Identification and Pathogenicity of Some Alaskan Isolates of *Armillaria*

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ABSTRACT

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Twenty-six isolates of *Armillaria* spp. were collected in Alaska from mushroom stipes, spores, decayed wood, and rhizomorphs and paired in culture with haploid tester strains of the known North American biological species (NABS) of *Armillaria*. The NABS of isolates obtained from wood and rhizomorphs could not be determined by these tests, but NABS V and IX were identified from material for which single-spore isolates were available. Several isolates also were tested for pathogenicity on seedlings of Alaska-cedar and Sitka spruce. None of the four isolates tested on Alaska-cedar infected any seedlings even though all of these isolates were obtained from dying Alaska-cedars and nearly all inoculum segments produced abundant rhizomorphs. On Sitka spruce, the three diploid isolates obtained from wood were significantly more pathogenic than the two diploid isolates obtained from sporophore stipes. In addition, single-spore isolates of both NABS V and IX caused more infections on Sitka spruce than did their diploid parent isolates. A statistical analysis of previously published but unanalyzed data also indicated that single-spore isolates may be as pathogenic, and in some cases more pathogenic, than field-collected, diploid isolates.

Additional keywords: biological species, *Chamaecyparis nootkatensis*, *Picea stichensis*, virulence.

Armillaria spp. commonly inhabit the old-growth rain forest of Sitka spruce (*Picea stichensis* (Bong.) Carr.) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) that dominates coastal southeast Alaska (23,24). After thinning in young stands that have regenerated following harvest of the old growth by clear cutting, fresh stumps are frequently colonized by *Armillaria* spp. (23,26,33). Although the fungus rarely has caused any significant disease problems in these young stands (23,26), there still is concern because problems with Armillaria root disease frequently become more noticeable after management actions (35).

The fungus also is common on dying Alaska-cedar (*Chamaecyparis nootkatensis* (D. Don) Spach), a valuable species that is currently in decline in southeast Alaska (6,27). *Armillaria* spp. are not considered to be a primary cause (27), but their role in the decline syndrome remains unclear.

We initiated this study because of the common occurrence of *Armillaria* spp. in these ecosystems, the known pathogenicity of *Armillaria* spp. in other western forest ecosystems (13,30,34,35), the lack of definitive identifications of *Armillaria* spp. (1,3) in Alaska, and their relative pathogenicity to indigenous forest species. Objectives of the study were to evaluate the pathogenicity to Sitka spruce and Alaska-cedar of isolates of *Armillaria* spp. obtained from forest types where these hosts are common, and to determine the biological species of these isolates. A preliminary report has been published (28).

MATERIALS AND METHODS

Identification of biological species. Biological species of unknown isolates of *Armillaria* can be determined by pairing them in culture with tester isolates of known biological species (3,7,11,13,16,34). We used these techniques with haploid tester isolates of the known North American biological species (NABS) of *Armillaria* (1,3) to identify the NABS of the isolates used in the pathogenicity test described below, a group of single-spore isolates obtained in a previous study (24), and various other isolates (Table 1).

Single-spore isolates used in the pathogenicity test were obtained from sporophores by suspending pieces of gill over water agar plates for 12 hr. A dilution series was made from spores washed off these plates, and 1-ml samples were placed on petri plates

TABLE 1. Origins and biological species of *Armillaria* isolates from Alaska

Isolate	Origin	Biological species[a]
A	Thinning stump, Hollis, AK	Unknown
B	Thinning stump, Hollis, AK	Unknown
C	Dead Alaska-cedar, Slocum Arm, AK	Unknown
D	Sporophore stipe, Lemon Creek, Juneau, AK	V
E	Single-spore isolate from D	V
F	Single-spore isolate from D	V
G	Sporophore stipe, Fritz Cove, Juneau, AK	IX
H	Single-spore isolate from G	IX[b]
I	Single-spore isolate from G	IX
J	Dead Alaska-cedar, Slocum Arm, AK	Unknown
	Rhizomorph on dead Alaska-cedar, Slocum Arm, UK	
L	Dead Alaska-cedar, Slocum Arm, AK	Unknown
M	Single-spore isolate, Cowee Creek, Juneau, AK	IX[c]
N	Single-spore isolates from sporophore	V[c]
O	collected at 14 mile Glacier Hwy, Juneau, AK	V
P[d]	Single-spore isolate, Palmer, AK	V
Q[d]	Single-spore isolate, Palmer, AK	V
R[d]	Single-spore isolate, Palmer, AK	V
S[e]	Single-spore isolate, Petersburg, AK	V[b,c]
T[e]	Single-spore isolate, Petersburg, AK	V[b,c]
U[e]	Single-spore isolate, Petersburg, AK	IX[b]
V[e]	Single-spore isolate, Petersburg, AK	V[b,c]
W[e]	Single-spore isolate, Petersburg, AK	V[b,c]
X[e]	Single-spore isolate, Petersburg, AK	IX[b]
Y[f]	Single-spore isolate, Fritz Cove, Juneau, AK	IX[b]
Z[e]	Diploid isolate, Hollis, AK	Unknown[g]

[a] Roman numerals correspond with those of Anderson and Ullrich (3).
[b] Identification confirmed by K. Korhonen.
[c] Identification confirmed by D. Morrison.
[d] Different sporophore collections on same site.
[e] Collected during bark-washing study; see reference 24.
[f] Different sporophore from same site as isolate G.
[g] Korhonen determined that this isolate was diploid and identified it as IX; we could not confirm the identification.

containing Kuhlman's medium (12). After germination, single-spore germlings were transferred individually to Kuhlman's medium and incubated at room temperature 3 wk. Similar dilution series were made to obtain other single-spore isolates (Table 1) after washings were obtained from spore prints. All isolates were maintained and tested on an enriched malt agar medium (30).

For testing, unknown isolates were paired in culture with haploid tester isolates of the known NABS (1,3). There were four different pairings in each 90-mm petri plate. All unknowns were paired three or four times with all known tester isolates. After cultures were incubated for 2 and 3 wk in the dark at 25 C, both authors and a third observer familiar with evaluating compatible and incompatible pairings of *Armillaria* isolates independently scored all cultures. In addition, representative isolates were sent to D. Morrison (Pacific Forest Research Centre, British Columbia, Canada) and K. Korhonen (Finnish Forest Research Institute, Helsinki, Finland) for their evaluation.

As summarized by Wargo and Shaw (34), compatible pairings between haploid isolates yield diploid colonies with dark, crustose mycelium; in incompatible pairings between haploid isolates, individual colonies remain distinct, white, and fluffy. In compatible pairings between diploid field isolates and haploid testers, the appearance of the cottony haploid changes to that of the crustose diploid; in incompatible pairings, the two isolates maintain their distinct appearance and identity, and a reaction corresponding to the Buller phenomenon (11) may develop.

Pathogenicity to Sitka spruce and Alaska-cedar. Inocula were prepared from 12 different isolates of *Armillaria* with branch segments of *Alnus rubra* Bong. as described by Shaw (22). Isolates A through I were used to inoculate seedlings of Sitka spruce, whereas C, J, K, and L were used for Alaska-cedar (Table 1). Container-grown seedlings of both hosts were about 18 mo old when they were individually transplanted into 19-L plastic pots containing a mixture of peat, vermiculite, and soil. An inoculum segment colonized by one of the 12 isolates, or an uncolonized control, was placed adjacent to each seedling's tap root during transplanting. Ten seedlings were inoculated for each of the nine isolates tested on Sitka spruce, and 15 were inoculated for each of the four isolates tested on Alaska-cedar. After inoculation in August 1982, seedlings were maintained in a greenhouse at Petersburg, AK.

Half of the seedlings were examined in 1984, 2 yr after inoculation, and the rest in 1985 for viability of inoculum segments, presence of rhizomorphs in the soil, seedling mortality, and symptoms or signs of infection by *Armillaria* on seedling roots. A resinous lesion on the root system accompanied by rhizomorph attachment and/or fungal mycelium were considered as evidence of pathogenic attack by *Armillaria* (22).

By host species, differences in percent infection among all isolates were analyzed by the comparison of many proportions procedure (5). On Sitka spruce, differences in percent infection among diploid isolates obtained from wood (A, B, and C) and those from sporophores (D and G) were similarly compared, as were differences between each parent, sporophore isolate, and the single-spore isolates from it. Differences were judged to be statistically significant at $P \leqslant 0.05$. Because seedlings were examined over 2 yr, an analysis of mortality levels was not made.

RESULTS

Identification of biological species. Biological species of the 26 isolates tested appear in Table 1; only NABS V and IX were identified. The identification of isolates sent to Korhonen or Morrison corresponded with our identifications (Table 1), except for isolate Z, which Korhonen considered to be NABS IX, but we could not identify. Only collections for which single-spore isolates were available could be identified consistently; the NABS of isolates collected directly from stumps or dying trees could not be determined by these tests.

NABS V and IX were about equally common in the Juneau area. Both species also occurred in the same stand near Petersburg, AK, where isolates were collected by washing spores from the bark of young trees (24); however, only NABS V was detected in the limited sampling conducted near Palmer, AK.

Pathogenicity to Sitka spruce and Alaska-cedar. No isolates infected any Alaska-cedar seedlings even though nearly all inoculum segments still appeared viable after 3 yr and more than 90% had produced abundant rhizomorphs that were intertwined with seedling roots. In contrast, eight of the nine isolates infected seedlings of Sitka spruce, and five of these isolates killed some seedlings (Table 2). Isolate C, the only one tested on both hosts, was nonpathogenic on Alaska-cedar but infected 70% of the Sitka spruce. Nearly all inoculum segments from the isolates tested on Sitka spruce also produced abundant rhizomorphs and still appeared viable after 3 yr.

Among all isolates tested on Sitka spruce, the comparison of many proportions (5) did not distinguish differences in ability to incite infection. Because of the limited sample size, this overall test is considered a weak comparison; the specific tests among grouped isolates are considered more powerful because they have a greater ability to detect differences when they are present. In the specific tests, there was a significant difference ($P \leqslant 0.05$) in the ability to incite infection between the diploid parent isolate D (NABS V) and the two single-spore isolates from it (E and F): The single-spore isolates caused significantly more infections. For parent isolate G (NABS IX) and the two single-spore isolates from it (H and I), the trend was the same in that the single-spore isolates caused markedly more infections, although this difference was marginally nonsignificant ($P = 0.09$). Among all diploid isolates, those obtained directly from wood, for which we were not able to determine the biological species (isolates A, B, and C), were, as a group, significantly better ($P < 0.01$) at causing infection than were the two diploid isolates from sporophores, D (NABS V) and G (NABS IX).

DISCUSSION

Our inability to determine the biological species of isolates for which we did not have single-spore material available might indicate that these isolates represent biological species new to North America. These results also might reflect difficulties in interpreting pairings between diploid field isolates and known haploid testers. Siepmann (31) noted that, with the European species of *Armillaria*, pairings between diploid field isolates and haploid testers often were not clear enough to identify species of the diploids. We, Kile and Watling (10), and Morrison (D. Morrison, *personal communication*) have had similar identification problems with diploid isolates from Australia and North America; these experiences reemphasize the need to develop a reliable method to sporulate an array of *Armillaria* spp. in vitro (34).

Results from the pathogenicity tests, although not conclusive, suggest that the unidentified field isolates may not belong to NABS V or IX because they did differ significantly from the two diploid

TABLE 2. Infection and mortality of Sitka spruce seedlings inoculated with various isolates of *Armillaria* spp.[a]

Isolate[b]	Trees infected/killed[c] (%)	North American biological species[d]
A	50/10	Unknown
B	70/20	Unknown
C	70/0	Unknown
D Parent	0/0	V
E Single spore	40/10	V
F Single spore	100/40	V
G Parent	10/0	IX
H Single spore	30/0	IX
I Single spore	50/10	IX
Control	0/0	None

[a] Cumulative for trees examined 2 and 3 yr after inoculation.
[b] See Table 1 for origin of isolates.
[c] Ten trees inoculated with each isolate.
[d] Roman numerals correspond with those of Anderson and Ullrich (3).

isolates of these NABS in pathogenicity to Sitka spruce. In addition, the limited data available suggest that NABS V and IX are, at best, weak pathogens (13,34), whereas these unknown isolates infected Sitka spruce seedlings in relatively high proportions. Because Sitka spruce is rather susceptible to infection by *Armillaria* (21,32), the relatively low levels of infection caused by the diploid sporophore isolates tend to confirm the rather limited pathogenic capabilities of NABS V and IX. Occurrence of these weak or nonpathogenic species in forests of southeast Alaska is ecologically significant because their occupancy of stump and root wood might provide a valuable deterrent to the spread of *Fomes annosus* (*Heterobasidion annosum*) from infected stumps to standing adjacent trees (14,15,23). Isolates of NABS V have been recovered from thinning stumps in adjacent areas of British Columbia, Canada (15).

Previous attempts to identify some of these unknown Alaskan isolates (25) indicated that they had a partial affinity to the European species B (*A. cepaestipes* Vel. subsp. *pseudobulbosa* Romagn. et Marxmuller). A similar affinity is shown by the new species F in British Columbia (13); however, Morrison considers that this species is, in contrast to these unknown Alaskan isolates, only a weak pathogen. Even though one isolate of this new species came from a coastal location near the Alaska-British Columbia border, we have not yet found this species in Alaska. NABS IV, which is interfertile with NABS V (1), and NABS X also have a partial compatibility with *A. c. pseudobulbosa* (2). This partial compatibility with such an array of North American material supports the suggestion that *A. c. pseudobulbosa* is an intermediate form (2). Continued efforts, including attempts to grow fertile sporophores in vitro (29) and to induce somatic segregation (4,16), are being made to identify the biological species of the isolates from thinning stumps and Alaska-cedar.

The inability of any of the isolates tested to infect Alaska-cedar was surprising, particularly because all of these isolates came from this host and one was highly pathogenic on Sitka spruce. Hennon (8) had similar experiences with field inoculations of Alaska-cedar with an *Armillaria* sp., results that tend to confirm earlier conclusions that this organism is not a primary cause of Alaska-cedar decline (27). We are unaware of any other pathogenicity tests with *Armillaria* spp. on this host, although Raabe (20) lists *C. lawsoniana* (A. Murr.) Parl., a close relative of *C. nootkatensis*, as moderately resistant to Armillaria root rot and the Ellwoodii cultivar of this species as immune or highly resistant.

The high levels of infection incited on Sitka spruce by single-spore isolates also were rather surprising because Raabe's tests (18,19) on three different hosts indicated that single-spore isolates were generally less pathogenic and virulent than their parents. Unfortunately, the biological species of Raabe's isolates cannot be determined because many of the cultures have been lost. However, we performed a statistical reevaluation of Raabe's data (17–19), from which he drew inference without statistical analysis, using a Kruskal-Wallis analysis of variance procedure (9) and an analysis of many proportions (5). These analyses indicated that single-spore isolates were generally as pathogenic, and in some cases even more pathogenic, than diploid isolates—a conclusion supported by our data for isolates of NABS V and IX.

Even though single-spore isolates do appear to have pathogenic capabilities, the natural significance of this ability to incite disease remains unclear because we are unaware of any confirmed recovery of haploid isolates from field-collected woody material or rhizomorphs.

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