CULTURAL CHARACTERISTICS AND PATHOGENICITY TO *PINUS RADIATA* OF *ARMILLARIA NOVAE-ZELANDIAE* AND *A. LIMONEA*

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New Zealand isolates of **Armillaria novae-zelandiae** and **A. limonea** were compared to determine if they could be distinguished in pure culture. Several consistent differences in cultural characters between isolates of **A. novae-zelandiae** and **A. limonea** could be used reliably (correct answers were obtained in > 90% of the comparisons) to distinguish between the 2 species and so provide a method to identify isolates obtained from diseased tissue. In pure culture, sporophores of **A. novae-zelandiae** were produced, but not those of **A. limonea**. Death of **P. radiata** seedlings inoculated with **A. novae-zelandiae** (23%) was higher than for those inoculated with **A. limonea** (18%), but the difference was not statistically significant. However, there were highly significant differences in virulence between individual isolates within each species.

INTRODUCTION

Damage caused by *Armillaria* root rot in plantations of *Pinus radiata* D. Don established on central North Island sites recently cleared of cut-over indigenous forest has been previously described (Shaw & Calderon 1977; MacKenzie & Shaw 1977; Roth *et al.* 1979). Sporophores of both *Armillaria novae-zelandiae* (Stevenson) Boesewinkel and *A. limonea* (Stevenson) Boesewinkel commonly occur on stumps of indigenous trees remaining in these plantations (MacKenzie & Shaw 1977) and are readily distinguishable from each other (Stevenson 1964).

Field evidence indicated more *P. radiata* seedlings died when planted around indigenous tree stumps bearing sporophores of *A. novae-zelandiae* than those bearing *A. limonea* sporophores (MacKenzie & Shaw 1977). However, relative pathogenicity of the 2 fungi to young *P. radiata* was not definitely known. In addition, during much of the year only mycelial fans and/or rhizomorphs of *Armillaria* spp. occur within roots of indigenous stumps and infected *P. radiata*. These vegetative structures give no indication of which fungal species is present.

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The objectives of this study were to determine: (1) the relative pathogenicity to *P. radiata* seedlings of isolates of *A. novae-zelandiae* and *A. limonea*; and (2) whether isolates of *A. novae-zelandiae* and *A. limonea* could be distinguished by cultural characteristics.

METHODS

Pathogenicity of A. novae-zelandiae *and* A. limonea *to* P. radiata

In October 1976, 160 *P. radiata* seedlings (18 months old) were obtained from the Forest Research Institute nursery and planted in pairs 20 cm apart in 10 litre plastic buckets. The buckets were filled with a potting mix of 2 parts nursery soil, 1 part fine pumice, and 1.5–2.0 parts bark mulch. A 2 × 25 cm test tube was inserted to a depth of 20 cm beside each seedling. Seedlings were maintained in a glasshouse for the 15 months of the experiment.

Three different isolates of *A. novae-zelandiae* and 4 of *A. limonea* were used. All isolates were cultured from sporophore stipes collected from stumps of *Beilschmiedia tawa* (A. Cunn.) Benth. et Hook. f. ex Kirk remaining on plantation sites in the central North Island. Inoculum was prepared using branch segments of *Alnus* sp. as described by Shaw (1977). Seedlings were inoculated in October 1976 by removing the test tubes and replacing each one with an inoculum segment which was then covered with soil. Ten buckets (20 trees) were used for each isolate.

Seedlings were examined every month for 15 months and dead ones removed and further examined. *Armillaria* was considered to have killed the seedlings when there were attached rhizomorphs and/or mycelial fans accompanied by host resinosis (MacKenzie & Shaw 1977).

In January 1978 live seedlings were removed from the containers and examined for root infections. Plants (1.1–1.3 m tall) were harvested at the end of a 14-week period of vigorous spring growth, during which no deaths occurred. Inoculum segments were also inspected for signs of viability as indicated by fresh mycelium and/or rhizomorphs.

Relative pathogenicity of the two species of *Armillaria* was compared statistically with a Student's t test. χ^2 tests were used to compare the pathogenicity of different isolates for each species.

Cultural characteristics of A. novae-zelandiae *and* A. limonea

Twenty isolates of both *A. novae-zelandiae* and *A. limonea* were obtained from sporophore stipes collected mainly from stumps of indigenous trees remaining in *P. radiata* plantations established in the central North Island between 1973 and 1976. Isolates were grown in 90 mm petri dishes with each dish containing 25–30 ml of the following autoclaved medium: 1000 ml distilled water, 19 g agar, 20 g dextrose, 30 g malt extract, and 5 g bacto-peptone. Cultures were incubated in the dark for 2 weeks at 25°C and examined for consistent and definitive characteristics.

After several characters which appeared useful for differentiating between the 2 species had been selected, their reliability was tested by 3 different observers who were all familiar with the characters selected. Each observer examined 2-week-old cultures of the 40 isolates and assigned them to species. Cultures were examined by the 3 observers independently on 3 different occasions.

Aseptic production of sporophores was attempted as this would provide a means of confirming species identity. Our medium was supplemented with 0.1 ppm sodium pentachlorophenol (Rykowski 1974) and flask cultures of isolates from both species were incubated at 20–23°C in full light for 2–3 months.

RESULTS

Pathogenicity of A. novae-zelandiae *and* A. limonea *to* P. radiata

Average mortality from isolates of *A. novae-zelandiae* and *A. limonea* was 23% and 18% respectively (Table 1). This difference is not statistically significant ($P > 0.05$). None of the remaining live seedlings inoculated with *A. novae-zelandiae* were infected by *Armillaria* root rot although 2 of those inoculated with *A. limonea* were infected. Nearly all of the inoculum segments appeared to be viable at harvesting.

However, there were significant differences in virulence between isolates of both *A. novae-zelandiae* ($\chi^2 = 26$, 2df, $P < 0.01$) and *A. limonea* ($\chi^2 = 24$, 3df, $P < 0.01$) and between all isolates taken together ($\chi^2 = 70$, 6df, $P < 0.01$). This indicates that there were virulent and moderately virulent isolates in both species.

TABLE 1—Mortality of **Pinus radiata** seedlings 15 months after inoculation with **A. novae-zelandiae** and **A. limonea**

Fungus isolate	Number of trees inoculated	% dead due to infection by **Armillaria**	% living but infected*	% dead from other causes
A. novae-zelandiae No. 24	20	5	0	5
A. novae-zelandiae No. 32	20	25	0	10
A. novae-zelandiae No. 3†	20	40	0	0
Average, **A. novae-zelandiae**	20	23	0	5
A. limonea No. 4†	20	15	12	0
A. limonea No. 34	20	40	17	0
A. limonea No. 33	20	5	0	5
A. limonea No. 26	20	10	0	0
Average, **A. limonea**	20	18	5	1
Control	20	0	0	0

* % of those living.

† Concurrent with these inoculations, but maintained in a different glasshouse, an additional 20 seedlings of **P. radiata** were inoculated with **A. novae-zelandiae** isolate No. 3, and 20 with **A. limonea** isolate No. 4. These inoculated seedlings had, respectively, 35% and 20% mortality. This agrees well with the table data for these isolates.

Cultural characteristics of A. novae-zelandiae *and* A. limonea

The following characters were found to differ consistently between isolates of the 2 species:

Surface mycelial characters (Fig. 1)

Brown crustose and fluffy white surface mycelium are both more abundant and grow higher above the agar surface in cultures of *A. limonea* than of *A. novae-zelandiae*.

The brown crustose surface mycelium of *A. limonea* develops from the centre outwards; in *A. novae-zelandiae* it is more uniform throughout the culture. Initial formation of brown crustose mycelium is usually earlier in *A. limonea* than *A. novae-zelandiae,* so that in cultures of similar age *A. limonea* usually has more brown crustose mycelium.

Rhizomorph characters (Fig. 1)

Rhizomorphs of *A. novae-zelandiae* which emerge above the agar are usually broad and flat with a blunt distal end (Fig. 1). Frequently, there are brown liquid droplets on these aerial rhizomorphs. In contrast, aerial rhizomorphs of *A. limonea* are generally more rounded and pointed and only rarely have brown liquid droplets. Overall rhizomorph diameter is greater for *A. novae-zelandiae* than for *A. limonea.*

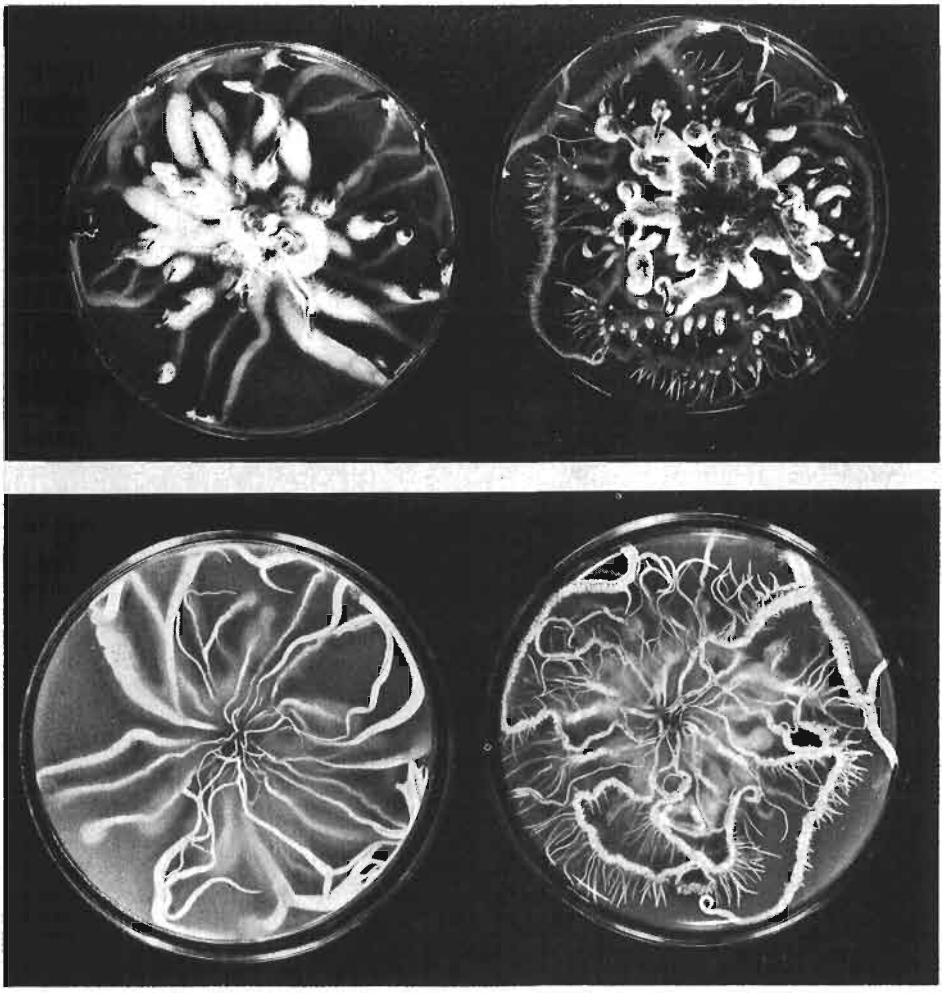

FIG. 1—Two-week-old cultures of **A. novae-zelandiae** (left) and **A. limonea** (right). Top from above, bottom from below.

In cultures viewed from below, rhizomorphs of *A. novae-zelandiae* have limited lateral branching; such branching is prolific in *A. limonea* (Fig. 1). Rhizomorphs of *A. limonea* developing along the bottom of the petri dish frequently have black-brown coloured edge lines or form mycelial patches which are similarly outlined against the bottom of the petri dish. These lines and patches are rare on rhizomorphs of *A. novae-zelandiae.*

General appearance

The combination of differences in rhizomorphs and mycelia gives cultures of *A. novae-zelandiae* a more lobed appearance than those of *A. limonea.* Two-week-old cultures of *A. limonea* are red-brown at the centre and white towards the margins when viewed from above, whereas those of *A. novae-zelandiae* are generally white throughout with, perhaps, reddish-brown islands appearing where rhizomorphs pass through surface mycelium.

FIG. 2—Mature sporophores of **A. novae-zelandiae** produced on artificial medium amended with 0.1 ppm sodium pentachlorophenol. Sporophores formed after incubation in full light for 2–3 months at 20–23°C.

All 3 observers consistently identified over 90% of the cultures to the correct species. No single isolate was consistently mis-identified.

Sporophore production

Our method produced sporophores of *A. novae-zelandiae* (Fig. 2), but none of *A. limonea.*

DISCUSSION

MacKenzie & Shaw (1977) suggested that *A. novae-zelandiae* may be more pathogenic than *A. limonea* to *P. radiata* seedlings. Although the results reported here show higher average mortality of *P. radiata* seedlings inoculated with isolates of *A. novae-zelandiae* than those inoculated with *A. limonea,* the difference is not statistically significant. Rather, these results indicate that pathogenicity to *P. radiata* varies between isolates within each species.

From the proximity of killed trees to stumps of indigenous trees bearing sporophores of *A. novae-zelandiae* and/or *A. limonea,* MacKenzie & Shaw (1977) drew inferences about the species of *Armillaria* which are responsible for seedling mortality. This indirect method was necessary as field symptoms or cultures of the fungi could not be identified to species at that time. The cultural characteristics of *A. novae-zelandiae* and *A. limonea* described here allow relatively reliable separation between isolates of these 2 *Armillaria* species growing on agar medium. This should provide a way of identifying the species of *Armillaria* present on trees attacked in the plantations. Confirmation of cultures identified as *A. novae-zelandiae* can be made through production of sporophores in the laboratory.

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REFERENCES

MacKENZIE, M.; SHAW, C. G. III 1977: Spatial relationships between **Armillaria** root rot in **Pinus radiata** seedlings and the stumps of indigenous trees. **New Zealand Journal of Forestry Science 7:** 374–83.

SHAW, C. G. III 1977: **Armillaria** isolates from pine and hardwoods differ in pathogenicity to pine seedlings. **Plant Disease Reporter 61:** 416–18.

SHAW, C. G. III; CALDERON, S. 1977: Impact of **Armillaria** root rot in plantations of **Pinus radiata** established on sites converted from indigenous forest. **New Zealand Journal of Forestry Science 7:** 359–73.

STEVENSON, G. 1964: The Agaricales of New Zealand: V. **Kew Bulletin 19:** 13–4.

ROTH, L. F.; SHAW, C. G. III; MacKENZIE, M.; CROCKETT, F. 1979: Early patterns of **Armillaria** root rot in New Zealand pine plantations — an alternative interpretation. **New Zealand Journal of Forestry Science 9:** 316–23.

RYKOWSKI, K. 1974: [Observations on the fructification of the honey fungus **Armillariella mellea** (Vahl.) Karst, in pure cultures.] **Prace Institute Badawczego Lesnictwa No. 427/432:** 147–69. (Translated by New Zealand Dept. of Internal Affairs, Wellington, 1975.)