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Cultural and other morphological studies of *Inonotus andersonii*

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The University of Arizona, 1988

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CULTURAL AND OTHER MORPHOLOGICAL STUDIES OF *INONOTUS ANDERSONII*

by

Mary Louise Fairweather

A Thesis Submitted to the Faculty of the

DEPARTMENT OF PLANT PATHOLOGY

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

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STATEMENT BY AUTHOR

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Robert L. Gilbertson
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June 2, 1988
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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

ABSTRACT

The nuclear behavior, vegetative incompatibility, and induction of basidiocarp formation and basidiospore germination of Inonotus andersonii was investigated. Safranin O-KOH and Hematoxylin stains were used to determine the number of nuclei in basidiospores, vegetative hyphae, and basidiocarp tissue. Both uni- and binucleate basidiospores were found in the tubes of basidiocarp tissue. The nuclei in the hyphal cells of presumptive heterokaryons typically numbered some multiple of two per cell which suggests that division is conjugate. Subhymenial hyphae and immature basidia were often uninucleate but tramal hyphae were multinucleate. Antagonistic hyphal interactions developed between different vegetative isolates on both 2% MEA and oakwood test blocks. Self-crosses were compatible. Basidiocarps formed in 12 wk on 2% MEA medium containing ground oakwood and wheat. Most attempts to induce spore germination failed. However, basidiospores obtained at the end of this study from actively sporulating basidiocarps germinated on 2% MEA.

INTRODUCTION

In 1890, Ellis and Everhart described Mucronoporus andersonii as a resupinate polypore fruiting under the bark of an oak log. In 1894 Underwood described a fungus similar to M. andersonii, found on the under side of poplar logs, and named it Polyporus xanthosporus. Neuman (1914) listed the Ellis and Everhart species as Poria andersonii with Polyporus xanthosporus as a synonym, but Murrill (1916) transferred M. andersonii into the genus Xanthoporia. In 1963, Cerny found this species in South Moravia and placed it in the genus Inonotus Karst. where it is known today as Inonotus andersonii (Ell. and Ev.) Cerny. Lloyd (1920) and Baxter (1929; 1933) considered I. andersonii a resupinate form of Inonotus glomeratus (Pk.) Murr., but Campbell and Davidson (1939) showed that I. andersonii and I. glomeratus are two different species with individual host specificities.

Inonotus andersonii (Aphyllophorales: Hymenochaetaceae) causes white spongy rot of several hardwoods. The fungus commonly enters through branch scars, at first decaying heartwood but it eventually moves into sapwood and kills the tree. Unlike most fungi that decay heartwood in oak, I. andersonii does not fruit on the living tree. The resupinate rusty-orange sporophore develops under the bark or outer layers of sapwood of recently killed standing or fallen trees, rupturing the host tissue to expose irregular spore bearing tubes (Fig. 1). Although reported on willow, poplar and hickory, it is most commonly found on

Figure 1. Basidiocarp of I. andersonii on Gambel oak.

species of oak (Quercus).

I. andersonii has been reported from North America, China, U.S.S.R., and Czechoslovakia (Domanski, 1972). In the U.S. it has been found throughout hardwood areas of the Midwest, Southeast, and Southwest to California and north to Oregon along the Pacific Coast (Gilbertson and Ryvarden, 1986). Studies conducted in southern and midwestern hardwood areas of the U.S. report that I. andersonii is the second most common fungus associated with decay in oak (Berry and Lombard, 1978; Toole, 1961), causing more trunk rot than any other fungus (Genaux and Kuenzel, 1939; Berry and Lombard, 1978). Cultural characteristics of I. andersonii were described by Davidson and Campbell (1939). Fruiting was reported to occur only on oak test blocks in flask cultures. Spore germination has not been reported, although Baxter (1929) mentioned spores collected in the field failed to germinate. Like all members of the family Hymenochaetaceae, I. andersonii lacks clamp connections which complicates distinguishing homokaryons from heterokaryons. Information on the sexuality and nuclear state of this fungus is lacking.

Cerny (1982) reports I. andersonii forms imperfect fruiting structures, prior to basidiocarps, on decaying oaks in Czechoslovakia. These have not been reported by other workers.

Our objectives in this study were to induce sporulation and spore germination in order to determine the mating system of I. andersonii, to observe the nuclear state of basidia, spores, and hyphae, to investigate vegetative incompatibility between isolates, and to examine decay capacity of vegetative isolates in the wood of oak and other trees.

MATERIALS AND METHODS

Cultural Studies

Five isolates of I. andersonii were obtained from the Center for Forest Mycology Research, U.S.F.S., Madison, WI: L 4937-rot isolate from willow oak (Quercus phellos L.), Tallulah, La.; FP 89835-rot isolate from decay started as heart rot, Gambel oak (Quercus gambelii Nutt.), South Rim, Grand Canyon, Arizona; FP 94289-rot isolate from decay in bur oak (Quercus macrocarpa Michx.), Spearfish, S.D.; CS 659214-rot isolate from black oak (Quercus velutina Lam.), Clark State Forest, Indiana; FP 72121-basidiocarp tissue isolate, unidentified oak log, Prince George County, Sunnyside, Md. Two isolates were obtained in this study: RLG 16145-from rot isolate, Gambel oak, Clear Creek, Coconino County, AZ.; MLF 56- from rot isolate, Emory oak (Quercus emoryii Torr.), Cave Creek, Chiricahua Mts., Coronado Nat. For., Cochise Co., AZ. Cultures were maintained on 2% malt extract agar (MEA) at approximately 25 C.

Temperature relationships of three vegetative isolates (L 4937, FP 89835, and FP 94289) were examined by growing isolates for 2 wk on 2% MEA at 25, 30, 35, and 40 C.

To determine the sexuality of I. andersonii, spores were collected from sporulating basidiocarps induced in culture and field-collected basidiocarps. Since I. andersonii does not fruit readily on 2% MEA, test blocks of silverleaf oak (Quercus hypoleucoides A. Camus) in Erlenmeyer flasks were inoculated with actively growing mycelium of isolates L 4937, FP 89835, FP 94289, and FP 72121, as described by

.Campbell and Davidson (1939). In addition, different types of media were tested for basidiocarp production. A 50-50 (v/v) oak-wheat mixture was made by grinding silverleaf oak and redwinter wheat in a Wiley Mill. Four grams of the oak-wheat mixture was added to 0, 1, and 2% MEA. (The wood-wheat mixture plus 2% MEA will be referred to as wood-wheat agar (WWA)). The fungus was also grown on PDA. All tests were incubated at 25 and 32 C.

Spore germination was attempted on media adjusted for pH and nutrient levels as follows:

2% MEA pH6

2% MEA pH5

2% MEA ph4

8% MEA pH5

8% MEA pH4

2% MEA + 4g/L of ground wheat-oak mixture

The pH of the media was adjusted by adding citrate/sodium phosphate (dibasic) buffer. Correlation between time of sporulation and germination was tested by collecting spores periodically from the same basidiocarp.

The effect of exposure to cold and hot temperatures on spore germination was tested. Spore prints collected on glass slides from basidiocarps produced in culture and in the field were placed in 0 C, for approx. 2, 4, or 6 wk. Spores dilutions were made in tubes of sterile distilled water, some of which were incubated for two wk at 40 C. Spores were streaked on the surface of 2% MEA medium and incubated

at approx. 25 C.

Nuclear Staining

Actively growing mycelium and sections from sporulating basidiocarps were cytologically stained in order to observe the nuclear state of hyphal cells, basidia and spores. Nuclear staining of hyphal cells was achieved by placing a plug of actively growing mycelium on sterile glass slides. The slides were stored in large petri dishes lined with moistened filter paper. After approximately 1 wk incubation at 25 C, Safranin O-KOH stain (Bandoni, 1979) was applied by methods of Yamamoto and Uchida (1982) . The number of nuclei in 20 randomly selected hyphal cells were counted for each isolate. The slides were observed on a compound microscope (640 X) with bright field illumination.

To determine nuclear state in basidiospores, small pieces of actively sporulating hymenium from basidiocarps were fixed in Formol-acetic-alcohol (FAA), embedded in paraffin, sectioned at 7 and 10 m and stained in Sass's hematoxylin (Johansen, 1940). Jensen's (1962) staining series was followed with modifications used by Hennon and Hansen (1987). Four percent ferric chloride (1 hr.) functioned as a mordant and 2% ferric chloride (15 sec) as a destainer. Also, due to the thick-walled basidiospores, the slides were exposed to the stain for 90 min. Slides were observed under bright field illumination using a compound microscope (640X and 1600X).

The nuclei of basidia were observed from the hematoxylin stained slides described above and also from fresh basidiocarp tissue stained

with Safranin O-KOH stain. In the latter method the tissue was teased apart with needles to separate the dense mass of basidia and hyphae.

Pairings in Culture

Heterokaryotic I. andersonii isolates were paired on 2% MEA in all possible combinations, with three replications of each pairing. Isolates were placed 5 cm apart on 25 ml of MEA in Petri dishes and incubated at 32 C. Self-crosses served as controls. Hyphal interactions between opposing cultures were noted after 10 weeks. Pairings between isolates L 4937, FP 89835, and FP 94289 were also made on silverleaf oak test blocks to observe and compare interactions on wood. Test blocks were prepared with the methods of Goldstein and Gilbertson (1981) and Adaskaveg and Gilbertson (1987) with slight modification. The isolates were placed on the medium before the woodblocks were added. Pairings were replicated three times. After 20 wk incubation at 32 C, mycelium was removed and blocks sawed in half lengthwise for examination of interaction zones.

Decay Study

The decay capacity of I. andersonii was evaluated using stock cultures L 4937, FP 89835, and FP 94289. Test blocks were prepared from four oak species, two associated hardwoods of Southern Arizona, and a conifer. The wood types used were: Quercus emoryi Torr. (Emory oak), Q. arizonica Sarg. (Arizona white oak), Q. gambelii (Gambel oak), Q. hypoleucoides A. Camus (silverleaf oak), Platanus wrightii S. Wats.

(Arizona sycamore), Fraxinus velutina Torr. (velvet ash), and Picea engelmannii Parry (Engelmann spruce). Collected wood was cut into 1/2 X 2 X 3 cm blocks. The test blocks were dried in a forced air oven at 110 C for 48 hr, placed in a desiccator for 2 hr, weighed, and autoclaved 1 hr. Decay chambers were made from 8oz. French square bottles containing 25 ml of MEA. The blocks were placed on glass rods and the agar was inoculated with a plug from an actively growing culture of I. andersonii. Five replications were prepared for each isolate-wood combination and five chambers of each wood type without the fungus served as controls. The chambers were incubated at 32 C for 28 wk. The decayed blocks were carefully stripped of the thick masses of hyphae, oven dried at 110 C for 48 hr, desiccated for 2 hr, and weighed. Percentage weight loss based on original oven dry weight served as a relative measure of decay capabilities of tested isolates.

Field Studies

Samples of fruiting body and decayed wood were collected in the field and isolations attempted on 2% MEA. Wood containing actively growing mycelium was incubated in a moist environment at 32 C in large beakers and plastic bags in an effort to induce fruiting and sporulation.

RESULTS

Temperature Relations

As reported by Campbell and Davidson (1939), the temperature optimum of I. andersonii isolates observed in this study ranged from 30 to 35 C (Table 1). Isolate FP 94289, originally obtained in South Dakota, had the lowest temperature optimum. Although growth of isolates FP 89835 and FP 94289 were slight at 40 C, which corresponds with results of Campbell and Davidson (1939), growth of isolate L 4937 was abundant and not significantly different than at 30 and 35 C. Isolate L 4937 was originally obtained from Louisiana.

Sporulation

Basidiocarps developing on oak blocks in flask cultures ruptured through thick masses of hyphae to expose spore bearing tubes. Fruiting occurred in 8-12 months at both incubation temperatures (25 and 32 C) regardless of exposure to light.

Two isolates of I. andersonii, MLF 56 and FP 89835, fruit readily on WWA medium containing ground oak-wheat mixture plus 2% MEA. The basidiocarps formed near the area of inoculation in 74-90 days and measured 2-3cm $\times$ 2-4cm. The tubes were vertical instead of angled downward like those found on trees in nature but the pores were of the same irregular shape. The plates were turned over at the time of sporulation so the spores would collect on the petri dish lid, making them accessible for germination tests. Isolate MLF 56 also infrequently

Table 1. Average growth of Inonotus andersonii isolates grown on 2% MEA for 7 days at 25, 30, 35, and 40 C.

Temperature	Isolate L4937	FP 89835	FP 94289
25	0.8a	3.2	2.0
30	2.2	3.6	3.6
35	3.2	4.6	3.2
40	2.6	---	---

a; diameter of culture in cm.

fruited on 2% MEA in petri plates and was the only isolate observed to fruit in slant tube cultures. In the latter, fruiting occurred deep in the tube, between the agar and glass.

Two types of chlamydospores, previously unreported for I. andersonii, were detected in culture and field samples. Smooth-walled intercalary chlamydospores were produced in the aerial mycelium of cultures grown on WWA medium and in mycelium surrounding decayed wood. Similar spores were observed within the cells of decayed wood. Chlamydospores produced in aerial mycelium are generally larger (10-18 X 15-22 μm) than spores produced in wood cells (7-12 X 12-18 μm), and the latter are also thicker walled and carry a hyphal appendage.

The second type of chlamydospore is infrequently found in the mycelium surrounding decayed wood and in mycelium growing on WWA medium. These spores are echinulate and measure 17-20 X 22-27 μm.

Spore Germination

Most attempts to induce basidiospore germination were unsuccessful. However, near the end of the study, basidiospores collected from recently sporulating basidiocarps of isolate FP 89835 (formed on WWA medium) germinated on 2% MEA after approximately 3 wk incubation at room temperature. The basidiospores were diluted in sterile distilled water and streaked onto 2% MEA medium in petri plates. Previous attempts using this standard method had failed.

Prior to streaking on MEA the spores from basidiocarp isolates FP 89835 and MLF 56 were examined. In addition to the normal (5-8 X 4-5

μm) thick-walled basidiospores of I. andersonii, smaller (4-7 X 3.5-4 μm), thin-walled spores, which also refracted light differently under phase-contrast microscopy, were observed. No spore germination was detected after 2 wk incubation but after 5 wk incubation germination had occurred and single spore isolates developed and interacted. Some of the interactions between single spore colonies resulted in the formation of pigmented zone lines, but the hyphae appeared to be fusing. Pairings between other colonies on these plates merged into one another with no indication of antagonism.

Cytological Staining

The number of nuclei per hyphal cell in heterokaryotic mycelium was variable. Actively growing hyphae stained readily with Safranin O-KOH (Fig. 2). The larger diameter (approx. 5 μm) hyphae typically contained 2, 4, or 8 nuclei per cell, dispersed randomly. An odd number or 6 nuclei per cell was rare. The nuclei were typically equidistantly spaced throughout a cell, but sometimes three nuclei were found clustered together and the fourth nucleus was alone at the other end of the cell. Nuclei were infrequently paired.

The number of nuclei in cells of the narrow diameter (2-3 μm), abundantly branched hyphae, was more difficult to determine. The cytoplasm was dense and the septa hard to distinguish in these elongated cells. Stained structures resembling nuclei were abundant, but their size was not consistent nor were they equidistantly spaced.

Spores stained with hematoxylin were found to be both uni- and

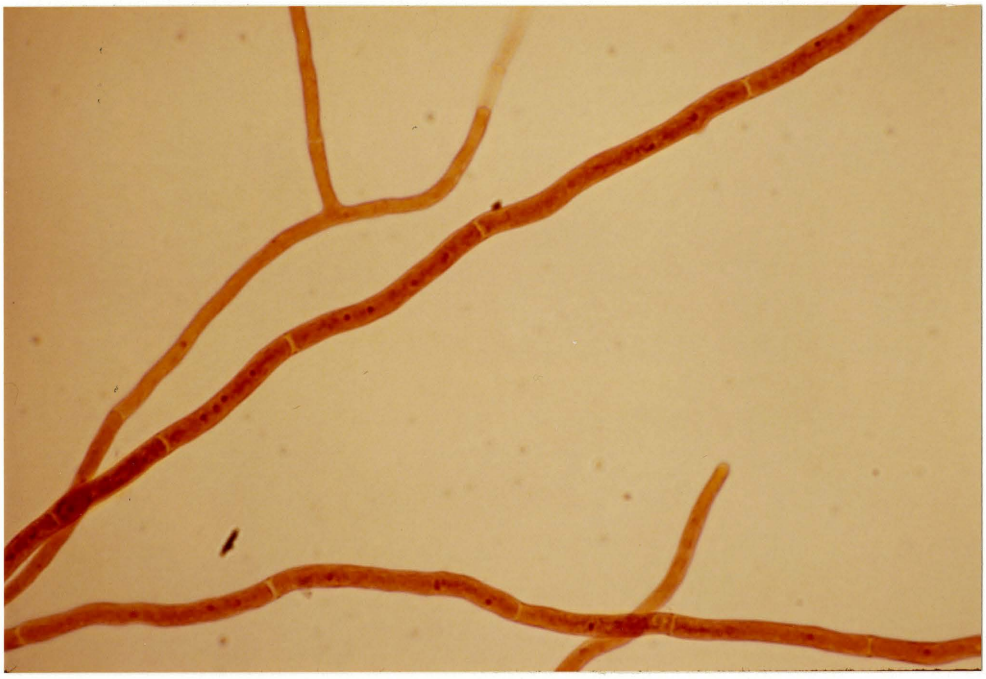

Figure 2. Safranin O-KOH stained nuclei in hyphae of presumptive heterokaryotic isolates of I. andersonii.

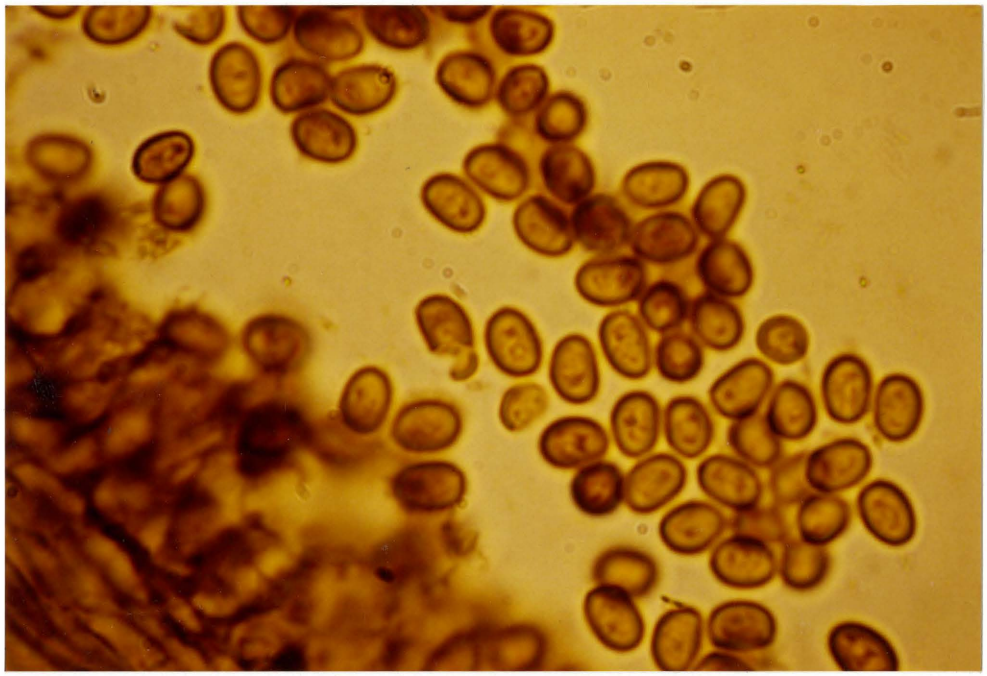

Figure 3. Hematoxylin stained basidiospores of I. andersonii containing one or two nuclei.

binucleate (Fig. 3). Approximately eighty percent of the spores examined were uninucleate. Binucleate spores were typically grouped together.

Although nuclei in hematoxylin stained basidia were detectable, the number per basidium was difficult to determine due to the overlap of basidia in the hymenium. The nuclear state of basidia was easier to detect when fresh basidiocarps were teased apart and stained with Safranin O-KOH. Immature basidia were uni- and binucleate and four nuclei were detected in mature basidia during the early stages of spore development. It was possible to distinguish between the multinucleate behavior of hyphal cells of tramal tissue and uninucleate subhymenial cells.

Vegetative Incompatibility

All pairings between different heterokaryotic isolates resulted in similar antagonistic interaction zones. The results are shown in Fig. 4 and Table 2. Inonotus andersonii isolates characteristically develop thick hyphal mats on 2% MEA. When paired, opposing cultures grew within 1-3mm (in rare instances 5-7mm) of one another. The hyphae were sparse in the narrow darkly pigmented zone between the two isolates. The interface margins of each opposing mat were characterized by a dense buildup of darkly pigmented hyphae which were swollen, contorted, and twisted together. In a few instances the entire area occupied by one isolate was darkly pigmented and the hyphae remained submerged. Self-paired isolates were compatible.

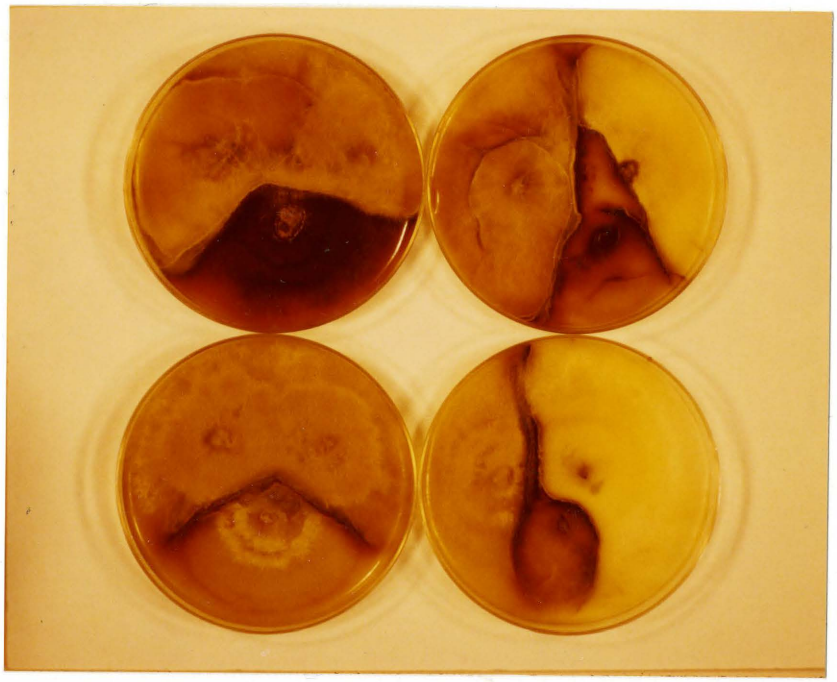

Figure 4. Vegetative imcompatibility reactions between different vegetative isolates of I. andersonii. Self-pairings are shown in top pairings of left row crosses.

Table 2. Interactions of heterokaryotic isolates of *Inonotus andersonii* on 2G MEA.

	RLG 16145	L 4937	FP 72121	FP 89835	FP 94289	CS 659214	MLF 56
RLG 16145	--	+	++	+	+	++	+
L 4937		--	+	+	+	+	+
FP 72121			--	+	+	+	+
FP 89835				--	+	+	+
FP 94289					--	+	+
CS 659214						--	+
MLF 56							--

--: no interaction zone; +: interaction zone 1-3mm; ++: interaction zone 5-7mm.

Microscopic examination of different heterokaryotic pairings on glass slides displayed little growth of either isolate in the interaction zone. Hyphal interaction between opposing isolates resulted in hyphal swellings and excessive branching. All self-pairings resulted in complete intermingling of isolates.

Results from pairing different heterokaryotic isolates in silverleaf oak blocks are shown in Figs. 5 and Table 3. Non-self pairings resulted in regions of undecayed wood where opposing isolates interacted, an area characterized by a zone of darker permanently stained wood. Compatible reactions between self-paired isolates were characterized by the uniform color of decay and the absence of dark interaction zones.

Decay Test

The results from the decay test are shown in Table 4. After 28 wk, the average percent weight loss for each wood type-isolate combination was determined only from the chambers containing moist wood in which the fungus was still alive and actively decaying. Although each wood type was decayed by I. andersonii, overall, the oaks were decayed at a significantly faster rate (P=0.05) than the other hardwoods and spruce. Decay by isolate FP 94289 resulted in significantly greater weight loss (P=0.05), overall, on most wood types than the other two isolates used in this experiment.

Field Studies

Inonotus andersonii forms annual resupinate basidiocarps with very

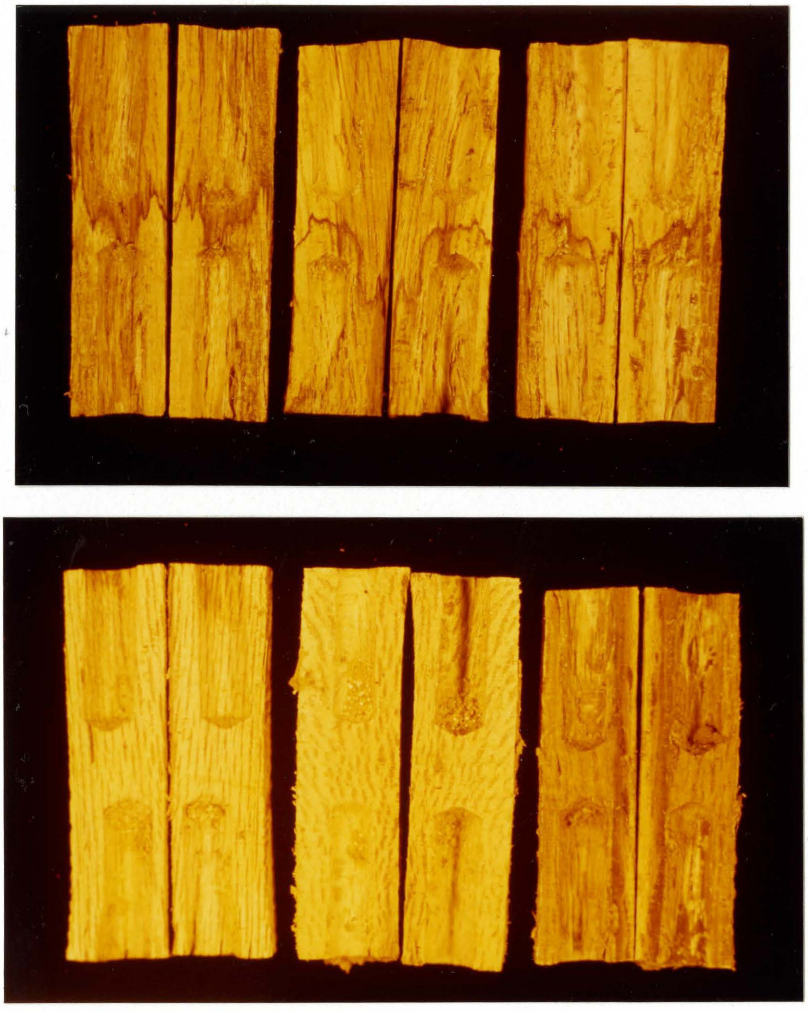

Figure 5. Antagonistic reaction between heterogenic matings of I. andersonii (top row); compatible reaction between homogenic matings of I. andersonii (left row).

Table 3. Interactions of heterokaryons of *Inonotus andersonii* in silverleaf oak test blocks.

	L 4937	FP 89835	FP 94289
L 4937	--	+	+
FP 89835		--	+
FP94289			--

--: compatible reaction, no interaction zone formed in wood block;
+: antagonistic reaction; formation of dark interaction zones formed between opposing cultures.

Table 4. Average weight loss of *in vitro* decayed wood by 3 isolates of *Inonotus andersonii* expressed as a percent of original oven-dry weight.*

		Isolate ***			
Substrate	**	L 4937 b	FP 89835 b	FP 94289 a	control
Gambel oak	A	35.73	37.53	52.13	3.43
Emory oak	A	30.47	36.20	48.20	1.30
silverleaf oak	A	39.16	27.96	36.10	3.43
Arizona white oak	A	35.52	38.22	52.88	1.81
Arizona sycamore	B	13.30	23.76	27.78	0.05
velvet ash	C	7.27	5.69	5.73	0.08
Engelmann spruce	C	12.39	14.02	16.50	1.50

*: Average percent weight loss determined from replications in which the fungus was still active after 28 wk.
**: Wood-types decayed, those followed by the same letter were not decayed at a significantly different rate at P=0.05, Duncan Analysis (based on total weight loss with 3 isolates).
***:Vegetative decay isolates, decay rate was not significantly different among isolates followed by the same letter at P=0.05, Duncan Analysis (based on total weight loss of 5 wood types).

thin context tissue so isolations are more easily obtained from decayed wood. Isolations are more successful if tried shortly after fruiting or before basidiocarp formation. Isolations were not successful from trees on which fruiting had occurred more than one year previously. Basidiocarp formation occurs only once on a tree killed by I. andersonii. The basidiocarps deteriorate progressively over several years.

Samples of typical I. andersonii decay collected from the stump of a newly wind-thrown Emory oak in early July of 1987 were incubated, without light, in moist chambers at 32 C. Basidiocarps began forming in approx. 2 wk between the outer layers, and other exposed areas of sapwood. In early September the same tree was observed for signs of basidiocarp formation. The bark was peeled back to expose developing tubes which covered a large perimeter of the tree. Samples taken at this time and incubated in the lab began forming basidiocarps within 2-3 days. The basidiocarps were variable in shape and were not restricted to the outer layers of sapwood.

DISCUSSION

Distinct zones of interaction developed between different heterokaryotic isolates of I. andersonii paired on MEA and wood test blocks. The substrate is pigmented and the hyphae adjacent to the interaction zone are knotted and swollen. Similar reactions have been described from intraspecific pairings of other wood decay fungi (Goldstein and Gilbertson, 1981; Hansen, 1979a; Rayner and Todd, 1977; Barrett and Uscuplic, 1971; and Childs, 1963), and vegetative incompatibility is considered widespread in fungi.

In wood test blocks, the area adjacent to the zone of interaction remains undecayed. Rayner and Todd (1977, 1978) observed similar antagonistic reactions and pigmentation in wood decayed by Trametes versicolor (=Coriolus versicolor (L:Fr.) Quel.). Adams and Roth (1969) examined the upper boles of damaged Douglas-fir trees and retrieved different genotypes of Fomitopsis cajanderi (Karst.) Kotl. et Pouz. separated in the wood by lines of demarcation. Both F. cajanderi and I. andersonii infect the host through dispersal and germination of basidiospores. In this study, pigmented interaction zones were observed in wood naturally infected and decayed by I. andersonii, suggesting this fungus exists as a population of different genotypes exhibiting vegetative incompatibility.

The mechanism which controls vegetative incompatibility is not well understood. It appears that antagonism is due to polygenic or multiallelic differences at the somatic incompatibility loci (Rayner and

Todd, 1979; Rayner et al., 1984; Rayner and Boddy, 1986). Melanin was extracted from the pigmented interaction zone of Phellinus weirii (Murr.) Gilbn. isolates by Li (1983) who suggests that melanin is responsible for inhibiting the growth of antagonistic organisms. The mechanism which controls vegetative incompatibility is overridden by genes which control homokaryotic incompatibility reactions.

Inonotus andersonii fruited in culture within 3 months on WWA medium, which is malt extract agar medium containing a mixture of ground oakwood and wheat (v/v). Light exposure neither induced nor inhibited basidiocarp formation. This was predicted because basidiocarp formation in nature begins beneath intact bark, between layers of outer sapwood.

Factors which induce germination of I. andersonii basidiospores were not determined. Spores collected from recently sporulating basidiocarps and streaked on 2% MEA germinated after approximately 3 wk incubation at room temperature. Previous attempts to induce spore germination with the same and other methods were unsuccessful. The spores from those trials had been collected from wood test block cultures a wk or more after the start of sporulation. However, this was the first time spores were sampled sequentially during various periods of sporulation.

Two basidiospore types were detected a few days after sporulation of I. andersonii was initiated. Small thin-walled spores were observed in addition to typical thick-walled basidiospores. It is not known from the experiment whether one or both spore types germinated.

Nuss (1982) reported some Ganoderma species produce two types of basidiospores from the same basidiocarp during different periods of

sporulation. Small thin-walled, 'proterospores', are produced at the beginning of sporulation and germinate easily on MEA. The typical thick-walled Ganoderma basidiospores are produced later and must pass through a fly larval gut in order to germinate. It is possible that the thin-walled spores produced by I. andersonii are a type of 'proterospore' that germinates more readily in vitro. Basidiospores of I. andersonii were found in insect frass in nature but attempts to germinate them on BSM medium (Castello et al., 1976) were unsuccessful.

Although basidiospores of many heartrotting Hymenomycetes are considered generally difficult to germinate under laboratory conditions (Merrill, 1970; and Fries, 1984), spore germination has been induced for some members of the group. The factors responsible for germination may be specific to one species or general for many species of a genus. Fischer (1987) reported successful spore germination of 20 Phellinus sp. and 4 Inonotus sp. (I. andersonii not included) was achieved at a pH of 4-5 on MEA medium. Similar alterations in pH made in this study had no effect on basidiospore germination of I. andersonii.

The heterokaryotic mycelium of I. andersonii isolates is plurinucleate. The nuclei apparently undergo conjugate division because they are found in multiples of two, typically 2, 4 or 8 nuclei per cell. Inonotus andersonii, like many hymenomycetes, fits Boidin's (1971) classification of fungi demonstrating holocenocytic behavior, characterized by basidiospores with one or two nuclei germinating into plurinucleate hyphal cells.

Variations in nuclear behavior within Hymenochaetaceae have been

reported but the mechanism which controls nuclear migration in the absence of clamp connections has not been determined. Hennon and Hansen (1987) observed the nuclear behavior of three Phellinus species and found a multinucleate condition in which the nuclei were unpaired and not consistently present in even numbers. Goldstein and Gilbertson (1981) reported 3 or 4 nuclei per cell in I. arizonicus Gilbn. isolates. They concluded the fungus to be homothallic because basidiocarps formed from single basidiospore isolates that were not morphologically different from tissue isolates. In contrast, the nuclei in the heterokaryotic mycelium of Phellinus weirii are paired and this fungus is considered heterothallic because single spore isolates do not fruit in culture (Hansen, 1979b). The nuclei in presumed heterokaryotic isolates of I. andersonii are typically found in even numbers and are infrequently paired.

Nuclear behavior in the hymenium was also examined. The cells of the tramal hyphae were multinucleate but a uninucleate condition was typical in subhymenial cells. Some areas of the trama contained hyphae in which the nuclei appeared larger. Variation in nuclear number and size may indicate nuclear fusion in areas outside the basidia.

Diploid and tetraploid nuclei have been detected in some fungi by microspectrophotometry which measures the DNA-content of nuclei. Armillaria mellea (Vahl ex Fr.) Kummer has a diploid vegetative state and the nuclei of cells in lamellar tramal tissue is tetraploid (Peabody, et al, 1978). Fischer (1987) examined the nuclear condition of twenty species of Phellinus Quel. and four of Inonotus Karst. Based

on nuclear DNA-content, the karyology of mycelium and hymenial elements, and the pattern of sexuality Fischer proposed three types of life cycles: heterothallically bipolar with a haplophase as the basic stage; homothallic and haploid; homothallic and having a diplophase as the basic stage. Fischer found nuclear fusions, resulting in diploid and tetraploid nuclei, were common in the heterokaryotic mycelium and fruitbody mycelium of all 3 groups. He suggests, because karyogamy and meiosis were observed within basidia, reduction division also occurs outside the basidium. Karyogamy and reduction division within basidia could not be observed by methods used in this study.

Among the fungi reported by Fischer to be homothallic and haploid is Inonotus obliquus (Pers.:Fr.) Pilat. Although this fungus decays mainly birch, it is very similar to I. andersonii in ecology and method of fruiting. Both fungi enter the host through branch stubs, decay the heartwood and move into the sapwood and eventually kill the tree. It is conceivable to imagine the two having similar life cycles. The importance of Fischer's finding for homothallic species is that recombination is made possible by the existence of heterogeneous nuclei within the vegetative mycelium.

The uninucleate condition found in the subhymenium and undeveloped basidia differs from the dikaryotic condition typical of 'model' basidiomycetes such as Schizophyllum commune Fr. (Raper, 1966). Hennon and Hansen (1987) suggest nuclear counts alone not be used as reliable criteria for determining sexuality in the hymenomycetes. Verrall (1937) concluded, based on differences in growth rate and nuclear state in

single spore and tissue isolates, P. tremulae (= Fomes igniarius (L.:Fr.) Kickx) was tetrapolar, but nuclear DNA-content analysis (Fisher, 1987) suggests this fungus is heterothallically bipolar.

Variation among isolates was detected in both decay and growth tests, but faster growth rate did not correspond with greater decay capacity. Merrill (1970) reported the optimum temperature for decay may be the same or a few degrees lower than the optimum temperature for enzyme activity and mycelial growth. However, the incubation temperature in the decay test was higher than the optimum growth temperature of the stronger decaying isolate. Although oaks were the only natural host tested, all wood types were decayed by I. andersonii.

The imperfect fruitbodies described by Cerny (1982) were not found near branch stubs of wood decayed by I. andersonii in Southern Arizona. They also failed to develop in test blocks in culture. Chlamydospores, unlike those reported by Cerny (1982), were observed in culture and field isolates. This is the first report of these spores and it is assumed they contribute to short range spread and act as resting structures.

In nature fruiting bodies develop only once on a tree killed by I. andersonii and the fungus dies out shortly after fruiting. Sporulation occurs in the late fall and early winter. All oak species in Southern Arizona are apparently susceptible to infection.

LITERATURE CITED

Adams, D. H., and L. F. Roth. 1969. Intraspecific competition among genotypes of Fomes cajanderi decaying young-growth Douglas-fir. Forest Sci. 15: 327-331.

Adaskaveg, J. E., and R. L. Gilbertson. 1987. Vegetative incompatibility between intraspecific dikaryotic pairings of Ganoderma lucidum and G. tsugae. Mycologia 79: 603-613.

Bandoni, R. J. 1979. Safranin O as a rapid nuclear stain for fungi. Mycologia 71: 873-874.

Barrett, D. K. and M. Uscuplic. 1971. The field distribution of interacting strains of Polyporus schweinitzii and their origin. New Phytol. 70: 581-598.

Baxter, D. V. 1929. Some Porias from the region of the Lake States. II. Pap. Mich. Acad. Sci. 9: 39-46.

Baxter, D. V. 1933. Some Resupinate Polypores from the region of the Great Lakes. IV. Pap. Mich. Acad. Sci. 18: 421-439.

Berry, F. H., and F. F. Lombard. 1978. Basidiomycetes associated with decay of living oak trees. U.S. Forest Serv. Res. Paper NE-413: 1-8.

Boidin, J. 1971. Nuclear behavior in the mycelium and the evolution of the Basidiomycetes. Pp. 129-148. In: Evolution in the higher Basidiomycetes. Ed. R. H. Peterson. Univ. of Tennessee Press, Knoxville, Tenn.

Campbell, W. A., and R. W. Davidson. 1939. Poria andersonii and Polyporous glomeratus, two distinct heart-rotting fungi. Mycologia 31: 161-168.

Castello, J. D., C. G. Shaw, and M. M. Furniss. 1976. Isolation of Cryptoporus volvatus and Fomes pinicola from Dendroctonus pseudosugae. Phytopathology 66: 1431-1434.

Cerny, A. 1963. Inonotus andersonii (Ellis et Everhart) Cerny comb. nov. A new polypore for Czechoslovakia. Ceska Mykol. 17: 1-8.

Cerny, A. 1982. Biology of Inonotus andersonii and its occurrence in Czechoslovakia. Ceska Mykol. 36: 11-23.

Childs, T. W. 1963. Poria weirii root rot. Phytopathology 53: 1124-1127.

Davidson, R. W., W. A. Campbell and D. Blaisdell Vaughn. 1942. Fungi causing decay of living oaks in the Eastern United States and their cultural indentification. U.S. D. A., Techn. Bull. 785: 1-65.

Domanski, S. 1972. Basidiomycetes:Aphyllophorales. Polyporaceae I. Mucronoporaceae I. In: Flora Polska (Grzyby) 2. 280 p.

Ellis, J. B., and B. M. Everhart. 1890. Mucronoporus andersoni. J. Mycol. 6: 79.

Fischer, M. 1987. Biosystematische Untersuchungen an den Porlingsgattungen Phellinus Quel. und Inonotus Karst. Biblioth. Mycol. 107: 1-133.

Fries, N. 1984. Spore germination in the higher Basidiomycetes. Proc. Indian Acad. Sci. (Plant Sci.) 93: 205-222.

Genaux, C. M., and J. G. Kuenzel. 1939. Defects which reduce quality and yield of oak-hickory stands in southeastern Iowa. Iowa Agric. Exp. Stn. Res. Bull. 269: 409-444.

Gilbertson, R. L., and L. Ryvarden. 1986. North American Polypores. V. 1. Abortiporus-Lindtnena. Fungiflora, Oslo. 433 p.

Goldstein, D., and R. L. Gilbertson. 1981. Cultural morphology and sexuality of Inonotus arizonicus. Mycologia 73: 167-180.

Hansen, E. M. 1979a. Sexual and vegetative incompatibility reactions in Phellinus weirii. Canad. J. Bot. 57: 1573-1578.

Hansen, E. M. 1979b. Nuclear condition and vegetative characteristics of homokaryotic and heterokaryotic isolates of Phellinus weirii. Canad. J. Bot. 57: 1579-1582.

Hennon, P. E., and E. M. Hansen. 1987. Nuclear behavior of Phellinus arctostaphyli, P. igniarius, and P. tremulae. Mycologia 79: 501-507.

Jensen, W. A. 1962. Botanical Histochemistry. W. H. Freeman, San Francisco. 408 p.

Johansen, D. A. 1940. Plant Microtechnique. McGraw-Hill, New York. 523 p.

Li, C. Y. 1983. Melanin-like pigment in zone lines of Phellinus weirii-

colonized wood. Mycologia 75: 562-566.

Lloyd, C. G. 1920. Mycological notes 6:1012.

Merrill, W. 1970. Spore germination and host penetration by heartrotting Hymemomycetes. Annual Rev. Phytopathol. 8: 281-300.

Murrill, W. A. 1916. A new genus of resupinate polypores. Mycologia 8: 56-57.

Neuman, J. J. 1914. The Polyporaceae of Wisconsin. Wis. Geol. and Nat. Hist. Survey Bul. 33. 207p.

Nuss, I. 1982. Die Bedeutung der Proterosporen: Schlu folgerungen aus Untersuchungen an Ganoderma (Basidiomycetes) Plant Syst. Evol. 141: 53-79.

Peabody, D. C., J. Motta, C. D. Therrien. 1978. Cytophotometric evidence for heteroploidy in the lifecycle of Armillaria mellea. Mycologia 70: 487-498.

Raper, J. R. 1966. Genetics of sexuality in higher fungi. The Ronald Press Co., New York. 283 p.

Rayner, A. D. M., D. Coates, A. M. Ainsworth, T. J. H. Adams, E. N. D. Williams and N. K. Todd. 1984. The biological consequences of the individualistic mycelium. Pp. 509-540. In: The ecology and physiology of the fungal mycelium. Eds., V. H. Jennings and A. D. M. Rayner. Cambridge University Press, Cambridge.

Rayner, A. D. M., and L. Boddy. 1986. Population structure and the infection biology of wood-decay fungi in living trees. Pp. 119-160. In: Advances in plant pathology. Ed. D. S. Ingram and P. H. Williams. V. 5, Academic Press, London.

Rayner, A. D. M. and N. K. Todd. 1977. Intraspecific antagonism in natural populations of wood-decaying basidiomycetes. J. Gen. Microbiol. 103: 85-90.

Rayner, A.D.M., and N.K. Todd. 1978. Polymorphism in Coriolus versicolor and its relation to interfertility and intraspecific antagonism. Trans. Brit. Mycol. Soc. 71: 99-106.

Rayner, A. D. M. and N. K. Todd. 1979. Population and community structure and dynamics of fungi in decaying wood. Pp. 380-420. In: Anvances in botanical research. Ed. H. W. Woolhouse. V.7, Academic Press, London.

Toole, E. R. 1961. Rot Entrance through dead branches of southern