# Armillaria root disease of *Pinus radiata* in New Zealand (3)

# ARMILLARIA ROOT DISEASE OF *PINUS RADIATA* IN NEW ZEALAND.
# 3: INFLUENCE OF THINNING AND PRUNING

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## ABSTRACT

Armillaria root disease persists in many seemingly healthy stands of *Pinus radiata* D. Don throughout New Zealand. Options for control or management remain limited, but one possibility may be to manipulate silviculture in a way that minimises chronic stand infection. A 3.1-ha trial in a second-rotation stand planted in 1985 was used to compare the effects of different thinning treatments approximately halfway through the rotation on a site not formerly covered in native forest. Treatments that created stumps at stand age 7 years appeared to increase the incidence of infection 5.5 years later (to 44% and 46% trees infected, for stocking levels of 500 and 250 stems/ha, respectively), compared with unthinned controls (30% infection, 810 stems/ha). Incidence was significantly greater (49%) among trees within 5 m of stumps derived from trees infected by *Armillaria* species before thinning, than among trees with no neighbourhood stumps (29%). Not all stumps could be found after 5.5 years, but of the 76% that were, at least 58% were colonised by species of *Armillaria*. Incidence and severity of infection appeared unaffected by pruning to 30% of tree height as measured at age 13 years. Infection was distributed unevenly across the trial area at ages 6 and 12.5 years. However, the overall spatial infection pattern did not alter over this period.

Cultural pairing between isolates of *Armillaria* species made from 23% of all infected or colonised trees prior to thinning identified a minimum number of 68 genets (at least 22 per hectare). Further cultures isolated 5.5 years after thinning from trees or stumps in six treatment plots belonged to 41 genets (44 per hectare, minimum), of which at least 20% were present prior to treatment. These high colony densities strongly imply a role for basidiospores in the dissemination of *Armillaria* species, whether or not colonies were established in first-crop stumps or earlier. All colonies were of *A. novae-zelandiae* (Stevenson) Herink except for one of *A. limonea* (Stevenson) Boesewinkel, which was present before thinning and subsequently re-isolated from the same location. Infection will be re-evaluated later in the rotation to determine the effect of larger stumps generated by a second thinning at age 13.5 years. In the meantime, caution appears warranted when thinning on heavily infested sites. Research is under way for an alternative control method for use in association with low-intensity thinning.

**Keywords**: root disease; thinning; pruning; silviculture; *Armillaria novae-zelandiae*; *Armillaria limonea*; *Pinus radiata*.

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## INTRODUCTION

Although chronic infection by pathogenic species of *Armillaria* is widespread in New Zealand forests of *Pinus radiata* (Hood 1989; Self *et al.* 1998), options for managing affected plantations remain limited. One possibility may be to manipulate stand silviculture in a way that discourages infection, especially if this can be integrated operationally with other forms of control currently under investigation. However, at present this approach is hampered by a lack of information about the effects of silviculture on the development of the fungi. Thinning boosts vigour by reducing competition stress, thereby conceivably enhancing disease resistance among residual host trees (e.g., Wargo & Harrington 1991; Filip & Goheen 1995; Filip *et al.* 1999). Conversely, the additional stump wood substrate made available to the pathogens may increase the inoculum pressure. In order to resolve this issue, a thinning trial was established in 1990 in a 5-year-old, second-rotation plantation of *P. radiata* affected by Armillaria root disease, which succeeded a 54-year-old stand of *P. nigra* ssp. *laricio* (Poir.) Maire (Hood & Sandberg 1993a). The results from this trial after the first pre-commercial thinning are reported in this paper, including the effects of different thinning treatments on disease incidence and severity, potential changes in the spatial distribution of the pathogen, and the results of cultural studies with isolates of *Armillaria* obtained from the trial site. This work was undertaken as part of an investigation to clarify how forest management influences the behaviour of populations of *Armillaria* species.

## MATERIAL AND METHODS
### Effect of Treatment on Incidence and Severity of Infection

The experimental stand was situated on a flat, level area in Cpt 365, Kaingaroa Forest, and was planted at ca. 850 stems/ha on parallel V-bladed mounds after burning of the slash from the previous crop. The trial (Fig. 1) was set up as a randomised complete block design, with four blocks of five nearly square plots (39 × 39.8 m) occupying a total area of 3.1 ha (Hood & Sandberg 1993a). The first pre-commercial thinning was undertaken in May 1992, at stand age 7 years. There were five thinning and pruning treatments:



FIG. 1–Trial design. Treatment numbers: 1 = unthinned, unpruned (control); 2 = thinned to 250 stems/ha, pruned; 3 = thinned to 250 stems/ha, pruned, no stumps; 4 = unthinned, pruned; 5 = thinned to 500 stems/ha, pruned. Solid lines indicate block boundaries. Trial dimensions, 195 × 159 m.

(1) Unthinned — 810 stems/ha, not pruned; control

(2) Thinned to 250 stems/ha, residual trees pruned

(3) Thinned to 250 stems/ha, by whole-tree extraction using a cable (no thinning stumps), residual trees pruned

(4) Unthinned, pruned (to be thinned to 250 stems/ha at age 13.5 years)

(5) Thinned to 500 stems/ha, pruned (to be thinned further to 250 stems/ha at age 13.5 years).

Criteria for selection were large size and straight form (dominants and codominants retained), but bigger trees with multiple leaders, forks, broken tops, or large branches were rejected. All trees in Treatments 2 to 5 were pruned to 2.2 m at the time of thinning. Some trees received further unintended pruning as part of forest operations up to stand age 11 years. Mean pruning heights (percentage tree height) on all residual trees at age 13 years were 34% in Treatment 2, 32% in Treatment 3, 27% in Treatment 4, and 29% in Treatment 5. Mean percentage green crown depths at the same age were 56% in Treatment 1, 69% in Treatment 2, 68% in Treatment 3, 60% in Treatment 4, and 66% in Treatment 5 (Kimberley *et al.* 2002). Stump diameters were measured immediately after thinning. All trial trees were evaluated for infection by *Armillaria* species at approximate age 6 years, shortly before thinning. Residual trees were again evaluated between November 1997 and January 1998 at age 12.5 years, 5.5 years after thinning. A light, short-handled grubber was used to expose the root collar zone with minimal bark disturbance in order to estimate the severity of infection, defined by the extent of girdling by a zone of externally visible resinosis, confirmed in each case by the presence of rhizomorphs. Each tree was assigned to one of five girdling classes: 0 = 0%; 1 = 1–25%; 2 = 26–50%; 3 = 51–100%; and 4 = dead, and the direction (aspect or azimuth) of the midpoint of the girdling zone was also recorded on the field sheets. Thinning stumps were exposed in the same way and partially dissected in February 1998. A record was made of stump colonisation by *Armillaria* species, identified by a characteristic decay pattern (Gilmour 1954) accompanied by rhizomorphs or white mycelial fans (rhizomorphs alone, growing through well-decayed stump wood, were not considered a sufficient criterion of colonisation).

A randomised complete block analysis of variance of plot means was used to compare, between silvicultural treatments, the percentage incidence of trees infected and the mean infection severity (root collar girdling score) during the second evaluation, using values from the first evaluation as covariates. Edge rows of each plot were regarded as buffer rows, and were not included in this analysis. The SAS procedure GLM (SAS Institute Inc.1989) was used for this analysis).

An individual tree analysis was also undertaken to test whether adjacent thinning stumps may have influenced the incidence of infection. For this analysis, individual trees were classified according to the presence or absence of thinning stumps within a specified radius into three classes:

(1) No thinning stumps within the neighbourhood radius

(2) At least one thinning stump, but none derived from trees infected prior to thinning

(3) At least one thinning stump derived from a pre-thinning infected tree.

A range of radii between 5 m and 10 m was tested. The mean incidence of infection was calculated for each class in each block. To improve the sensitivity of this analysis, means

were adjusted to take account of the pre-thinning localised incidence of the disease. This was achieved by fitting a logistic regression model using the procedure LOGISTIC (SAS Institute Inc. 1989), with the dependent variable being presence/absence of infection in each living tree in the second evaluation. The independent variables were the presence/absence of infection in the subject tree before thinning and the mean infection incidence of trees within the specified radius of the subject tree before thinning. Adjusted means were obtained by subtracting the incidence predicted by the regression equation from the actual incidence, and adding the overall mean incidence. Adjusted means for each class in each block were obtained in this way, and classes were compared using a randomised complete block analysis of variance.

## Spatial Effects

To appreciate how Armillaria root disease progresses in a stand, it was of value to know if infection altered in magnitude and direction around individual trees during the study period. Comparisons were therefore made between each evaluation for relative proportions of trees in different categories of infection severity and direction of root collar girdling, as estimated by the zone of resinosis. Girdling scores (severity) were tested using a $4 \times 5$ contingency table (omitting trees that had died by the time of the first evaluation). The directional aspect was examined by comparing frequencies of occurrence of the girdling midpoint in each of four quadrants around the tree (N/NW, E/NE, S/SE, and W/SW). Possible movement around trees was investigated by testing the association between the presence or absence of infection in each of the four quadrants, for each of the two evaluations. Presence in each quadrant was derived from the data recording the extent of root collar girdling (from the girdling score) and the midpoint direction of the girdled zone. A two-way analysis of variance was used to test the significance of quadrant differences, incorporating terms for tree and quadrant. This analysis was restricted to trees with a clear directional aspect to infection in both evaluations (i.e., data were excluded from trees with infection either present or absent in all four quadrants during either evaluation, or if there was more than one infection point around the root collar in the same evaluation). Infection present in the same quadrant in both evaluations was defined as a change of zero (0); movement to a neighbouring quadrant on either side indicated a change of 1, and to the opposite quadrant a change of 2. Actual frequencies in each of these classes were compared with expected theoretical values for the null hypothesis that the directions between evaluations were independent (0: 25%; 1: 50%; 2: 25%). The actual and expected numbers of trees in each class were tested using a $\chi^2$ goodness-of-fit test.

## Nature of Infection

Isolates of *Armillaria* species from dead trees (Hood & Sandberg 1993a), and from rhizomorphs collected from living infected trees between 1990 and 1993 (stand age 5–8 years, up to 12 months after the first thinning, buffer trees included) were made on to 3% malt agar. All except two isolates, which were not tested, were identified to species using colony appearance after culturing for 3 weeks under a 24-hour fluorescent tube photoperiod at 8–11 $\mu E/m^2/sec$, or compatibility with single-basidiospore-isolate tester cultures of different *Armillaria* species, or both (Benjamin 1983; Hood & Sandberg 1993a). Field isolates were also separated into genets (vegetative compatibility groups, somatic incompatibility groups)

by pairing together on 3% malt agar and noting the presence or absence of mutual antagonism after 3–6 weeks. Incompatibility was indicated by the formation of a typical thin brown interaction zone, or sometimes by the development of a dividing seam or furrow associated with disparate morphology between paired cultures (Hood & Sandberg 1987, 1993a). Results were sorted and genets delineated with the aid of the SAS procedure PROC SORT (SAS Institute Inc. 1989). A further series of cultures of *Armillaria* species was isolated from rhizomorphs collected from stumps or residual infected trees or both in six selected plots during February and March 1998 (stand age 12.5 years), nearly 6 years after thinning. Sampling was undertaken in Plots 14, 17, 21 (Treatment 2; thinned to 250 stems/ha) and Plots 6, 12, 16 (Treatment 3; thinned to 250 stems/ha, no stumps). These isolates were also typed in the same way into genets, one culture of which was identified to species. At least one culture of each genet isolated during the second series was tested against one culture of each genet isolated from nearby trees (located within half a plot length radius) during the first series. The distributions of genets (colonies of *Armillaria* species) were mapped and compared within and between both isolation series.

## RESULTS
### Effect of Treatment on Incidence and Severity of Infection

Infection by *Armillaria* species was widespread in the trial stand during the study period (Fig. 2 and 3). Incidence of mortality recorded at age 6 years was 3%, and a further 22% of living, green-crowned trees were infected at the root collar. Mortality after age 6 years was negligible, but incidence of chronic infection had generally increased by age 12.5 years. Incidence and severity of infection are shown in Tables 1 and 2 and Fig. 4 for all thinning treatments during each evaluation. Overall treatment was significant for incidence ($F_{4,11}$ = 3.83, p = 0.035) but not for severity ($F_{4,11}$ = 2.12; p = 0.15). However, a stronger trend for both was indicated when specific treatments were compared with the unthinned, unpruned control (Treatment 1). Treatments that left stumps in the ground (i.e., Treatment 2, thinned to 250 stems/ha, $F_{1,11}$ = 10.80; p = 0.007; Treatment 5, thinned to 500 stems/ha, $F_{1,11}$ = 6.31, p = 0.029) appeared to result in a greater increase in incidence of infection than those that did not (Treatment 3, thinned to 250 stems/ha, no stumps, $F_{1,11}$ = 1.34, p = 0.27; Treatment 4, unthinned, pruned, $F_{1,11}$ = 0.23, p = 0.64). The pattern for infection severity appeared similar (Table 2), although the results were statistically less significant (Control *v.* Treatment 2, $F_{1,11}$ = 5.36; p = 0.041; Control *v.* Treatment 5, $F_{1,11}$ = 4.34, p = 0.061). The comparison between all treatments that left stumps in the ground against those that did not (Treatments 2 and 5 *v.* 1, 3, and 4) gave an even stronger result ($F_{1,11}$ = 13.31, p = 0.0038 for incidence of infection; $F_{1,11}$ = 8.05, p = 0.016 for infection severity). Pruning appeared to have little effect (Treatments 1, 4, Fig. 4).

Exploring the treatment trend further using individual tree analysis, strong evidence was found of a higher incidence of infection in trees adjacent to thinning stumps which had been recorded with root collar infection during the pre-thinning assessment 5.5 years earlier. Of the several neighbourhood radii tested, the strongest effect was apparent for the smallest radius of 5 m (Table 3). This analysis included an adjustment for localised pre-thinning incidence of infection by *Armillaria* species. The increased incidence in trees close to thinning stumps was consistent across the four blocks in the trial. Compared with trees with no neighbourhood stumps, which had a mean incidence of 29.2%, those near stumps derived
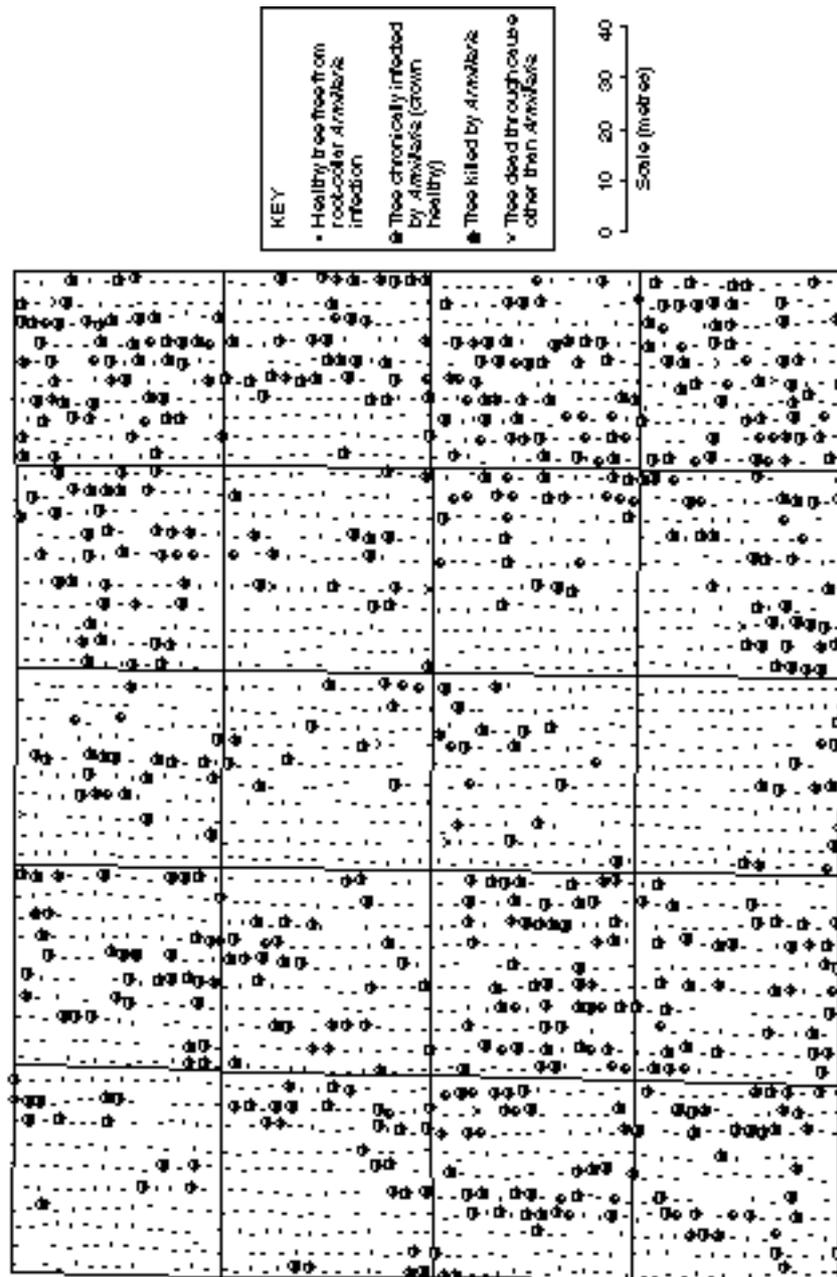
FIG. 2–Distribution of trees infected by *Armillaria* species at stand age 6 years, ca. 1 year prior
to thinning (tree positions mapped using aerial photography prior to crown closure).

from trees without root collar infection had an incidence of 37.0%, which was higher, but not
significantly so ($F_{1,6} = 3.67$, $p = 0.10$). However, those near stumps from previously infected
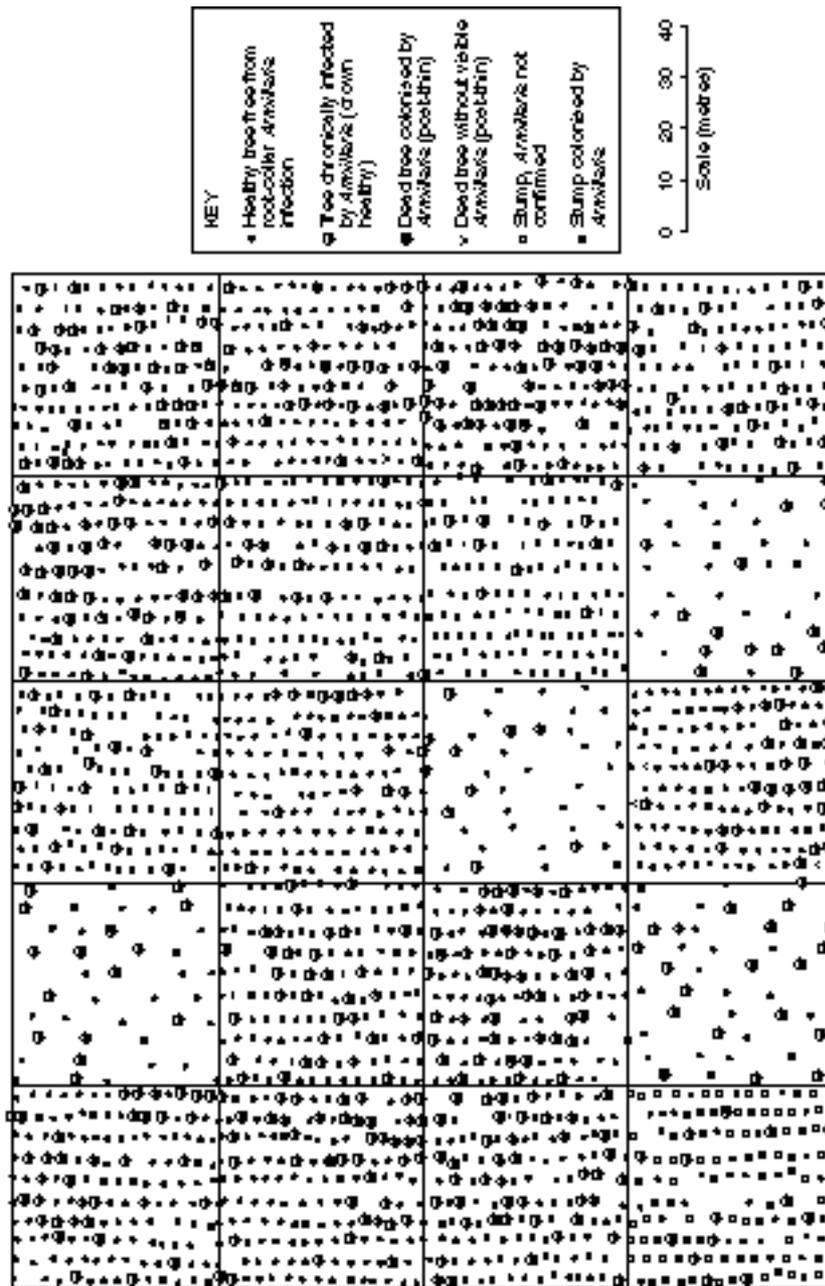trees had an even higher mean incidence of 48.9% (tested against trees without neighbouring

FIG. 3–Distribution of trees infected by *Armillaria* species at stand age 12.5 years, ca. 5.5 years after thinning (for treatments, refer Fig. 1; all thinning stumps shown, but presence of *Armillaria* based on field inspection of residual stumps, not on root collar infection in parent tree).

stumps, $F_{1,6} = 23.61$, $p = 0.0030$). The test between trees near infected stumps against those near uninfected stumps was also significant ($F_{1,6} = 8.66$, $p = 0.026$).

TABLE 1–Mean percentage incidence of trees infected by *Armillaria* species, by silvicultural treatment

| Treatment | First, pre-treatment evaluation, age 6 years | | Second evaluation, 5.5 years after treatment, age 12.5 years | | |
|---|---|---|---|---|---|
| | All trees | Residual, post-thin trees only | Unadjusted | Adjusted for covariate | s.e. |
| (1) Control, unthinned (810 stems/ha), unpruned | 22.1 | 21.7 | 28.9 | 29.0 | 4.7 |
| (2) Thinned to 250 stems/ha, pruned | 23.0 | 20.4 | 49.8 | 50.8 | 4.7 |
| (3) Thinned to 250 stems/ha, pruned; no stumps | 22.8 | 19.9 | 35.3 | 36.7 | 4.7 |
| (4) Unthinned, pruned | 19.9 | 21.8 | 32.2 | 32.1 | 4.7 |
| (5) Thinned to 500 stems/ha, pruned | 22.8 | 24.6 | 47.9 | 45.7 | 4.7 |

Post-thin values exclude buffer rows

TABLE 2–Mean percentage severity of infection by *Armillaria* species (mean girdling score), by silvicultural treatment

| Treatment | First, pre-treatment evaluation, age 6 years | | Second evaluation, 5.5 years after treatment, age 12.5 years | | |
|---|---|---|---|---|---|
| | All trees | Residual, post-thin trees only | Unadjusted | Adjusted for covariate | s.e. |
| (1) Control, unthinned (810 stems/ha), unpruned | 0.428 | 0.413 | 0.439 | 0.451 | 0.091 |
| (2) Thinned to 250 stems/ha, pruned | 0.479 | 0.472 | 0.773 | 0.751 | 0.091 |
| (3) Thinned to 250 stems/ha, pruned; no stumps | 0.453 | 0.361 | 0.476 | 0.517 | 0.091 |
| (4) Unthinned, pruned | 0.416 | 0.435 | 0.520 | 0.520 | 0.091 |
| (5) Thinned to 500 stems/ha, pruned | 0.457 | 0.487 | 0.752 | 0.722 | 0.091 |

Post-thin values exclude buffer rows; unadjusted scale, 0–3

Mean stump diameter after thinning was 17 ± 5 cm (Treatments 2 and 5; with standard deviation; n = 530). Many thinning stumps had already disintegrated when they were assessed 5.5 years after cutting, and 24% were not found. At least 58% of the residual thinning stumps were colonised by *Armillaria* species, representing a minimum incidence of 44% of all original stumps.

## Spatial Effects

The covariates in the analyses undertaken in the previous section were found to be statistically significant. Infection incidence ($F_{1,11}$ = 13.68, p = 0.0035) and severity ($F_{1,11}$ = 8.51, p = 0.014), respectively, at age 6 years were each significantly associated with those at age 12.5 years (cf. Fig. 2 and 3). However, with the covariate from the first evaluation incorporated into the analysis, there was no significant difference between blocks, confirming that there was little spatial variability between assessments, other than that already evident at age 6 years.
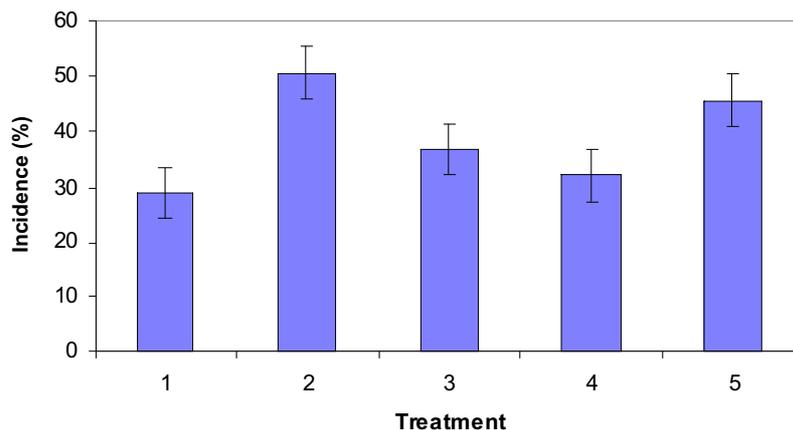
FIG. 4–Mean percentage incidence of trees infected by *Armillaria* species at age 12.5 years, adjusted for pre-thinning incidence at age 6 years (error bars indicate standard errors). Treatment numbers: 1 = unthinned, unpruned; 2 = thinned to 250 stems/ha, pruned; 3 = thinned to 250 stems/ha, pruned, no stumps; 4 = unthinned, pruned; 5 = thinned to 500 stems/ha, pruned. Treatments 2 (p, 0.007) and 5 (p, 0.029) differ significantly from Treatment 1 (control); Treatments 3 and 4 do not.

TABLE 3–Mean percentage incidence of trees infected by *Armillaria* species at age 12.5 years *v.* presence/absence of thinning stump within a 5-m radius within each block in the trial. Mean incidence values at 12.5 years were adjusted for localised pre-thinning incidence of infection at age 6 years.

|  | Block | | | | Mean | Total number of trees |
| --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | | |
| No stump within 5 m | 33.7 | 25.0 | 25.2 | 32.8 | 29.2 | 868 |
| Stump within 5 m, derived from uninfected tree | 43.1 | 36.4 | 31.1 | 37.3 | 37.0 | 351 |
| Stump within 5 m, derived from infected tree | 50.8 | 54.2 | 51.7 | 38.9 | 48.9 | 272 |

Changes in infection severity with stand age are demonstrated in Table 4, in which the post-thinning percentage distribution of girdling class at age 12.5 years for trees within each pre-thinning girdling class at age 6 years is given. Although there is a statistically significant association between scores from both evaluations ($\chi^2_{16} = 271$, p < 0.001), there were also considerable differences among individual trees between severity classes. For example, 55% of Class 1 trees, 39% of Class 2 trees, and 24% of Class 3 trees were not found infected in the second evaluation. The directional distribution of infection around tree root collars showed virtually no overall net pattern within or between evaluations (Fig. 5), but this analysis does not discriminate for circumferential movement in individual trees. To test this, the percentages in each of the directional change classes between evaluations were determined, respectively, as 33% (Class 0), 52% (Class 1), and 15% (Class 2). Compared with the expected values for total association between evaluations (Class 0 = 100%; Class 1 = 0%; Class 2 = 0%), or for total independence (Class 0 = 25%; Class 1 = 50%; Class 2 = 25%), the results are close to the latter. Although the hypothesis of total independence is rejected

TABLE 4–Percentage distribution of severity of infection at stand age 12.5 years (5.5 years after thinning) for each pre-thinning severity class (age 6 years)

| Girdling score at age 6 years* | Girdling score* at age 12.5 years | | | | | Total number of trees |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | |
| 0 | 73 | 18 | 6 | 3 | 0.1 | 1311 |
| 1 | 55 | 27 | 14 | 4 | 0 | 138 |
| 2 | 39 | 34 | 21 | 6 | 0 | 97 |
| 3 | 24 | 24 | 24 | 28 | 0.9 | 115 |
| Total No. trees | 1093 | 341 | 142 | 82 | 3 | 1661 |

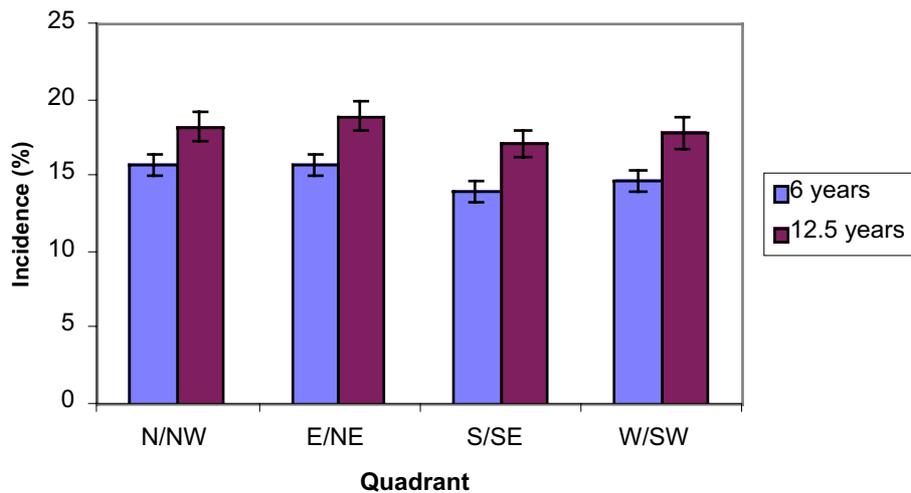*0 = no girdling; 1 = 1–25%; 2 = 26–50%; 3 = 51–100%; 4 = 100% girdled and dead



FIG. 5–Direction of infection around trees at stand age 6 years (1991–92) and, after thinning, at 12.5 years (1997–98) (percentage girdling midpoint incidence for each quadrant; error bars indicate standard errors).

($\chi^2_2$ = 8.82, p = 0.012), the directional association between the two assessments is clearly extremely weak, implying considerable movement of infection around root collars during the study period.

## Nature of Infection

In the first isolation series, cultures of *Armillaria* species were obtained from each of 142 dead or living trees, 23% of those colonised or infected in the first evaluation. Of these, 75 were isolated no later than July 1992, not more than 2 months after thinning, and all but two of all the post-thinning isolates were from trees known to have been infected before thinning. These cultures were paired in more than eight thousand combinations (80% of all possible arrangements), and most (90%) were also self-paired (all proving self-compatible). Computer sorting identified 68–82 compatibility groups among these isolates, representing a minimum

*Armillaria* species population density of 22–27 genets or colonies per hectare in the study area (Fig. 6). Results of the majority of interactions were unambiguous. However, some pairings yielded unclear or doubtfully compatible results, even when tests were repeated, indicating possible non-homogeneity among 13 of the 68 genets in the initial selection. These results accounted for up to 14 further potential genets, or an alternative minimum genet number of 82, as noted above (indicated by ' signs in Fig. 6). In addition, Genets 19 and 64, although clearly distinct, showed an element of apparent compatibility between some isolates. Colonies were typically small in size (e.g., Genet 3, Plot 1; Genet 11, Plot 14; Genet 18, Plots 16, 17, 21, 22; Genet 23, Plot 24; Genets 48, 49, Plot 7; Genets 10, 58, Plot 9) (Fig. 6), but isolates of some were dispersed over a wider area (e.g., "Genet" 31/31', Plots 1, 22; "Genet" 40/40', Plots 1, 2, 22; Genet 37, Plots 2, 8, 16). Results of cultural tests to identify species were unambiguous and concordant between methods where both were performed, with one exception (the single-spore compatibility test result is given for Genet 58 Plot 9, because of uncertainty with the 24-hour photoperiod result). All 85 colonies and
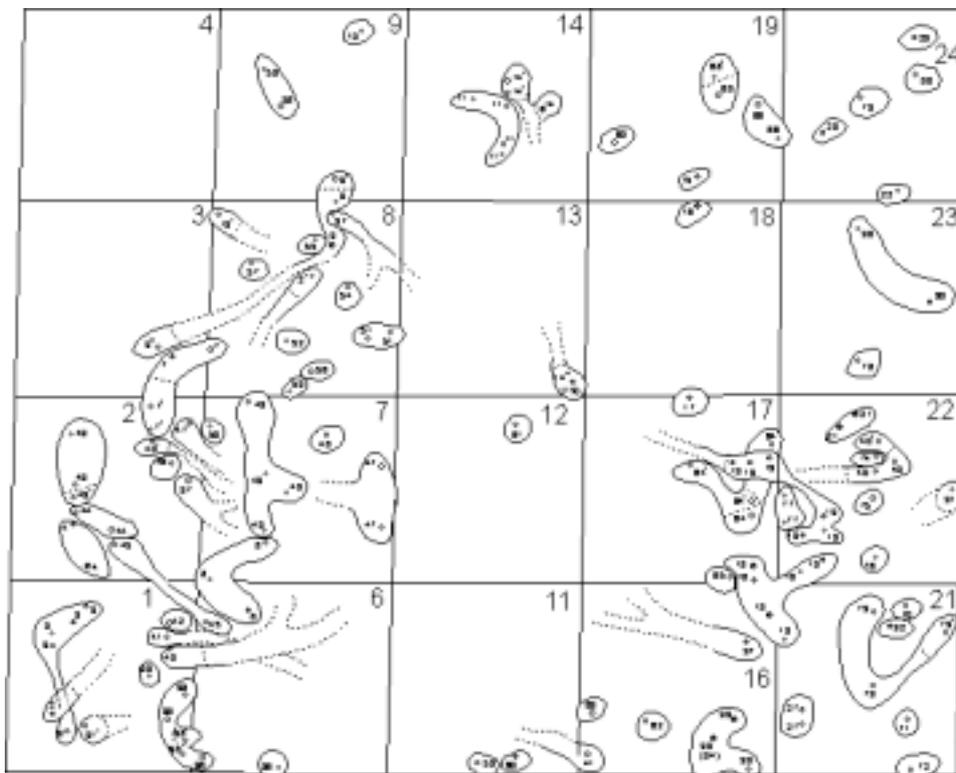


FIG. 6–Distribution of *Armillaria* isolates taken from trees, dead (solid circles) or living (hollow circles), between 1990 and 1993 (stand age 5–7 years; first isolation series). Large numerals are plot numbers. Small figures denote separate compatibility groups or colonies (genets; those shared with Fig. 7 are the same; numbers with ' signs indicate possible additional genets suggested during cultural pairing). Groups, as known, are delineated by encircling solid or (where more distant) solid and broken lines.

potential colonies but one were identified in this way as *A. novae-zelandiae*. Genet 71 (Plot 21) was of *A. limonea*.

In the second isolation series, nearly 6 years after thinning, cultures of *Armillaria* species were obtained from 26 trees and 60 stumps in three selected Treatment 2 plots, and from 20 trees in three selected Treatment 3 plots. These were sorted by cultural pairing into 41–51 genets, equivalent to a minimum of 44 colonies per hectare (or 69 colonies per hectare, Treatment 2, with stumps; 26 colonies per hectare, Treatment 3, no stumps) (Fig. 7). Of these, isolates of only eight genets were compatible with still-extant first-series cultures obtained from the same vicinity (75–80% of these remained held in stock), indicating that a minimum of 20% of second-series genets isolated were present as colonies before thinning. These eight genets came from 28 thinning stumps and 11 trees, mostly in Treatment 2 plots (only four trees in two Treatment 3 plots yielded two pre-treatment genets during the second
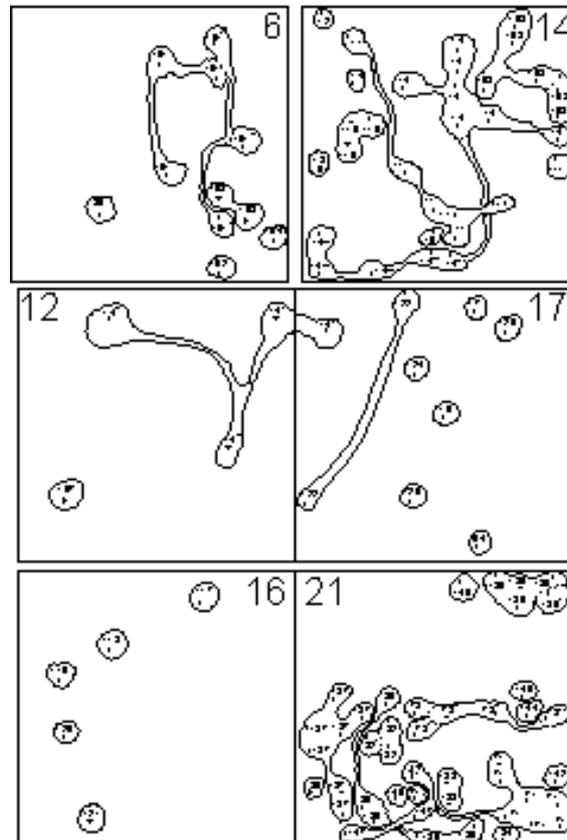


FIG. 7–Distribution of *Armillaria* isolates taken from infected trees or colonised stumps in six selected plots in February and March 1998 (stand age 12.5 years, nearly 6 years after thinning to 250 stems / ha; second isolation series). Large numerals are plot numbers (Treatment 3 plots, no stumps, are to the left and Treatment 2 plots, with stumps, to the right). Small figures denote separate compatibility groups or colonies (genets; numerals <100 shared with Fig. 6; numbers with ' signs indicate possible additional genets suggested during cultural pairing). Solid circles represent thinning stumps (Plots 14, 17, 21, only) and hollow circles living trees

isolation series). All second-series genets were identified culturally as *A. novae-zelandiae*, except for the pre-thinning Genet 71 of *A. limonea* which was re-isolated from trees and stumps in Plot 21.

## DISCUSSION

Infection is not static in stands of *P. radiata* affected by Armillaria root disease. As previously demonstrated (MacKenzie 1987) and confirmed here, girdling in individual root collars may increase or decrease in severity, and apparently show some circumferential movement during the course of the stand rotation period. The dynamic nature of the host-pathogen interaction has recently been verified through morphological examination of root systems and root collar sections from *P. radiata* trees infected by *Armillaria* species (van der Kamp & Hood 2002). This study sought to identify a thinning regime that would shift the aggregate balance towards a reduction in the general level of stand infection. However, initial results, midway through the rotation period, indicate that thinning appears to encourage both the incidence and severity of infection, seemingly by providing additional substrate for *Armillaria* species in the form of new stumps. Although treatment trends were not fully conclusive because of high plot-to-plot variation, individual tree analysis strongly supported this finding. This outcome will be tested further in about 5 years' time, after the final non-commercial thinning which was undertaken in December 1998, shortly after the 12.5 year evaluation. The stumps created during this operation are substantially greater in volume than those from the first thinning, and might therefore be expected to generate a stronger effect (Morrison *et al*. 2000), but this remains to be seen. The results so far obtained from this study suggest that any tree resistance induced by increased vigour (as opposed to intrinsic genetic resistance) may be overcome with comparative ease, given a sufficient increase in inoculum potential.

The individual tree analyses to examine the effect of thinning used data from stumps created from trees infected in the first evaluation. It was reasonable to assume that thinned, infected trees would yield stumps colonised by *Armillaria* species, and this procedure was justified by the significance with which it explained the distribution of infection. It was not possible to use stumps actually recorded as colonised by species of *Armillaria* because many stumps had decomposed completely after nearly 6 years, and could no longer be located. The high proportion of stumps colonised by *Armillaria* species among those that were found agrees with earlier work (Hood & Sandberg 1993b), and confirms that pine stumps are readily invaded on infested sites. In a typical situation, decay and accompanying pseudosclerotial zone plates characteristic of Armillaria root disease were present in the basal portion of the stump, with white mycelial fans extending beneath the bark along primary roots, and often into the upper part of the stump. Live primary roots from adjacent trees frequently grew within the space occupied by the stump root system. An unknown additional number of missing stumps is also likely to have been colonised in this way. Because of the generally advanced state of wood decomposition, and their comparatively small size, it is not considered that the destructive sampling of some stumps will influence the course of the trial. A small number (6%) of thinning stumps were still alive, either in entirety or as small remnant segments, apparently as a result of root grafting with adjacent residual trees. Infection by *Armillaria* species in such material was usually accompanied by resinosis, indicating parasitic attack.

Pruning varied in intensity between trees in different plots, but appeared to have little effect on either incidence or severity of infection. However, this treatment is likely to increase the stress and symptom response in trees already infected. In young stands mortality is sometimes observed soon after severe pruning of trees infected by *Armillaria* species at the time of first thinning (I. Hood, unpubl.). In a young *P. radiata* plantation incidence of inoculated trees infected by *Sphaeropsis sapinea* (Fries) Dyko & Sutton was eight times greater after 40% or 50% of the crown was removed by pruning than after a reduction by only 25% (Chou & MacKenzie 1988).

Although infection incidence and severity were greater among trees nearer to newly created stumps, this trend occurred within the context of the "natural" background distribution pattern, which did not change significantly between evaluations (Fig. 2, 3). This is not unexpected, given that only a component of the trial stand was thinned in such a way as to leave stumps (eight of the 20 plots, distributed across the whole area). There was also no overall change in the directional distribution of infection around tree root collars, which showed no overall pattern in either of the two evaluations (Fig. 5). This is probably because previous crop stumps, the presumed source of infection, would be distributed independently of second-rotation trees. Any movement around individual root collars will probably balance out over the whole stand.

In this work it was assumed that the incidence and severity of tree infection are reliably estimated by the presence and extent, respectively, of root collar resinosis. Without destructive sampling it is not possible to assess the relationship between level of girdling and possible unseen viable infection hidden in roots or in tissues callused over at the root collar (G.M.Filip, D.J.Morrison, pers. comm.; Whitney *et al.* 1989; Morrison *et al.* 2000). However, it is believed that the impact of any such hidden infection is not great in New Zealand *P. radiata* plantations. In a study which involved excavating and dissecting the root systems of a number of trees adjacent to the study area in the same stand at age 13 years, barely two root infections by *Armillaria* species were found, on average, per tree (van der Kamp & Hood 2002). These appeared relatively innocuous when compared with lesions caused by *Armillaria ostoyae* (Romagn.) Herink on conifer hosts in British Columbia (B.J.van der Kamp, pers. comm.; Morrison *et al.* 2000). Lesions on *P. radiata* were contained, and only smaller roots were completely girdled. Infection points had rapidly callused in larger roots, in which functioning and translocation appeared to continue unimpaired. It may also be significant that mortality caused by *A. ostoyae* persists longer in British Columbia, particularly in the interior where trees continue to die throughout the life of the stand, whereas in New Zealand chronic infection, only, persists in *P. radiata* right until harvest. The association found between incidence of trees with root collar resinois (always accompanied by rhizomorphs) and density of stumps from infected trees also implies that root collar girdling is a reliable measure of infection. Similarly, it is unlikely that the significant association between spatial infection distribution in both surveys would be so apparent if root collar resinosis was not a stable indicator of tree infection.

Cultural pairing demonstrated that the pathogen population is composed of a comparatively high density of separate colonies of *Armillaria* species, a finding that conforms to earlier work in stands planted on sites converted from indigenous forest (e.g., Hood & Sandberg 1993b). Even more colonies may be present than were detected, because isolates were

obtained from only 23% of all infected trees and colonised stumps. A small proportion of pairing tests yielded consistently indeterminate results where, although cultures seemed to merge, there was sometimes evidence of apparent incompatibility (e.g., a crease or fine groove between cultures that otherwise appeared quite homogeneous). Some genets, more distant in Fig. 6 (e.g., "Genet" 2/2', Plots 1, 8), may represent distinct genotypes not clearly distinguishable by cultural pairing. Other less dispersed genets (e.g., "Genet" 7/7', Plots 2, 3, 8), may be partially related (e.g., sharing one parent in common; cf. Hood & Sandberg 1993b). Disturbance during ground preparation prior to planting may also cause some displacement of colonies.

The densities of genets or colonies detected among cultures from the second isolation series were also high, particularly in the plots that retained thinning stumps. At least 20% of the second-series genets were already present before treatment, most having colonised thinning stumps by vegetative growth, the others continuing to infect residual trees. In the first isolation series, genets identified among the cultures isolated in the period up to 1 year after thinning were also already present beforehand. Only one colony (potential Genet 38', Plot 1) was not confirmed as being from at least one tree already infected when trees were thinned. Two explanations are possible for the appearance of new genets in the second series, making the reasonable assumption that few or none have arisen by genotype mutation during vegetative growth in either the plantation or among laboratory culture stocks since the first series of isolations (Hood & Sandberg 1987). The new genets may also have been present earlier as undetected colonies, since cultures were obtained from only a proportion of the infected trees during the first isolation series. But some genets may be genuine new colonies introduced by means of basidiospores to fresh thinning stumps (Hood & Sandberg 1993b). Either way, the high colony density strongly suggests a significant role for spore dispersal, regardless of whether or not *A. novae-zelandiae* may have been present in the treeless heath or scrub vegetation that preceded the first pine crop (Gilmour 1954; Hood & Sandberg 1993a). Only one genet was culturally identified as *A. limonea*, and this species does not appear to invade pine stands in this way as readily as *A. novae-zelandiae*. Work currently in progress is aimed at a better understanding of the significance of basidiospores in colonising stumps, in order to clarify the extent to which *Armillaria* species may be invading other pine plantations established on non-forest sites (Hood *et al*. 2002).

Recommendations conflict on the use of thinning to reduce the impact of Armillaria root disease in forests outside New Zealand. The few studies so far undertaken suggest that the effect of this treatment may depend on the nature of the stand (Pielou & Foster 1962; Filip *et al*. 1989, 1999; Wargo & Harrington 1991; Entry *et al*. 1991; Morrison *et al*. 1992; Filip & Goheen 1995; Morrison & Mallett 1996; Cruickshank *et al*. 1997). At this interim stage, it seems that excessive thinning and pruning may not be appropriate in *P. radiata* stands severely infested by *Armillaria* species in this country, but results after the second thinning will give a clearer idea of the significance of the effect of silviculture further on in the rotation. There is a need for an alternative control method that restricts the amount of wood substrate available to the pathogen. Promising approaches include stump or whole-tree extraction (Arnold 1981; Morrison *et al*. 1988; Morrison & Mallett 1996) or perhaps some form of stump treatment (e.g., Yang & Hood 1992). The lower incidence of stand infection resulting from thinning by removal of the whole tree (Treatment 3) suggests that pushing trees over or pulling them from the ground before cutting the log length during harvest, if

feasible, may be an effective and long-term means of control on sites known to be substantially infested by this pathogen.

## ACKNOWLEDGMENTS

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