Mycologia, 87(5), 1995, pp. 707-712.

Identification of *Armillaria* species from Wisconsin and adjacent areas[1]

Mark T. Banik
Jennifer A. Paul
Harold H. Burdsall, Jr.

USDA Forest Service, Forest Products Laboratory, One Gifford Pinchot Drive, Madison, Wisconsin 53705-2398

Abstract: Single-spore isolates from 218 basidiomata of *Armillaria* from Wisconsin, the upper peninsula of Michigan, and eastern Minnesota were identified to species by pairing with known haploid testers. Collections were made from 25 different host species and were identified as *A. ostoyae, A. gallica, A. calvescens, A. mellea,* or *A. sinapina.* The most frequently collected species were *A. ostoyae* and *A. gallica.* Within the sampling area, *A. ostoyae* and *A. sinapina* were collected only in the northern portions, and *A. mellea* was collected predominantly in the southern portions. *Armillaria gallica* and *A. calvescens* were collected throughout the area. *Armillaria sinapina* and *A. ostoyae* basidiomata were found equally on gymnosperms and angiosperms, whereas the other three species were collected predominantly from angiosperms. In addition to the basidiomata collections, 71 cultures of *Armillaria* were isolated from roots and rhizomorphs in seven 10- to 20-year-old quaking aspen stands in northern Wisconsin. These were subsequently identified by pairing with known haploid testers; *A. sinapina* and *A. ostoyae* were recovered most often, whereas *A. gallica* was isolated less frequently. In comparison with the other species, *A. ostoyae* was more frequently isolated from lesions on otherwise healthy roots and from roots of recently killed trees. On this basis, *A. ostoyae* appeared to be more pathogenic on aspen than either *A. sinapina* or *A. gallica.*

Key Words: *Armillaria,* aspen, basidiomata, Michigan, Minnesota, Wisconsin

INTRODUCTION

Knowledge of the geographical distribution and preferred substrate of a fungus can be useful for taxonomy and identification. This information has become more meaningful in the tree root pathogen genus *Armillaria* (Fr.:Fr.) Staude since the work of Korhonen (1978) and Anderson and Ullrich (1979) delimited biological species within the genus in Europe and North America, respectively. Most of the eight currently accepted North American biological species (NABS) have been given species names (Anderson et al., 1980; Bérubé and Dessureault, 1988, 1989). In general, these species have somewhat different hosts and substrates (Blodgett and Worrall, 1992a; Dumas, 1988; Morrison et al., 1985).

Limited information is available concerning the species of *Armillaria* that occur in the upper midwestern United States. Smith et al. (1990, 1994) identified *A. gallica* Marxmüller & Romagn. and *A. ostoyae* (Romagn.) Herink genets from the upper peninsula of Michigan. Proffer et al. (1987) reported *A. ostoyae, A. mellea* (Vahlifr.) Kummer, and *A. calvescens* Bérubé & Dessureault from fruit orchards in lower Michigan. Darmono et al. (1992) reported the occurrence of *A. gallica* and *A. mellea* on oak in southern Wisconsin. Using isolates of *A. ostoyae, A. calvescens, A. sinapina* Bérubé & Dessureault, and *A. gallica* collected from Wisconsin and the upper peninsula of Michigan, Larsen et al. (1992) found clamp connections from single-spore pairings. Stanosz and Patton (1987) extensively sampled aspen stands in Wisconsin but did not identify their isolates to species. Their research indicated that *Armillaria* species, using colonized stumps as a food base, posed a threat to aspen sucker stand development.

These reports allude to the presence of five species of *Armillaria* in the upper midwestern United States. The work reported here documents the substrates of origin and distribution of the *Armillaria* species that occur in Wisconsin, the upper peninsula of Michigan, and eastern Minnesota, based on basidiomata collections. In addition, the species of *Armillaria* that occur on quaking aspen *(Populus tremuloides* Michx.) are documented using isolates from wood, rhizomorphs, and mycelia.

MATERIALS AND METHODS

Basidiomata collections, — Basidiomata were collected in a nonsystematic manner from 1984 to 1994 through-

Accepted for publication May 9, 1995.

[1] **The Forest Products Laboratory is maintained in cooperation with the University of Wisconsin. This article was written and prepared by U.S. Government employees on official time, and it is therefore in the public domain and not subject to copyright.**

out Wisconsin, the upper peninsula of Michigan, and eastern Minnesota. Single-spore isolates were obtained from each collection by suspending small pieces of lamellae over petri plates containing 1.5% malt extract/2.0% agar (MEA). Spores deposited on the agar surface were allowed to germinate and then transferred singly to new MEA plates with the aid of a dissecting scope at 60–100 ×. The resulting single-spore isolates were maintained on MEA tubes at 6 C. Collections were identified by pairing these isolates with four known haploid tester strains of each of the eight NABS using the technique of Larsen et al. (1992). Known haploid tester strains were either those identified by Anderson (1986) or isolates identified via pairings with Anderson's tester isolates, A list of the tester isolates used is available from the authors upon request.

Aspen isolations. —Isolations were made from seven 10- to 20-year-old quaking aspen stands in three northern Wisconsin counties during the fall of 1992 and 1993 (FIG. 1). Samples were taken from four substrate classes: stumps left after harvest and regrowth trees that were either dominant, suppressed, or dead. Five to ten individuals of each class were sampled from every stand. Isolations were made from rhizomorphs, root lesions, pseudosclerotia, and mycelial fans and tufts. Rhizomorphs were surface-sterilized in 0.5% sodium hypochlorite or 70% ethanol for 10 min, cut in 4-mm pieces, and placed on Taylor's medium (TM) (Taylor, 1971) modified with the addition of 80 mg/L of chlorotetracycline. Woody material from lesions and pseudosclerotia was quickly passed over a flame, cut apart with a sterile scalpel, and placed on TM. Small pieces of mycelial fans and tufts were aseptically transferred directly to TM. All incubations were conducted at room temperature.

The field isolates were identified by pairing them with the known haploid tester strains using the method of Rizzo and Harrington (1992). Haploid tester and field isolate plugs 5 mm in diam were placed 5 mm apart on MEA in 90-mm plastic petri plates, and incubated at 24 C. After 4 wk, 4- to 5-mm-diam plugs were subcultured from the haploid tester side of the confrontation line: two plugs were taken from 2 mm past the confrontation line and another two plugs from the advancing margin. Subcultures were placed on enriched medium (Larsen et al., 1992) and incubated at 24 C for 4 wk, at which point morphological characteristics of the subcultures were observed. Fluffy, white subcultures were recorded as incompatible; appressed, dark, sometimes crustose subcultures were recorded as compatible. At the time of subculturing, all haploid testers were plated individually to be used as controls for incompatible matings. Unknown field isolates were paired with themselves as positive controls.

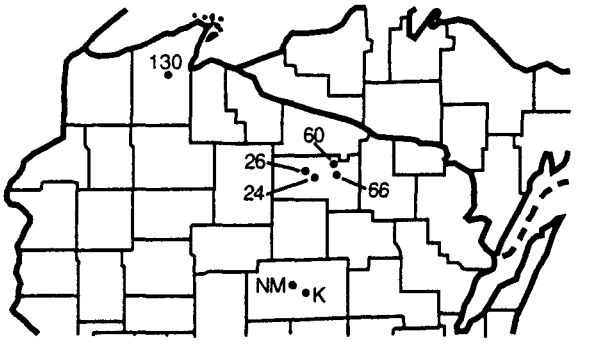

FIG. 1. Location and designation of aspen stands in northern Wisconsin sampled for presence of *Armillaria* species.

RESULTS AND DISCUSSION

Basidiomata collections. — Single-spore isolates obtained from 218 collections of *Armillaria* basidiomata were identified as the following species: *A. ostoyae, A. mellea, A. calvescens, A. sinapina,* and *A. gallica* **(TABLE** I). Basidiomata collections were obtained from seven coniferous and 18 deciduous host species in 34 counties (FIG. 2).

Armillaria ostoyae was the most commonly found species, representing 39% of the collections. Of the *A. ostoyae* basidiomata found in association with a substrate, about half were from gymnosperm hosts and the remainder from angiosperm hosts. The most common hosts were *Abies balsamea, Populus tremuloides, Tsuga canadensis, Betula papyrifera,* and *Quercus rubra* (TABLE I).

Armillaria gallica was the second most commonly collected species, constituting 36% of the collections. About 90% of the collections came from angiosperms, mostly *Quercus velutina, Acer sacchurinum,* and *Q. rubra* (TABLE I).

Armillaria calvescens, A. mellea, and *A. sinapina* each constituted about 12% or less of the collections identified. *Armillaria calvescens* was found exclusively on angiosperms (TABLE I). *Armillaria mellea* was also found fruiting only on angiosperm hosts, almost exclusively on *Quercus velutina* (TABLE I). *Armillaria sinapina,* the least collected species, was found fruiting equally on gymnosperm and angiosperm hosts **(TABLE** I).

Armillaria ostoyae and *A. sinapina* were found only in the northern portion of the sampling area (FIG. 2). Their range seems to approximate the native range of a majority of northern conifer species. This is similar to the findings of other researchers for these species (Blodgett, 1992a; Guillaumin et al., 1989). *Armillaria*

TABLE I. Basidiomata collections from Wisconsin and adjacent area

Host/substrate	Number of basidiomata collected per *Armillaria* species				
	gallica	*ostoyae*	*calvescens*	*mellea*	*sinapina*
Abies balsamea (L.) Mill.	3	11	0	0	1
Picea glauca (Moench) Voss	0	1	0	0	0
Pinus banksiana Lamb.	0	1	0	0	0
P. resinosa Ait.	1	5	0	0	0
P. strobus L.	1	2	0	0	0
Thuja occidentalis L.	0	4	0	0	3
Tsuga canadensis (L.) Carr.	0	8	0	0	1
Acer rubrum L.	6	4	0	0	1
A. saccharinum L.	7	0	0	0	0
A. saccharum Marsh.	5	1	5	1	0
Betula alleghaniensis Britton	0	3	0	0	1
B. papyrifera Marsh.	3	7	1	0	0
Carya ovata (Mill.) K. Koch	1	0	0	0	0
Fraxinus nigra Marsh.	0	1	0	0	2
F. pennsylvanica Marsh.	1	0	0	0	0
Lonicera L. spp.	0	0	1	0	0
Populus tremuloides Michx.	4	9	1	0	2
Prunus serotina Ehrh.	1	0	0	0	0
Quercus alba L.	3	0	0	1	0
Q. ellipsoidalis E. J. Hill	1	0	0	0	0
Q. macrocarpa Michx.	2	0	0	0	0
Q. rubra L.	6	7	1	2	1
Q. velutina Lam	13	1	4	10	0
Tilia americana L.	0	0	1	0	0
Ulmus L. spp.	6	2	6	0	0
Undetermined angiosperm	2	0	5	0	0
Duff	5	13	0	1	0
Unknown substrate	7	4	1	2	1
Total	78	84	26	17	13

gallica was common in both the northern and southern parts of the sampling area. This was the most widespread species; it was also the species most often found in the southern part of the sampling area (FIG. 2). This distribution is consistent with that reported elsewhere (Blodgett and Worrall, 1992a; Dumas, 1988; Harrington and Rizzo, 1993).

Armillaria calvescens was widely scattered but uncommon throughout the sampling area (FIG. 2). Its distribution seems to be similar to that of *A. gallica,* but *A. calvescens* was found much less frequently and is presumably less common than *A. gallica. Armillaria mellea* was found predominantly in the southern region of the sampling area. This species is much more common in southern areas, as reported in the literature (Blodgett and Worrall, 1992a; Guillaumin et al., 1989; Hat-ring-ton and Rizzo, 1993). In fact, this species is undoubtedly more common in southern Wisconsin than the data suggest (FIG. 2) because it is easily recognized in the field and often was not collected when found. However, the occurrence of several fruiting body collections of *A. mellea* from areas more northerly than previously recorded suggests that a northern ecotype of this species may exist.

Aspen isolations. — A total of 71 isolates were identified from the aspen stands sampled; 47% of these were *A. sinapina,* 33% were *A. ostoyae,* and 20% were *A. gallica*

TABLE II. Number of isolates of various *Armillaria* species obtained from aspen stands

Species	Isolates per designated stand by county[a]							
	Oneida Co.				Ashland Co.	Marathon Co.		Total
	24	16	60	6	130	K	NM	
A. sinapina	0	3	21	4	0	0	5	33
A. ostoyae	3	3	8	1	9	0	0	24
A. gallica	2	0	0	0	9	3	0	14
Total	5	6	29	5	18	3	5	71

[a] See FIG. 1 for location of designated stands in counties.

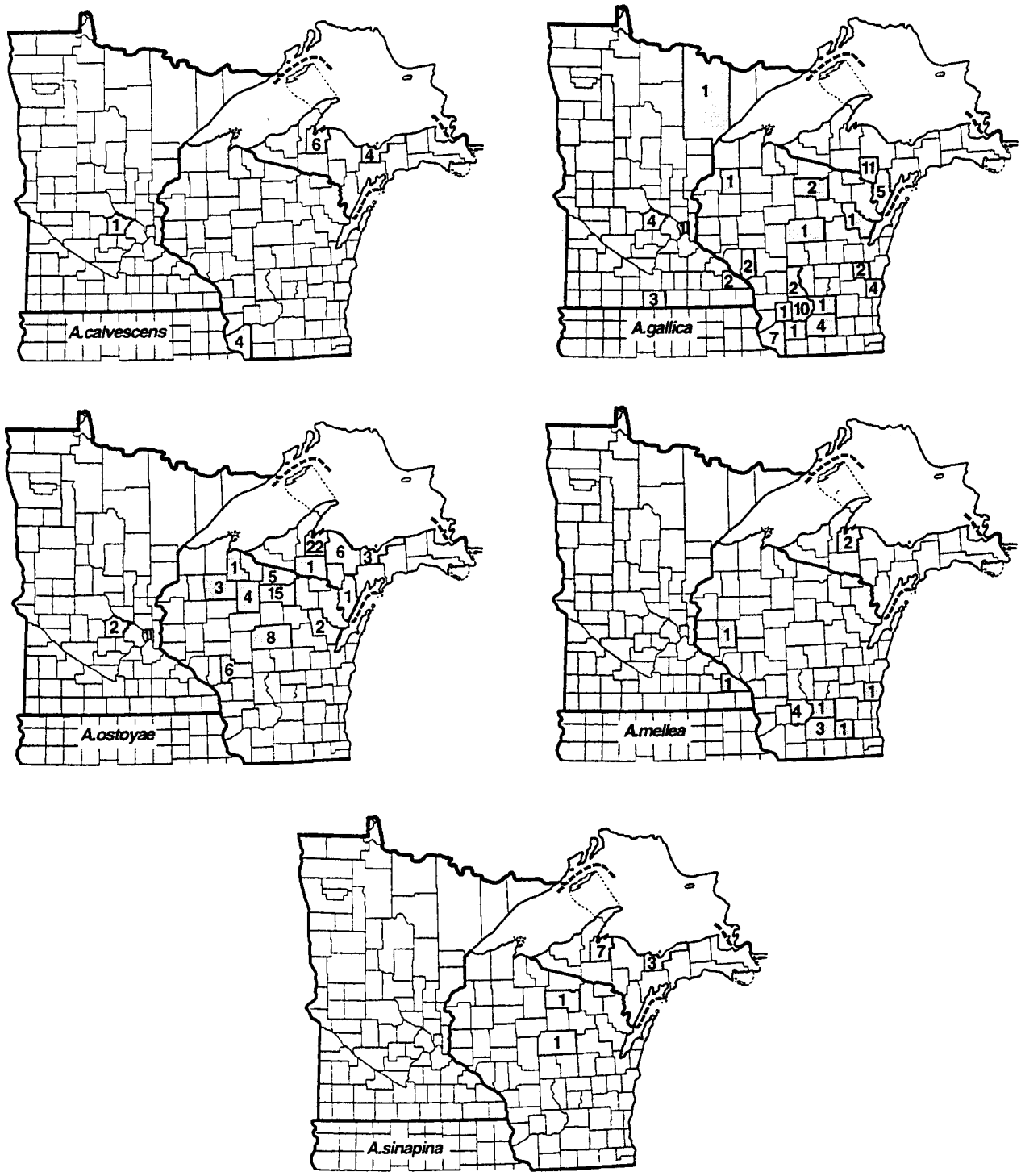

FIG. 2. Counties in Wisconsin, Michigan, and Minnesota in which basidiomata of *A. calvescens, A. gallica, A. ostoyae, A. mellea,* and *A. sinapina* were collected. Numbers indicate the number of collections of a given species in that county.

TABLE II). The relative abundance of *A. sinapina* may be overestimated as a result of the large number of collections coming from a single stand (21 of 33). *Armillaria mellea* and *A. calvescens* were not recovered from any of the aspen stands. *Armillaria ostoyae* was found in five stands, *A. sinapina* in four stands, and *A. gallica* in three stands (TABLE II). In five of the seven stands, more than one species of *Armillaria* was recovered.

Rhizomorphs were the source of a majority of *A.*

TABLE III. Number of isolates of three *Armillaria* species obtained from differing classes of aspen trees[a]

	A. ostoyae					*A. sinapina*					*A. gallica*				
Host class	rhiz	rot	myc	les	T	rhiz	rot	myc	les	T	rhiz	rot	myc	les	T
Dominant	1	0	0	2	3	9	0	1	1	11	5	0	0	0	5
Suppressed	4	0	0	3	7	7	0	0	1	8	0	0	0	0	0
Dead sucker	4	5	3	0	11	5	2	0	0	7	5	0	0	0	5
Old stump	0	1	1	0	2	4	2	1	0	7	2	1	1	0	4
Total	9	6	4	5	24	25	4	2	2	33	12	1	1	0	14

[a] Rhiz = isolated from rhizomorphs; rot = isolated from rotted wood; myc = isolated from mycelial tufts or fans; les = isolated from visible root lesions; T = total number of isolates from a given source.

sinapina isolates (76%) and *A. gallica* isolates (86%) (TABLE III). *Armillaria ostoyae* isolates from rhizomorphs accounted for only a little more than a third (38%) of the isolates obtained for this species (TABLE III). This observation supports the findings of other researchers that *A. gallica* and *A. sinapina* form rhizomorphs more abundantly than does *A. ostoyae* (Blodgett and Worrall, 1992b; Rishbeth, 1985).

Lesions were the source of 21% of the *A. ostoyae* isolates but only 6% of the *A. sinapina* isolates (TABLE III). *Armillaria gallica* was not isolated from lesions on living trees. *Armillaria ostoyae* was also isolated more frequently (33%) from rot and mycelia in association with dead trees than were *A. sinapina* (6%) and *A. gallica* (0%). Since these trees were often recently killed, the presence of *A. ostoyae* as mycelia on the wood or in rotted wood may indicate that this fungus was the cause of death. Rhizomorphic association, such as observed almost exclusively for *A. gallica* and *A. sinapina,* could be a consequence of saprobic activity. These data suggest that *A. ostoyae* is more pathogenic towards aspen than *A. sinapina,* which in turn is more pathogenic than *A. gallica.* This conclusion coincides with those of many other researchers (see Gregory et al., 1991) regarding the relative pathogenicity of these three species.

The relative abundance of *A. sinapina* in aspen is in contrast to the scarcity of fruiting bodies of this species collected on aspen and in the region as a whole. Only two basidiomata of *A. sinapina* were collected from aspen, even though it readily colonizes this substrate. It is apparent that fruiting frequency does not necessarily correlate with fungal abundance. Factors that influence fruiting may have little to do with a species' ability to colonize a substrate. Fruiting is a highly variable phenomenon among species as well as individuals. This must be recognized when drawing conclusions on the distribution of *Armillaria* species, as well as other fungi, based solely on where basidiomata have been collected.

ACKNOWLEDGMENTS

The authors thank Dr. Thomas Volk for assistance in collecting specimens and for comments on this manuscript; Cindy Bergman for her critique of the manuscript and technical assistance; and Fran Silver and Rita Rentmeester for technical assistance. The assistance of Dr. Glen Stanosz is gratefully acknowledged. Dr. David Rizzo graciously provided information on the collection from Minnesota.

LITERATURE CITED

Anderson, J. B. 1986. Biological species of *Armillaria* in North America: Redesignation of groups IV and VII and enumeration of voucher strains for other groups. *Mycologia* 78: 837–839.

———, K. Korhonen, and R. C. Ullrich. 1980. Relationships between European and North American biological species of *Armillaria mellea*. *Exp. Mycol.* 4: 87–95.

———, and R. C. Ullrich. 1979. Biological species of *Armillaria mellea* in North America. *Mycologia* 71: 402–414.

Bérubé, J. A., and M. Dessureault. 1988. Morphological characterization of *Armillaria ostoyae* and *Armillaria sinapina* sp.nov. *Canad. J. Bot.* 66: 2027–2034.

———, and ———. 1989. Morphological studies of the *Armillaria mellea* complex: two new species, *A. gemina* and *A. calvescens*. *Mycologia* 81: 216–225.

Blodgett, J. T., and J. J. Worrall. 1992a. Distribution and hosts of *Armillaria* species in New York. *Pl. Dis.* 76: 166–170.

———, and ———. 1992b. Site relationships of *Armillaria* species in New York. *Pl. Dis.* 76: 170–174.

Darmono, T. W., M. T. Banik, and H. H. Burdsall, Jr. 1992. Time and space partitioning between *Armillaria mellea* and *Armillaria gallica*. *Inoculum* 43: 30.

Dumas, M. T. 1988. Biological species of *Armillaria* in the mixedwood forest of northern Ontario. *Canad. J. Forest Res.* 18: 872–874.

Gregory, S. C., J. Rishbeth, and C. G. Shaw, III. 1991. Pathogenicity and virulence. Pp. 1–9. *In:* Armillaria *root disease*. Eds., C. G. Shaw, III, and G. A. Kile. Agriculture Handbook 691. USDA Forest Service, Washington, D.C.

Guillaumin, J. J., C. Mohammed, and S. Berthelay. 1989. *Armillaria* species in the northern temperate hemisphere. Pp. 27–43. *In: Proceedings of seventh international conference on rott butt rots.* Ed., D. J. Morrison. Forestry Canada, Victoria, British Columbia.

Harrington, T. C., and D. M. Rizzo. 1993. Identification of *Armillaria* species from New Hampshire. *Mycologia* 85: 365–368.

Korhonen, K. 1978. Infertility and clonal size in the *Armillaria mellea* complex. *Karstenia* 18: 31–42.

Larsen, M. J., M. T. Banik, and H. H. Burdsall, Jr. 1992. Clamp connections in North American *Armillaria* species: occurrence and potential application for delimiting species. *Mycologia* 84: 214–218.

Morrison, D. J., D. Chu, and A. L. S. Johnson. 1985. Species of *Armillaria* in British Columbia. *Canad. J. Pl. Pathol.* 7: 242–246.

Proffer, T. J., A. L. Jones, and G. R. Ehret. 1987. Biological species of *Armillaria* isolated from sour cherry orchards in Michigan. *Phytopathology* 77: 941–943.

Rishbeth, J. 1985. Infection cycle of *Armillaria* and host response. *Eur. J. Forest Pathol.* 15: 332–341.

Rizzo, D. M., and T. C. Harrington. 1992. Nuclear migration in diploid–haploid in pairings of *Armillaria ostoyae*. *Mycologia* 84: 863–869.

Smith, M. L., J. N. Bruhn, and J. B. Anderson. 1994. Relatedness and spatial distribution of *Armillaria* genets infecting red pine seedlings. *Phytopathology* 84: 822–829.

Smith, M. L., L. C. Duchesne, J. N. Bruhn, and J. B. Anderson. 1990. Mitochondrial genetics in a natural population of the plant pathogen *Armillaria*. *Genetics* 126: 575–582.

Stanosz, G. R., and R. F. Patton. 1987. *Armillaria* root rot in Wisconsin aspen sucker stands. *Canad. J. Forest Res.* 17: 995–1000.

Taylor, J. B. 1971. A selective medium for the isolation of basidiomycetes from diseased roots, mycorrhizas, and soil. *Trans. Brit. Mycol. Soc.* 56: 313–314.