Armillaria root disease of *Pinus radiata* in New Zealand (1)
ARMILLARIA ROOT DISEASE OF PINUS RADIATA IN NEW ZEALAND.  
1: BASIDIOSPORE DISPERSAL

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ABSTRACT

As part of an investigation into the manner in which Armillaria root disease develops in Pinus radiata D. Don plantations in New Zealand, studies were undertaken to examine the ability of spores to colonise freshly cut bark-encased billets of wood in which moisture content was maintained by partial burial in soil. Billets were enclosed in plastic cylinders to protect them from colonisation by soil rhizomorphs, and in different studies were located as spore traps within second-growth indigenous podocarp/broadleaved forest, sometimes adjacent to fruitbody clusters of Armillaria species, or in the open. In two studies, natural airborne inoculum of Armillaria species was supplemented by treatment with spores or aqueous spore suspensions. After periods of 7 to 44 months, studies were terminated by exhuming billets and removing bark in order to determine which were colonised by species of Armillaria, as indicated by the presence of characteristic mycelial fans or rhizomorphs. Many billets of P. radiata (28% of 83 traps) and willow (Salix sp.; 83% of 12 traps with billets pre-treated using 2,4-D herbicide) were colonised by Armillaria species. Cultures isolated from three pine and six willow billets were identified as A. novae-zelandiae (Stevenson) Herink. In one study 65% of 20 pine traps were colonised instead by Phlebiopsis gigantea (Fries) Jülich, a possible candidate as a biological control agent for Armillaria species in pine stumps. Three (9%) of 35 traps placed in the open became colonised by Armillaria species. If typical, this incidence may be sufficient to account for many of the colonies of these pathogens observed in pine plantations, and suggests that new infection centres may become established by means of airborne basidiospores. However, further work is under way to compare the receptiveness

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of billets and pine stumps to spore colonisation. In separate microscope studies, germination of basidiospores of two *Armillaria* species, followed by hyphal penetration of the xylem, was demonstrated on the surfaces of discs of *P. radiata* and *Beilschmiedia tawa* (A. Cunn.) Kirk incubated in a moist environment.

**Keywords:** basidiospores; colonisation; root disease; *Pinus radiata*; *Armillaria novae-zelandiae*; *Armillaria limonea*

**INTRODUCTION**

There are grounds for inferring that basidiospores play a role in the spread and establishment of *Armillaria* species in plantations of *Pinus radiata* in New Zealand (Hood 1989). Colonisation of previous-crop stumps by this means has been invoked as a possible explanation for significant infection in second-rotation stands replacing plantations in which infection was apparently negligible, on sites not formerly covered in indigenous forest (Gilmour 1954). Secondly, there is now ample evidence that populations of *Armillaria* species in New Zealand pine plantations are made up of a high incidence of spatially small colonies, whether or not trees were established on land previously supporting indigenous forest (Benjamin & Newhook 1984; Hood & Sandberg 1987, 1989, 1993a, b; Hood et al. 2002; cf. Northern Hemisphere work of Rishbeth 1988, and Lung-Escarmant et al. 1998). This is in marked contrast to the low density of larger colonies present in forests in some other parts of the world, resulting from ongoing vegetative spread over many decades or even centuries, and a low frequency of establishment of new colonies by means of basidiospores (Adams 1974; Kile 1983; Thompson & Boddy 1983; Hood & Morrison 1984; Siepmann 1985; Durrieu & Chaumeton 1988; Siepmann & Leibiger 1989; Smith et al. 1992). Finally, cultural pairing has been used to demonstrate indirectly the natural initiation of new infection centres by means of basidiospores of *Armillaria novae-zelandiae* in kiwifruit orchards in the Bay of Plenty district (Horner 1991). Dispersal by means of basidiospores may contribute to the widespread occurrence of Armillaria root disease in many urban parks and gardens throughout New Zealand, and in willows protecting river embankments in some rural areas.

However, there is still no direct evidence indicating that basidiospores of *Armillaria* species are able to initiate new infection centres in New Zealand pine forests. Knowledge of the degree to which this may actually occur is important, because it will help to indicate whether or not *Armillaria* species may be establishing in new plantations, and if so, whether the incidence of the disease may be increasing in successive rotations. This paper reports the results of a preliminary series of studies investigating the capacity of spores to disperse, and also to colonise freshly cut wood.

**METHODS**

Spore traps consisting of fresh, bark-encased billets of pine (*Pinus radiata*) or willow (*Salix* sp.) were partially buried vertically in soil with one end exposed. Billets from trees or saplings felled 0–1(–2) weeks earlier were cut to size less than 24 hours before burial. Different-sized billets were used in separate studies. Large billets measured 600 mm long × 150–200 mm diameter over bark. Small billets were 180–200 mm long × either 60–70 mm or 20–30 mm diameter. In one study (Study 6b), the exposed ends of small willow billets were painted with 2,4-dichlorophenoxy (2,4-D) acetic acid herbicide to prevent shooting. All
billet were shielded from colonisation by rhizomorphs of *Armillaria* species potentially present in the soil by enclosing them within open-ended plastic cylinders, the space between the sides of the cylinder and the billets being filled with sieved soil (c. 3–4 mm mesh) or coarse moist sand. Shields for large traps were cut from 300-mm-diameter plastic pipe, and extended 150 mm beneath the trap base. Shields for most small traps were cut from lengths of 100-mm-diameter plastic pipe which extended 120 mm below the trap base. Traps 20–30 mm in diameter were buried either in pairs or threes within each shield, while 60–70 mm diameter traps were placed singly. For two preliminary studies, small billets 70–80 mm diameter \( \times 200 \) mm long were partially buried vertically in soil in square plastic pots with drainage holes, measuring 170 mm wide \( \times 190 \) mm tall. These were placed on plastic sheeting on the ground surface, either beneath tree cover for the full period, or under shade cloth for 4 weeks and then in the open (Studies 3 and 4, Table 1). The other traps were situated either in the open, or beneath forest cover or shade cloth (Table 1).

Traps were set up in winter (May or June), during the natural fruiting season for *Armillaria* species (late March to July), at three locations within 30 km of Rotorua, in Rotorua itself, on the Mamaku Plateau, and at the Hinehopu Reserve between Lakes Rototiti and Rotoehu (Table 1). In some studies colonisation by *Armillaria* species relied solely on natural airborne spore inoculum, while in others this was supplemented by placing traps near clusters of fruitbodies of *Armillaria* species (Fig. 1a). In two studies spores were applied directly to billet surfaces, either as a dry deposit (Study 3, Table 1) or as an aqueous suspension (Study 4b; 12 \( \times 10^6 \) spores/ml, equivalent to c. 1.2 \( \times 10^6 \) spores/100 mm\(^2\) of cut billet surface). Spores applied as a suspension were still viable on a water agar surface after dry storage at 4\(^\circ\)C for 3–4 weeks prior to treatment. Traps were initially maintained for periods of between 10 and 44 months (Studies 1–4), but later were harvested after 7–10 months (Studies 5–7). At harvest, billets were exhumed and the soil or sand within the cylinder was carefully sieved, note being taken of any rhizomorphs present in the space within the shield beneath each billet (data were excluded from two traps where such evidence of possible colonisation by soil rhizomorphs was found). In the laboratory, a record was kept of any external rhizomorphs of *Armillaria* species or signs of other fungi present on the bark or cut billet surface. Bark was removed, and the appearance of mycelial fans or rhizomorphs was recorded by means of sketches and photography. In some studies isolation attempts were undertaken from mycelium or wood on to 3% malt agar.

Three subsidiary experiments were conducted over two consecutive seasons to examine the ability of basidiospores of *Armillaria* species to germinate and penetrate wood of *P. radiata* and *Beilschmiedia tawa*, a species commonly colonised by species of *Armillaria* in indigenous forests. Detached caps of fruitbodies of *Armillaria novae-zelandiae* or *A. limonea* (Stevenson) Boesewinkel were fastened with vaseline, gill-surface downwards, to separate lids of 50-mm-tall Petri dishes, and held above discs of each tree species placed on moist filter paper within the base of each dish. Discs measured 15–30 mm diameter \( \times 5–8 \) mm thick, with bark retained, and were prepared by transverse sectioning of whole branches or stems. Pine discs were cut from freshly collected branch wood in each of the three studies. Discs of *B. tawa* were prepared fresh in one study, and retained dry for a second study 2 weeks later. After 6–10 days’ incubation, radial and tangential longitudinal sections were cut from selected discs and examined under the microscope for evidence of basidiospore germination and hyphal penetration of wood cells, using Nomarski interference contrast illumination.
TABLE 1—Details and results of *Armillaria* spore trap studies

<table>
<thead>
<tr>
<th>Study No.</th>
<th>Location</th>
<th>Site type</th>
<th>Spore source</th>
<th>Date establ.</th>
<th>Period to harvest (months)</th>
<th>Billet size</th>
<th>Pine traps*</th>
<th>Willow traps*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No. traps</td>
<td>No. traps</td>
</tr>
<tr>
<td>1a</td>
<td>Mamaku</td>
<td>Open</td>
<td>Natural (nearest indig. forest edge ≥ 100 m)</td>
<td>5/1985</td>
<td>19–20</td>
<td>Large</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>1b</td>
<td>Mamaku</td>
<td>Forest</td>
<td>Natural</td>
<td>5/1985</td>
<td>19–20</td>
<td>Large</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Mamaku</td>
<td>Forest†</td>
<td>Fruitbodies of <em>A. novae-zelandiae</em> ≤ 5 m</td>
<td>5/1987</td>
<td>17†</td>
<td>Large</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Rotorua</td>
<td>Exotic forest</td>
<td>Detached fruitbodies of <em>A. novae-zelandiae</em> above billets</td>
<td>5/1987</td>
<td>10, 16½</td>
<td>Small (pots)</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>4a</td>
<td>Rotorua</td>
<td>Open‡</td>
<td>Natural</td>
<td>6/1988</td>
<td>44</td>
<td>Small (pots)</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>4b</td>
<td>Rotorua</td>
<td>Open</td>
<td>Aqueous pore suspension</td>
<td>6/1988</td>
<td>44</td>
<td>Small (pots)</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>5a</td>
<td>Mamaku</td>
<td>Forest</td>
<td>Fruitbodies of <em>A. limonea</em> ≤ 2 m</td>
<td>5/1998</td>
<td>8–9</td>
<td>Small, 2-billet</td>
<td>2</td>
<td>2(2)</td>
</tr>
<tr>
<td>5b</td>
<td>Hinehopu</td>
<td>Forest</td>
<td>Fruitbodies of <em>A. novae-zelandiae</em> ≤ 2 m</td>
<td>6/1998</td>
<td>8–9</td>
<td>Small, 2-billet</td>
<td>6</td>
<td>4(6)</td>
</tr>
<tr>
<td>6a</td>
<td>Hinehopu</td>
<td>Forest</td>
<td>Fruitbodies of <em>A. novae-zelandiae</em> ≤ 20 m</td>
<td>5/1999</td>
<td>10</td>
<td>Small, 2-billet</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>6b</td>
<td>Hinehopu</td>
<td>Forest</td>
<td>Fruitbodies of <em>A. novae-zelandiae</em> ≤ 20 m</td>
<td>6/1999</td>
<td>9½</td>
<td>Small, 3-billet§</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>Mamaku</td>
<td>Open</td>
<td>Natural (traps in line perp. to indig. forest edge, 0–200 m distant)</td>
<td>6/2000</td>
<td>7</td>
<td>Small</td>
<td>20</td>
<td>2</td>
</tr>
</tbody>
</table>

TOTAL | 83 | 23 | 13 | 10

* No. of single-, paired-, or 3-billet traps (No. billets with *Armillaria* in multi-billet traps, in parentheses)
† 6 months beneath mixed residual indig. forest with exotic plantings, followed by a further 11 months reburied within plastic shields in an open nursery bed beneath shade cloth
‡ After 4 weeks beneath shade cloth (results not distinguished between a and b billets)
§ Treated with 2,4-D herbicide
RESULTS

*Armillaria* species colonised 23 of 83 (28%) pine traps and 10 of 13 (77%) willow traps (Table 1). Colonisation was verified by the presence of external rhizomorphs attached to the billet bark or cut surface, or more frequently by characteristic mycelial ribbons beneath the bark associated with black zone lines (Fig. 1b). Where traps were exposed to natural airborne inoculum only, *Armillaria* species colonised the one pine trap placed in indigenous forest (Study 1b), and at least three of 35 pine traps (9%) placed in the open (Studies 1a, 4a, 7). The three colonised traps located in the open were sited 20 m, 60 m, and more than 100 m,
respectively, from the nearest indigenous forest. Evidence of Armillaria species was found in only one of the 30 small billets in pots (Studies 3 and 4a and b). Soil appeared heavy and very wet in these traps. Cultures isolated from three pine and six 2,4-D-treated willow billets were identified as *A. novae-zelandiae* by diploid-haploid pairing or cultural appearance after growth for 3 weeks under a 24-hour photoperiod (Hood & Sandberg 1987; Studies 1b, 6b, and 7). Diploid-diploid pairing was used to demonstrate that the cultures of *A. novae-zelandiae* from each of the two single-billet pine traps colonised by this species in Study 7 belonged to different clones or genets (Hood & Sandberg 1987). In the same way, one to three genets of *A. novae-zelandiae* were confirmed in each of six of the three-billet willow traps in Study 6b (one genet in each of two traps, two in each of two others, and three in each of two further traps). More than one genet was present in the same billet in several of these traps.

Other fungi were identified on several billets. These included fruitbodies of *Skeletocutis alutacea* (J. Lowe) Jean Keller on *P. radiata*, and *Chondrostereum purpureum* (Persoon: Fries) Pouzar on sprouting willow that had not been treated with herbicide. Isolations made from 13 of 20 traps (65%) in Study 7 yielded cultures of *Phlebiopsis gigantea* which produced white, wispy, branching strands composed of hyaline hyphae with crystalline warts or protuberances on many of these billets (Fig. 1c). Billets yielding *P. gigantea* were not colonised by *Armillaria* species.

In the laboratory studies, basidiospores of both species of *Armillaria* were deposited in large numbers as a white powder on the upper surfaces of the discs. Longitudinal sectioning demonstrated that spores of both species had germinated and penetrated the wood of each tree species (Fig. 1d). Growth of germ tubes of *A. novae-zelandiae* along tracheid lumina in pine disc xylem was substantial after 7 days.

**DISCUSSION**

The results of this investigation supported an earlier study (Hood & Sandberg 1987), and confirmed that *A. novae-zelandiae*, at least, colonises freshly cut wood of *P. radiata* by means of basidiospores. This result was strengthened by the microscope studies which demonstrated invasion of cut pine wood by the extension of growing germ tubes of both species of *Armillaria*. Willow billets killed by herbicide application were also frequently colonised by means of basidiospores, verifying an earlier study by Horner (1991). This conclusion was reinforced by the demonstration of multiple diploid genets of *Armillaria novae-zelandiae* in single billets. The pine data may be conservative, since additional colonies consisting of narrow, white, mycelial ribbons typical of *Armillaria* species radiating out from a central point were often seen on otherwise-sound wood, but their identity was not confirmed and they are not included with the other data. The observed patterns of colonisation suggest that mycelial fans and ribbons in the cambial region may originate as a result of hyphal fusion, either from direct spore colonisation at the exposed cut end, or indirectly from within, after initial internal growth through the tracheids from the exposed surface. In all traps where cultural identification was undertaken, the colonising species was determined as *A. novae-zelandiae*, a result consistent with those from other studies in pine plantations on non-native forest sites and in kiwifruit orchards (e.g., Horner 1991; Hood et al. 2002). The predominance of this one species in these situations is also suggestive of spore dispersal. It is difficult to explain the absence of *A. limonea* in such sites without suggesting
that *A. novae-zelandiae* spreads more readily in this manner, since both species can be grown saprophytically in pine wood and are common in indigenous forests and in the pine plantations which replace them (Hood & Sandberg 1987, 1989). Results are expressed as numbers of traps rather than billets, because it was assumed that vegetative spread was possible between billets within multiple-billet traps, and a rhizomorph connection was observed between colonised billets in one such trap. On the other hand, it was common in paired and three-billet traps to find only one billet colonised by *Armillaria* species, and multiple genets occurred in some traps.

Incidence of colonisation by *Armillaria* species varied between studies. Successful occupation of billets requires available spores, absence of effective competition by other wood-colonising fungi, and suitable conditions allowing invasion and mycelial growth to proceed. It may be significant that in the traps consisting of small billets placed in pots (Studies 3 and 4), which were not colonised by *Armillaria* species, soil appeared generally heavy and poorly drained. *Armillaria* species were also absent from a small number of open-ended traps in which free water appeared to linger after a period of rain, apparently because soil was excessively compacted around billets. *Armillaria* species are capable of growing in very moist conditions (Rishbeth 1970), but saturation of billets in water-logged soil may be prohibitive. On the other hand, Rishbeth (1970) believed that desiccation is a likely reason preventing spore colonisation of stumps by species of *Armillaria*. The pot studies were unintentionally retained for an unduly long period (Table 1), and although periodically watered may also have experienced drying during summer. Other fungi besides *Armillaria* species colonised billets in the spore traps. Of particular interest was the high frequency of occurrence of *Phlebiopsis gigantea* over a wide area in Study 7, almost certainly by means of spores (basidiospores or arthrospores). This species is a common coloniser of logs and slash of *P. radiata* in New Zealand (Butcher 1967; Butcher & Drysdale 1991). It is used operationally in some European forests as a biological control agent to prevent stump colonisation by the root disease fungus *Heterobasidion annosum* (Fries) Brefeld, following the early pioneering work of Rishbeth (Rishbeth 1979; Korhonen et al. 1994). It appears to be an aggressive coloniser, causing rapid stump breakdown (Käärik & Rennerfelt 1957; Butcher 1967), and may be an appropriate biological control candidate for countering invasion of pine wood by species of *Armillaria* in this country.

Spores of *Armillaria* species colonised 9% of 35 pine billets placed in the open, at distances between 20 m and more than 100 m from the indigenous forest edge. If this incidence is typical over wider areas, it may be sufficient to account for the frequency of colonies of *Armillaria* species found in pine plantations in this country (Hood et al. 2002). It is not yet clear, however, if *P. radiata* stumps are as susceptible to colonisation as detached segments of stems. Rishbeth (1964, 1970) had difficulty inoculating conifer stumps, while Kile (1983) found basidiospores ineffective on eucalypt and acacia stumps, despite application of 2,4,5-T to kill tissues, although herbicide treatment is effective in facilitating colonisation of willows by *A. novae-zelandiae* in New Zealand (Horner 1991). Significant mortality caused by Armillaria root disease has recently been observed in a number of young second-rotation plantations of *P. radiata* and *Eucalyptus fastigata* Deane & Maiden established on sites previously stocked in *E. fastigata* in the central North Island (J. Pascoe, B. Poole, L. Renney, pers. comm.). Studies are now under way to investigate the receptiveness of *P. radiata* stumps to basidiospores of *A. novae-zelandiae*. Even so, it may be that colonisation
by spores in moist pine debris partially buried in mounds during site preparation is enough to make possible the introduction of new infection centres in succeeding rotations. Rhizomorphs and mycelial fans are usually not difficult to find in pine slash where the pathogen is known to occur, confirming that this type of material provides a suitable habitat.

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