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In vitro Fruiting of *Armillaria* Species

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Abstract

Thirty-three different isolates of various *Armillaria* species were grown on sterilized orange slices under a controlled temperature and light regime. Nine isolates of *A. ostoyae* (North American Biological Species I, NABS I), three of NABS VII, one of NABS IX, one of NABS X, and two unidentified isolates formed basidiomes. Most of the basidiomes had uncharacteristic and inverted caps. Three different basidiomes contained viable spores, two from *A. ostoyae* and one from an unidentified species. Thirteen (9 NABS I, 3 NABS VII, 1 NABS X) of the identified and two of the unidentified isolates that fruited were obtained from conifers and one (NABS IX) from a hardwood. This seems to be the first report of *in vitro* production of basidiomes by NABS VII, IX, and X.

Keywords: Biological species, basidiomes, basidiocarps, sporophores.

Introduction

Ten reproductively isolated groups (biological species[1]) of *Armillaria* have been identified in North America (Anderson and Ullrich 1979), five in Europe (Mohammed and others 1989), and four in Australia (Kile and Watling 1988). The pairing of haploid isolates on an agar-based medium and observing compatibility-incompatibility reactions have been the primary means of identification for these biological species (Wargo and Shaw 1985).

Identification of an *Armillaria* isolate species is reliable when known haploid isolates are paired with unknown haploid isolates, but less reliable when known haploid isolates are paired with unknown diploid isolates, such as those commonly recovered from *Armillaria*-colonized woody tissue or rhizomorphs collected *in vivo* (Siepmann 1985). Fruiting of *Armillaria* is sporadic in forests of the interior of Western North America where *Armillaria* is recognized as a serious pathogen (Wargo and Shaw 1985). If single-spore isolates are not available, species determination through haploid-diploid pairings may not clarify which *Armillaria* species are present in any particular area (Siepmann 1985).

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[1] Biological species are groups that are reproductively isolated, and the intersterility between groups is absolute (Anderson and Ullrich 1979).

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In vitro fruitification of *Armillaria* provides a means to reduce the uncertainty of haploid-diploid pairings. Raabe (1972) successfully fruited *A. mellea* on branches of fig trees that were ground, moistened, and autoclaved, and Shaw and others (1981) were successful in getting *A. novae-zelandiae* (Stevenson) Herink. to fruit by using a defined medium. Recently, species of *Armillaria* from Africa, North America, and Europe fruited on orange slices *in vitro*[2] (Guillaumin and others 1989, Mohammed and others 1989, Shaw 1989).

Objectives of this study were to evaluate the feasibility of producing spore-bearing basidiocarps of various *Armillaria* species *in vitro*. Origins of the diploid isolates of *Armillaria* we used are shown in table 1.

Materials and Methods

Four medium-sized oranges were cut into ca. 7.6-centimeter slices and placed into ca. 1-liter jars or 1-liter flasks. Distilled water was poured into the containers up to the top of the orange slices (Mohammed and others 1989). Containers were sterilized in an autoclave for 15 minutes at 15 pounds per square inch.

Containers were then seeded by placing 5-millimeter mycelial plugs (five of only one isolate in each container) of *Armillaria* from 21-day-old pure cultures of each of 32 different isolates into each container. Containers were incubated in the dark at ambient room temperature (20-23 °C) until the orange slices were thoroughly colonized by mycelia and rhizomorphs of *Armillaria* (ca. 3-4 weeks). They were placed in an environmental chamber incubator with fluorescent lights, where they were incubated under alternating 12-hour light and dark intervals at 12 °C until basidiomes developed (ca. 2-3 months).

We tried to recover single spores by selecting the best basidiome from each container, cutting the cap of the basidiome into small sections, sticking (with petroleum jelly) a section of the cap (three replications per isolate) to a petri dish top that covered a bottom filled with the enriched medium of Shaw and Roth medium (SRM) below (Shaw and Roth 1976). The petri dish tops were replaced with sterile tops after the sections of basidiomes cast spores for 1, 2, 4, or 8 hours and were incubated at 25 °C until spores germinated. A sterile needle was used to carefully remove germlings from the medium surfaces as soon as they formed. Germlings from each isolate were placed on fresh SRM and incubated in the dark at 25 °C for 21 days.

Results and Discussion

Basidiomes formed in 16 of 33 containers (table 1). In most instances, basidiome caps were inverted, thereby revealing gills to the outside (fig. 1). Typical basidiomes developed, but in only a few containers (fig. 2). The uncharacteristic shape of the basidiome caps may have resulted from the high humidity present in the containers. In containers with typical basidiomes, there was no excess water.

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[2] Personal communication, G. Filip, research plant pathologist, Forestry and Range Sciences Laboratory, La Grande, OR 97850.

Table 1—The production of basidiomes by *Armillaria* isolates

Armillaria isolates	Hosts	Location	Year isolated	Biological species[a]	Basidiomes[b]	Viable spores[b]
JR-D-DF-24	Douglas-fir *Pseudotsuga menziesii* (Mirb.) Franco)	Bull Run, OR	1985	NABS 1	+	–
JR-D-DF-33	Douglas-fir	Glenwood, WA	1986	NABS 1	+	–
Sudan #4	Douglas-fir	Coast Range, OR	1988	NABS 1	+	–
Sp-83-47	Douglas-fir	Salmon Arm, BC	1983	NABS 1	+	–
JR-D-LR-25	Western larch (*Larix occidentalis* Nutt.)	Sisters, OR	1987	NABS 1	–	–
JR-D-PP-15	Ponderosa pine *Pinus ponderosa* Dougl. ex Laws.	Glenwood, WA	1972	NABS 1	–	–
JR-D-PP-34	Ponderosa pine	Glenwood, WA	1986	NABS 1	+	+
JR-1953-HL	Western hemlock (*Tsuga heterophylla* (Raf.) Sarg.)	Glenwood, WA	1986	NABS 1	–	–
JR-1953-SP	Sugar pine (*P. lambertiana* Dougl.)	La Pine, OR	1981	NABS 1	–	–
JR-D-WF-K-4	White fir (*Abies concolor* (Gord. & Glend) Lindl. ex Hildebr.)	Klamath Falls, OR	1988	NABS 1	+	–
JR-PP	Ponderosa pine	Glenwood, WA	1988	NABS 1	+	+
JR-1-P	Ponderosa pine	Glenwood, WA	1986	NABS 1	+	–
GF-MTE-87[c]	Grand fir (*A. grandis* (Dougl. ex D. Don) Lindl.)	La Grande, OR	1987	NABS 1	+	–
SP-81-13[d]	Subalpine fir (*A. lasiocarpa* (Hook.) Nutt.)	Blue River, BC	1981	NABS V	–	–
SP-82-29[d]	White oak (*Quercus garryana* Dougl. ex Hook.)	Victoria, BC	1981	NABS VII	–	–
SP-82-72[d]	Oak (*Quercus*) sp.	Victoria, BC	1981	NABS VII	–	–
JR-D-WF-1	White fir	Weed, CA	1985	NABS VII	–	–
JR-D-WF-9	White fir	Mount Shasta, CA	1985	NABS VII	–	–
BR-8	Douglas-fir	Bull Run, OR	1987	NABS VII	+	–
BR-9	Douglas-fir	Bull Run, OR	1987	NABS VII	+	–
JR-D-SRF-K3	California red fir (*A. magnifica* A. Murr.)	Klamath Falls, OR	1988	NABS VII	+	–
JR-D-OK-5[e]	Shingle oak (*Q. imbricaria* Michx.)	Pennsylvania		NABS IX	+	–
SP-84-15[d]	Birch (*Betula* sp. Laws.)	Fernie, BC	1984	NABS X	–	–
GF-MC-87[c]	Grand fir	Cove, OR	1987	NABS X	+	–
Tree 4674	Douglas-fir	Bull Run, OR	1985	NA	+	–
JR-D-CD-20	Western red-cedar (*Thuja plicata* Donn. ex D. Don.)	Tillamook, OR	1987	NA	–	–
JR-D-LP	Sierra lodgepole pine (*P. contorta* var. *murrayana* (Grev. & Balf.) Engelm.)	Sisters, OR	1986	NA	–	–

Table 1—continued

Armillaria isolates	Hosts	Location	Year isolated	Biological species[a]	Basidiomes[b]	Viable spores[b]
JR-D-PP-K2	Ponderosa pine	Klamath Falls, OR	1988	NA	–	–
JR-D-OK0-27	Oregon white oak	Lebanon, OR	1985	NA	–	–
GF-KF-77[c]	Incense cedar (*Librocedrus decurrens* Torr.)	Klamath Falls, OR	1977	NA	–	–
GF-LS-88[c]	Grand fir	Elgin, OR	1988	NA	–	–
GF-S-79[c]	Ponderosa pine	Sisters, OR	1979	NA	+	+

NA = not available.
[a] Biological species were determined as described by Morrison and others (1985). NABS = North American Biological Species.
[b] Plus (+) = present; minus (–) = absent.
[c] *Armillaria* isolates obtained from Gregory Filip, USDA Forest Service, Pacific Northwest Research Station, La Grande, Oregon.
[d] *Armillaria* isolates obtained from Duncan Morrison, Canadian Forestry Service, Pacific Forestry Centre, Victoria, British Columbia.
[e] *Armillaria* isolates obtained from Philip Wargo, USDA Forest Service, Northeast Forest Experiment Station, Hamden, Connecticut.

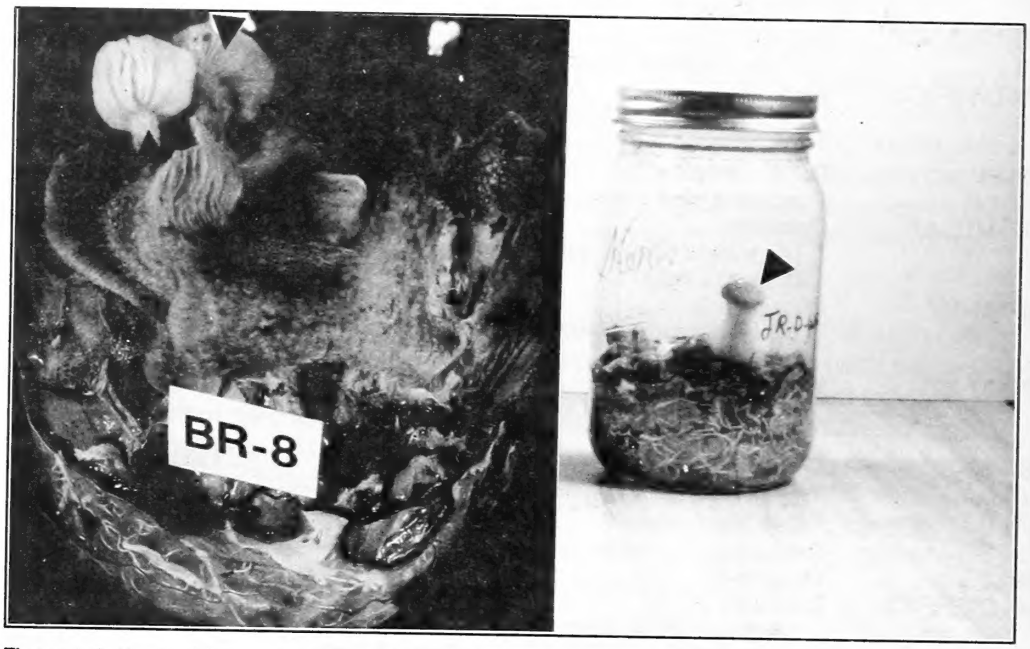

Figure 1 (left)—Basidiomes (arrowsheads) of NABS VII (BR-8) forming on the surface of orange slices. The caps are turned inside out thereby exposing the gills and the curve stalks.

Figure 2 (right)—Basidiome (arrowhead) of NABS I (JR-D-WF-K-4) forming on the surface of orange slices. The cap and stalk are characteristic.

Viable spores were recovered from only three fruiting bodies, two from NABS I isolates of and one from an unidentified species. Of those basidiomes yielding single spores, one had characteristic morphology and two had uncharacteristic morphology. Perhaps some basidiomes were infertile, or basidiomes with the exposed gills cast their spores as soon as they opened. Consequently, when we tried to obtain single spores, the gills were depleted.

Nine of the isolates that produced basidiomes were NABS I (*A. ostoyae* [Romang.] Herink.), three were NABS VII (*A. bulbosa* (Barla) Kile et Watling), one was NABS IX, one was NABS X, and two were from unidentified species (table 1). Of the 16 *Armillaria* isolates that produced basidiomes, 15 were isolated from conifers and one from a hardwood (table 1). To our knowledge, this is the first report of successful *in vitro* fruiting of known isolates of NABS VII, IX, and X of *Armillaria*. We have no definitive reason as to why NABS VII, IX, and X fruited. We did notice, however, that if the containers were not exposed to adequate light (they were shadowed by other containers), fruiting was absent or delayed. Also, in the containers where the orange slices were not completely colonized by *Armillaria* and excess water remained in the container, basidiomes did not form, perhaps because of near or actual anaerobic conditions.

If this method can be refined so that fertile basidiomes can be produced reliably by different *Armillaria* species and single spores can be consistently recovered from them, then we may be able to more accurately identify field collected isolates of *Armillaria* (Wargo and Shaw 1985).

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