	1.56	N.S.		
[P x R x C	114	5.114	<1	N.S.]		
Tubes:LRC + P x R x C	1108	6.595	1.16	**		
Tubes:LRC + L x R x C	1114	6.615	1.17	**		
Tubes:LRC	994	6.727	1.19	***		
Within tubes	1222	5.667				

TABLE 9: Analysis of variance for Score c
(For meaning of asterisks, etc., see Table 6 footnotes)

Effect	Degrees of freedom	Mean squares	Assuming fixed effects		Assuming random effects	
			F	P	F	P
L	20	185.86	5.58	***	2.57	**†
P vs Co	1	674.37	20.25	***	5.38	0.1+
P	19	160.14	4.83	***	2.34	**†
R	3	439.48	13.20	***	2.36	N.S.
C	2	688.44	20.67	***	3.60	N.S.
L x R	60	42.44	1.27	N.S.	1.27	N.S.
(P vs Co) x R	3	73.81	2.22	N.S.	1.26	N.S.
P x R	57	40.79	1.23	N.S.	1.23	N.S.
L x C	40	42.78	1.28	N.S.	1.28	N.S.
(P vs Co) x C	2	62.51	1.88	N.S.	1.06	N.S.
P x C	38	41.75	1.26	N.S.		
R x C	6	157.49	4.73	***		
[L x R x C	120	29.39	<1	N.S.]		
(P vs Co) x R x C	6	58.79	1.51	N.S.		
[P x R x C	114	27.84	<1	N.S.]		
Tubes:LRC + P x R x C	1108	33.16	1.16	**		
Tubes:LRC + L x R x C	1114	33.30	1.17	**		
Tubes:LRC	994	33.77	1.18	***		
Within tubes	1222	28.56				

TABLE 10: Analysis of variance for Score d
(For meaning of asterisks, etc., see Table 6 footnotes)

Effect	Degrees of freedom	Mean square	Assuming fixed effects		Assuming random effects	
			F	P	F	P
L	20	52 691	5.89	***	2.67	***†
P vs Co	1	235 694	26.35	***	6.25	*†
P	19	43 059	4.84	***	2.34	**†
R	3	105 283	11.77	***	2.17	N.S.
C	2	188 146	21.03	***	3.62	*
L x R	60	10 631	1.19	N.S.	1.19	N.S.
(P vs Co) x R	3	20 298	2.27	N.S.	1.06	N.S.
P x R	57	10 122	1.14	N.S.	1.14	N.S.
L x C	40	12 438	1.39	N.S.	1.39	N.S.
(P vs Co) x C	2	20 475	2.29	N.S.	1.07	N.S.
P x C	38	12 015	1.35	N.S.		
R x C	6	41 963	4.69	***		
[L x R x C	120	7 973	<1	N.S.]		
(P vs Co) x R x C	6	19 201	2.14	*		
[P x R x C	114	7 382	<1	N.S.]		
Tubes:LRC + P x R x C	1108	8 889	1.16	**		
Tubes:LRC + L x R x C	1114	8 945	1.16	**		
Tubes:LRC	994	9 063	1.18	***		
Within tubes	1222	7 683				

TABLE 11: Analysis of variance for Score e
(For meaning of askerisks, etc., see Table 6 footnotes)

Effect	Degrees of freedom	Mean square	Assuming fixed effects		Assuming random effects	
			F	P	F	P
L	20	48 252	6.01	***	2.81	***†
P vs Co	1	200 651	25.00	***	11.49	*‡
P	19	40 230	5.05	***	2.49	**†
R	3	85 887	10.70	***	2.03	N.S.
C	2	138 897	17.31	***	3.05	N.S.
L x R	60	9 061	1.13	N.S.	1.13	N.S.
(P vs Co) x R	3	16 055	2.00	N.S.	[<1	N.S.]
P x R	57	8 693	1.09	N.S.	1.09	N.S.
L x C	40	10 970	1.37	N.S.	1.37	N.S.
(P vs Co) x C	2	16 674	2.08	N.S.	[<1	N.S.]
P x C	38	10 670	1.34	N.S.		
R x C	6	37 265	4.64	***		
[L x R x C	120	7 293	<1	N.S.]		
(P vs Co) x R x C	6	18 427	2.30	*		
[P x R x C	114	6 686	<1	N.S.]		
Tubes:LRC + P x R x C	1108	7 970	1.16	**		
Tubes:LRC + L x R x C	1114	8 026	1.17	**		
Tubes:LRC	994	8 117	1.18	**		
Within tubes	1222	6 870				

TABLE 12: Means of progenies and controls within individual runs and chambers

Response	Main effect	Class	Controls	Progenies	Difference
Infection (%)	Runs	1	76.9	68.9	8.0
		2	79.7	62.9	16.8
		3	88.0	80.8	7.2
		4	73.3	66.2	7.1
	Compartments	1	87.8	80.6	7.2
		2	77.0	68.2	8.2
		3	71.0	60.3	10.7
Dieback (%)	Runs	1	41.3	32.8	8.5
		2	53.2	36.9	16.3
		3	47.2	45.4	1.8
		4	36.3	32.4	3.9
	Compartments	1	43.9	44.1	-0.2
		2	43.8	34.2	9.6
		3	45.6	32.3	13.3
Score e (100 SD)	Runs	1	-12.1	10.7	22.8
		2	-27.9	9.8	37.7
		3	-25.9	-14.1	11.8
		4	0.0	13.4	13.4
	Compartments	1	-21.7	-12.3	9.4
		2	-16.8	10.2	27.0
		3	-10.7	17.1	27.8
Dieback/ Infection (%)	Runs	1	53.7	47.6	6.1
		2	66.8	58.7	8.1
		3	53.6	56.2	-2.6
		4	49.5	48.9	0.6
	Compartments	1	50.0	54.7	-4.7
		2	56.9	50.1	6.8
		3	64.2	53.6	10.6

NOTE: Means of Run and Compartment means are not quite identical to overall means because of the slight imbalance of the classification.

TABLE 13: Estimates of variance components, of narrow-sense heritabilities and repeatabilities of inoculation responses (infection, dieback, Score d, Score e), assuming fixed and random effects for compartments and runs. Data from controls not included in this analysis, except for purposes of arriving at appropriate transformation in the cases of Scores d and e. Values in brackets are narrow-sense heritabilities adjusted for all-or-nothing expression of traits

Effect	Component	Score d		Score e		Infection		Dieback	
		Fixed	Random	Fixed	Random	Fixed	Random	Fixed	Random
Progenies (P)	σ^2_p	358	309	338	299	0.00492	0.00408	0.00762	0.00596
[Runs (R)	σ^2_r	180	86	145	61	-	-	-	-]
[Chambers (c)	σ^2_c	297	223	222	155	-	-	-	-]
P x R	σ^2_{pr}	58		36		0.0008		0.00376	
P x C	σ^2_{pc}	145		89		0.0020		0.00217	
[R x C	σ^2_{rc}	272		248		-		-]	
(P x R x C) Tubes:PRC	σ^2_t	460		452		0.0100		0.01163	
Within tubes	σ^2_w	7799		6932		0.1973		0.203	
Narrow-sense heritability (h^2)									
$\hat{h^2} = \frac{4\hat{\sigma^2_p}}{\hat{\sigma^2_p} + \hat{\sigma^2_t} + \hat{\sigma^2_w}}$		0.17***	-	0.18***	-	0.10(0.17)***	-	0.14(0.22)***	-
$\hat{h^2} = \frac{4\hat{\sigma^2_p}}{\hat{\sigma^2_p} + \hat{\sigma^2_{pr}} + \hat{\sigma^2_{pc}} + \hat{\sigma^2_t} + \hat{\sigma^2_w}}$		-	0.14**	-	0.15**	-	0.08(0.14)*	-	0.10(0.17)*
Repeatability of progeny means (H^2)									
$\hat{H^2} = \frac{4\hat{\sigma^2_p}}{\hat{\sigma^2_p} + \hat{\sigma^2_{t/48}} + \hat{\sigma^2_{w/96}}}$		0.80***	-	0.81***	-	0.66***	-	0.76***	-
$\hat{H^2} = \frac{\hat{\sigma^2_p}}{\hat{\sigma^2_p} + \hat{\sigma^2_{pr/4}} + \hat{\sigma^2_{pc/3}} + \hat{\sigma^2_{t/48}} + \hat{\sigma^2_{w/96}}}$		-	0.67**	-	0.72**	-	0.58*	-	0.60*

* denotes significant ($P < 0.5$)

** denotes highly significant ($P < 0.01$)

*** denotes very highly significant ($P < 0.001$)

TABLE 14: Correlations between progeny mean values for different criteria of inoculation response

	Dieback (%)	Dieback/ Infection	Score				
			a	b	c	d	e
Infection (%)	0.861	0.634	0.903	0.909	0.932	-0.945	-0.936
Dieback (%)		0.934	0.979	0.991	0.984	-0.973	-0.974
Dieback/ Infection			-	-	-	-	-
Score a				0.993	0.992	-0.987	-0.986
b					0.998	-0.991	-0.992
c						-0.996	-0.996
d							0.997

TABLE 15: Analysis of variance for dieback with sums of squares adjusted for individual covariance on infection. Controls pooled to represent a single lot

Effect	Degrees of freedom	Mean square	Assuming fixed effects		Assuming random effects	
			F	P	F	P
Lots (L)	20	.4374	2.38	**	1.29‡‡	N.S.
Runs (R)	3	.9703	5.28	**	2.07‡‡	N.S.
Chambers (C)	2	.1843	1.00	N.S.	<1‡‡	N.S.
L x R	60	.2311	1.26	N.S.	1.26	N.S. (P ≅0.1)
L x C	40	.2516	1.37	N.S.	1.37	N.S. (P ≅0.1)
R x C	6	.3249	1.77	N.S.	1.77	N.S. (P ≅0.1)
L x R x C Tubes:LRC	1114	.1838	1.13	*†	1.13	*†
Within tubes	1222	.1631				

* denotes significant (P <0.05)

** denotes highly significant (P <0.01)

N.S. denotes not significant (P >0.05)

† indicates rough interpolation of significance level

‡‡ Satterthwaite approximation for F and degrees of freedom

TABLE 16: Correlations between mean inoculation responses (by several criteria) of progenies and levels of individual monoterpenes (% total monoterpenes) in cortical oleoresin of seed parents, and between levels of different monoterpenes

	α-pinene	camphene	β-pinene	sabinene	Δ^3 carene	myrcene	α-terpinene	limonene	β-phellandrene	γ-terpinene	p-cymene	terpinolene
Infection	.069	-.065	.056	-.328	.216	-.292	-.157	.072	-.288	-.067	-.334	-.227
Dieback (dbk)	.158	-.004	-.021	-.296	.327	-.415	-.237	-.033	-.310	-.019	-.353	-.168
Dbk / Infection	.206	.030	-.063	-.214	.329	-.464	-.257	-.095	-.278	-.013	-.361	-.092
Score d	-.172	-.018	.000	.339	-.288	.359	.211	.001	.314	.036	.334	.215
α-pinene		.915	.127	.183	-.292	-.023	.096	-.669	-.129	.354	.100	.040
camphene			.111	.063	-.279	-.071	.128	-.695	.032	.418	.368	.115
β-pinene				-.339	-.360	.011	-.248	-.570	-.111	-.247	-.141	-.327
sabinene					-.470	.160	.062	.160	-.264	.521	.241	.957
Δ^3 carene						-.538	.324	.047	-.038	-.022	.006	-.338
myrcene							-.104	.278	.505	-.003	.103	-.031
α-terpinene								-.176	-.074	.131	.186	.043
limonene									.117	-.294	-.142	.079
β-phellandrene										-.402	-.113	-.369
γ-terpinene											.466	.581
p-cymene												.317
terpinolene												

P = 0.05, r_{18} = 0.444 P = 0.001, r_{18} = 0.679

P = 0.01, r_{18} = 0.561

This will be repeated in the report specifically dealing with relationship between dieback and monoterpene composition

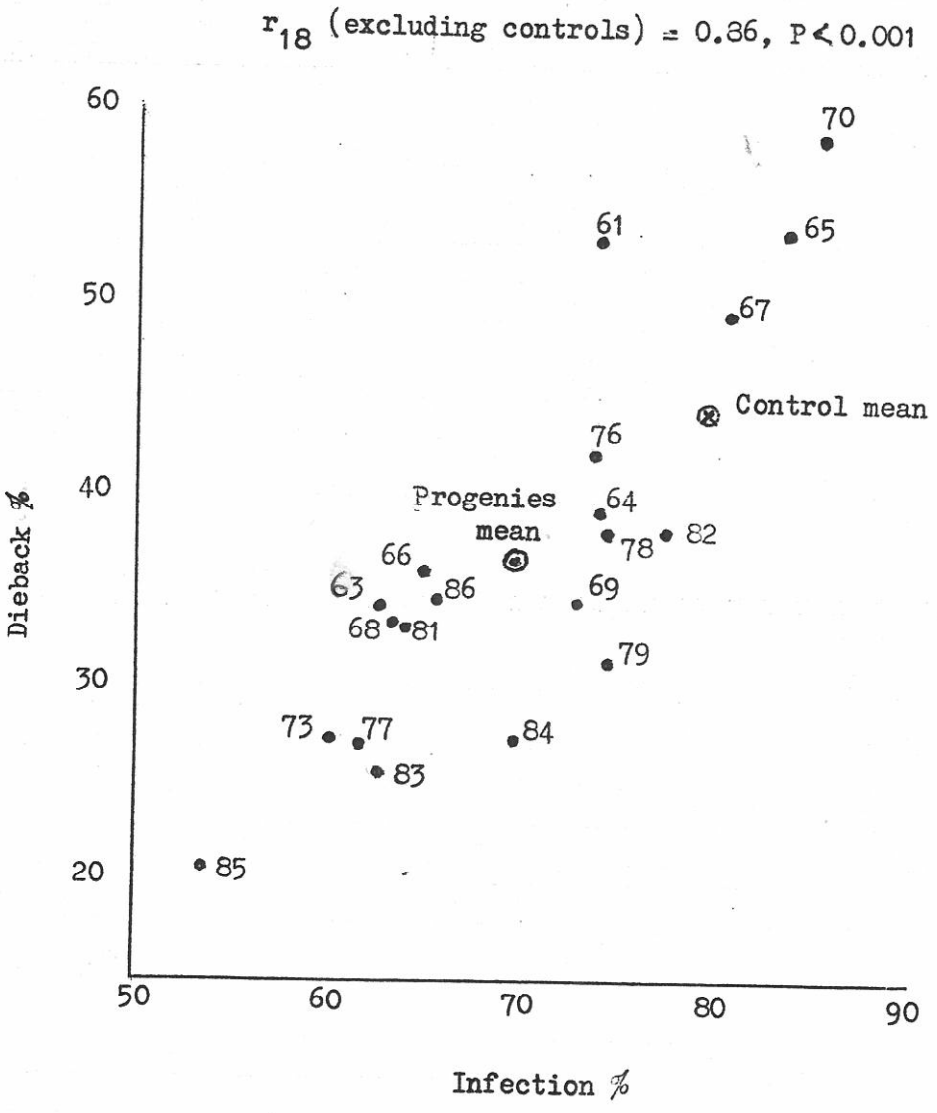
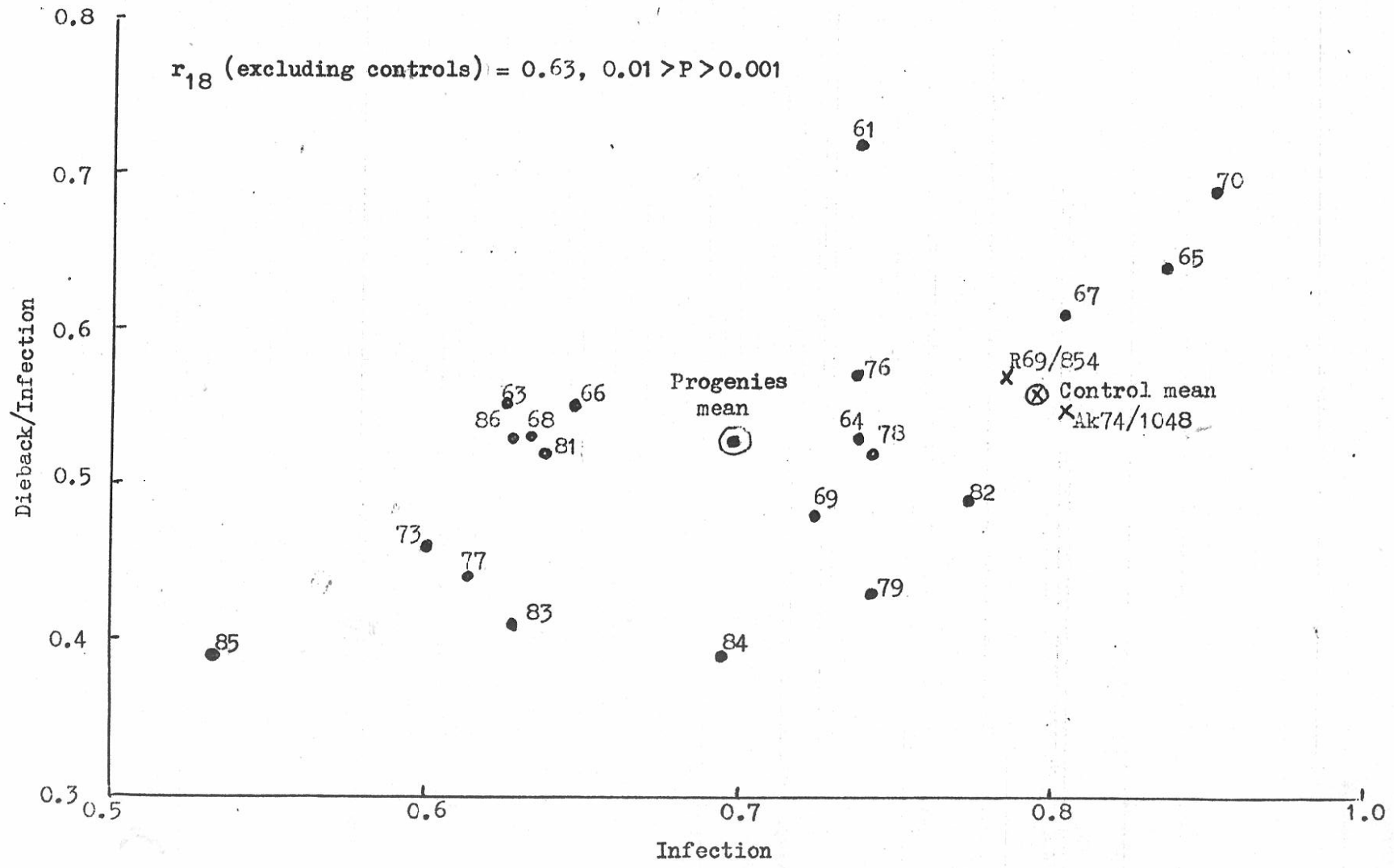


FIG. 1 - Dieback % vs Infection %, by Lots

FIG. 2 - Dieback/Infection vs Infection, by Lots



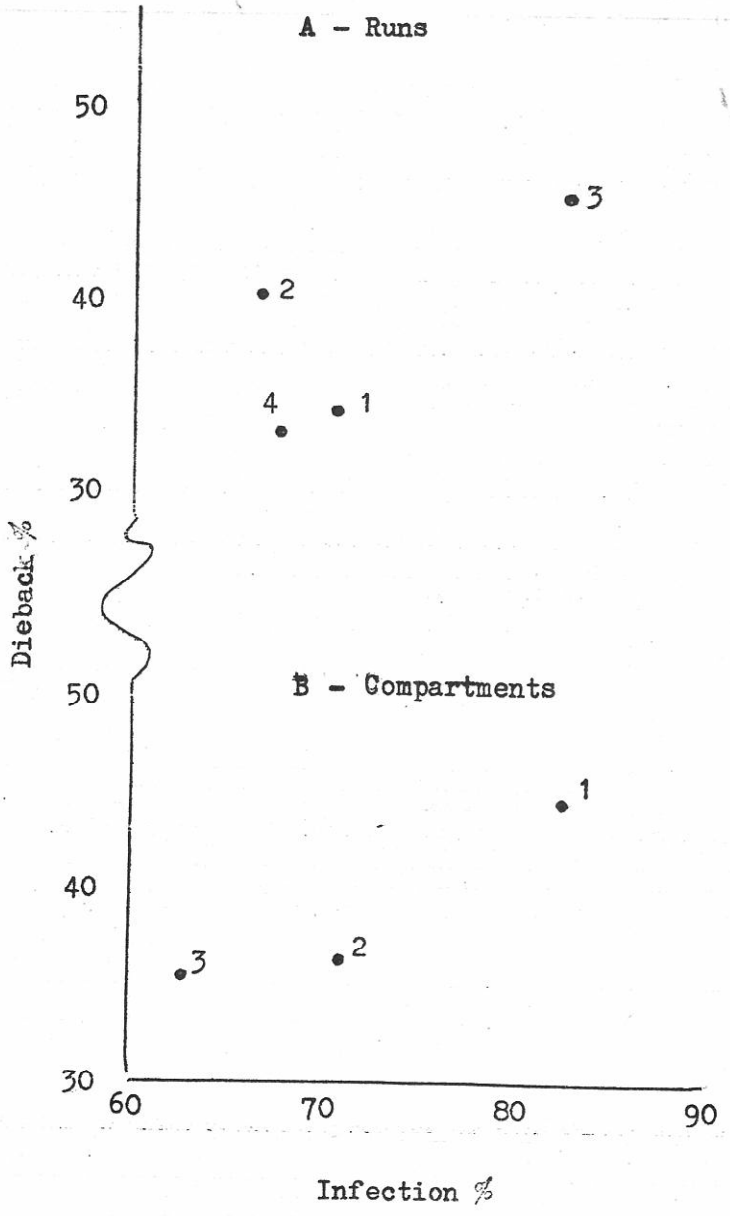


FIG. 3 - Dieback % vs Infection % (A) by Runs
(B) by Compartments

APPENDIX I: Examination of experimental error items in Controls and Progenies

The strict validity of analysis of variance depends on homogeneity of error variance. Mean squares for the tubes and within-tubes effects are shown for the control and the progenies respectively (Table (i)). Differences in corresponding mean squares between the two groups of materials were not great. There does appear to be significant heterogeneity in the within-tube mean squares for infection, but this is presumably a scalar effect, since the controls which showed the lower mean square had an infection rate which was beginning to approach 1. Overall, it is considered that all results could be satisfactorily pooled in a single analysis of variance for each criterion of response.

TABLE (i): List of experimental error mean squares for controls and progenies respectively

Response criterion	Between tubes within-LRC M.S.		Within-tubes M.S.	
	Controls	Progenies	Controls	Progenies
Dieback	.286	.231	.208	.203
Infection	.163	.200	.148	.179
Score a	7.44	6.45	5.24	5.80
Score b	36.25	32.82	26.19	29.27
Score c	0.796	0.761	0.609	0.684
Score d	8378	8016	6669	6931
Score e	9406	8930	7301	7799

NOTE: The between-tubes mean squares are only slightly altered if the lots x runs x chambers sums of squares are pooled with the tubes mean squares.

APPENDIX II: Adjustment of estimated heritability of all-or-nothing characters

Where the heritability is estimated by analysis of variance with only two possible numerical values (normally 0 and 1) for the trait, the adjustment provides an estimate of heritability for relating to an underlying continuously varying scale. This represents one measure of the heritability of the predisposition to the disease phenomenon.

The adjustment is given by

$$h_x^2 = h^2 \times \frac{p(1-p)}{Z^2}$$ (Dempster and Lerner, 1950; Van Vleck, 1972)

where h^2 = apparent heritability as estimated from analysis of variance

h_x^2 = heritability on the underlying continuously varying scale

p = proportion of individuals exhibiting the phenomenon

Z = height of the ordinate of the normal distribution at the threshold percentile point.

The value for Z is obtained in two steps. First, obtain x from Fisher and Yates (1963), Table 1. P in this table corresponds to 2p (or 2(1-p), whichever is the smaller), since we are concerned with only one tail of the distribution. For the appropriate x, Z can then be obtained from Fisher and Yates, Table 2.

From simulation studies (Van Vleck, 1972) it appears that the adjustment causes little if any bias for the frequencies of infection and dieback which were actually encountered. Not covered, however, is how the validity of the correction is affected by the existence of a blocked layout.

Dempster and Lerner have noted that the use of the adjustment to give $\hat{h}_x^2$ independent of p involves the assumption that a single predisposing factor is involved. If there are several predisposing factors, of differing heritabilities, then h_x^2 as estimated above can be expected to vary as environmental conditions interact with different factors to vary p.

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