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Title: INCOMPATIBILITY REACTIONS AND NUCLEAR CONDITION OF THREE PHELLINUS SPECIES

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Phellinus arctostaphyli (Long) Niemelä, P. igniarius (L. ex Fr.) Quél., and P. tremulae (Bond.) Bond. et Boriss., heartrotting hymenomycetes in the family Hymenochaetaceae (Aphyllophorales), all conform to a multi-allelic heterothallic mating system. Both compatible and incompatible reactions result from pairings of homokaryons from the same basidiocarp. Homokaryons isolated from different basidiocarps, but the same species, are fully compatible. Cultural characteristics are not useful in distinguishing between homokaryons and heterokaryons. However, heterokaryons exhibit significantly faster growth rate in culture than homokaryons. Antagonistic responses between paired homokaryotic isolates serve as an indicator of sexual incompatibility; sexually compatible pairs acquire the faster growth rate of heterokaryons. Antagonistic characteristics similar in appearance to sexual incompatibility occur between paired heterokaryotic isolates displaying vegetative incompatibility. These three species are similar in the variation of the nuclear condition throughout their life cycles. Basidiospores

are produced with one nucleus, but are ordinarily binucleate prior to germination. Both homokaryons and heterokaryons have a variable number of unpaired nuclei between septa. The dikaryophase is delayed until basidiocarp formation, when the dikaryon can be found in young basidia and mycelium underlying basidia. Incompatible reactions in hybridization attempts support the segregation of these three closely related species.

Incompatibility Reactions and Nuclear Condition of Three *Phellinus* Species

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INTRODUCTION

Species of the genus *Phellinus* Quél. are among the most destructive wood-inhabiting fungi. Despite their economic importance, their life cycles are largely unknown. The objective of this study is to contribute to the comparatively little information that exists concerning their nuclear behavior and mating systems. Three closely related species were chosen for investigation: *Phellinus arctostaphyli* (Long) Niemelä, *P. igniarius* (L. ex Fr.) Quél., and *P. tremulae* (Bond.) Bond. et Boriss.

The consistent lack of clamp connections, a feature that these species share with other members of the Hymenochaetaceae, has impeded sexual incompatibility studies. The clamp connection has been the most frequently utilized indicator of sexual compatibility, aiding in studies demonstrating several hymenomycete mating patterns. Homokaryotic mycelium is typically the result of the germination of a single, haploid basidiospore. A species is considered homothallic if this mycelium is self-fertile, capable of completing its entire life cycle, including karyogamy and meiosis, without anastamosis with another mycelium. The homokaryons of heterothallic species, however, are self-sterile, requiring an anastamosis with another mycelium before achieving fertility. The sexual compatibility or incompatibility of this interaction is genetically governed at one locus for bipolar (or

unifactoral) species and two unlinked loci for tetrapolar (or bifactoral) species. The interacting pair is compatible only if different alleles occur at the mating locus (for bipolar) or at both mating loci (for tetrapolar species). In hymenomycetes, these loci are multiallelic; there are many potential alleles for each mating locus. The mycelium resulting from compatible pairs is heterokaryotic, i.e., composed of genetically distinct nuclei. A special heterokaryon, the dikaryon, ordinarily possesses clamp connections to facilitate conjugate division of its closely paired nuclei.

Because Phellinus spp. lack clamp connections, it is not easy to determine the compatibility of matings. Consequently, the mating systems of only three species (P. gilvus, P. weirii, and P. igniarius) have been investigated. Hirt (1928) determined P. gilvus to be homothallic based on basidiocarp formation occurring in single spore isolates in culture. The lack of basidiocarp formation in single spore isolates and its presence in heterokaryotic isolates led Gillette (1975) and Hansen (1979b) to conclude that P. weirii is heterothallic. Utilizing additional criteria such as cultural characteristics of homokaryons and heterokaryons and antagonistic responses in pairings, Hansen (1979b) found that P. weirii fit neither the bipolar nor the tetrapolar pattern of sexual compatibility. Verrall (1937) judged P. igniarius to possess a tetrapolar, heterothallic mating system; this is the only report of the number of incompatibility loci in Phellinus. Insufficient numbers of single

spore isolates and uncertain genetic origins were used, however. Three mating studies involving only three, four, and five homokaryons paired in all combinations were conducted, and each of the latter two included homokaryons taken from two separate basidiocarps. Nonetheless, tetrapolarity of P. igniarius is accepted in the literature (Nobles, 1965; Day, 1978).

The nuclear condition of Phellinus spp. has received somewhat more attention. Although methods are not mentioned, Kühner (1950) found six species of Hymenochaetaceae to possess binucleate hyphal cells and twenty species, including P. igniarius, to have a variable number of nuclei. Apparently single spore isolates were not examined. Hansen (1979a) established that homokaryotic hyphal cells of P. weirii contain a variable number of nuclei, but heterokaryotic isolates (from basidiocarp context and decaying wood) are irregularly binucleate.

P. arctostaphyli, P. igniarius and P. tremulae cause a white rot of the heartwood in their living hosts. Because of its common occurrence and wide host range, P. igniarius (Fig. 1a) causes a greater loss than any other fungal wood-destroyer of living park and forest hardwoods (Boyce, 1961). The most important cause of heartrot in aspens, P. tremulae (Fig. 2a, b), is common in both eastern and western hemispheres (Schmidtz and Jackson, 1927). P. arctostaphyli (Fig. 1b), occurring on manzanita species in the western United States (Gilbertson, 1979), has received very little attention due to its confusion with P. igniarius and the

Fig. 1a. P. igniarius basidiocarps on Salix sp. (top).
Fig. 1b. P. arctostaphyli on Arctostaphylus patula (bottom).

Fig. 2a. P. tremulae basidiocarp on Populus tremuloides (left).

Fig. 2b. Longitudinal section through a P. tremulae basidiocarp revealing features characteristic of Phellinus spp.: brown context, black stain with KOH, white mycelium of inactive tube layers, and its perennial nature (right).

economic insignificance of its hosts.

Taxonomic treatment of these three closely related species has changed over the years. From early taxonomic studies until the present P. igniarius was split first into varieties and then into distinct species. Linneaus (1753), applying the name Boletus igniarius to a specimen collected from a Betula sp., first acknowledged a member of the P. igniarius group. Upon erection of the genus Phellinus, Quélet (1886) established P. igniarius as the type. The recognition of P. arctostaphyli as a distinct species (Long, 1917) has been largely overlooked until recently. Long and Harsch (1918) suggested that isolates collected from Alnus sp. and Populus sp. were not the same species on the basis of differing cultural characteristics. Fritz (1923) argued that cultural differences were not sufficient characters for species division. Campbell (1938), Nobles (1948, 1965) and Overholts (1953) accepted these distinctions to be significant at the variety level. Overholts (1953) perceived P. arctostaphyli to be an exact duplicate of P. igniarius, except for smaller size and did not recognize it as a separate species. Based on gross basidiocarp features and, again, cultural characteristics, Verrall (1937) divided P. igniarius into three groups: 1) the aspen type, 2) the birch type, and 3) the type from miscellaneous hosts. Probably because his collections were from the Lake States (except one from Colorado), P. arctostaphyli was not included in Verrall's work.

Microscopic investigations of basidiocarp morphology

increased the number of distinguishing features. Bondartzev (1953) created P. tremulae for aspen isolates formerly assigned to P. igniarius var. populinus. Neuman (1914) first recognized this variety but assigned it to Fomes nigricans. Campbell (1938) later transferred it to F. igniarius. Niemelä (1972, 1974, 1975, 1977a) compiled a detailed taxonomic treatment of P. igniarius and allied species. Niemelä (1974) provided further microscopic evidence for segregating P. tremulae, and Gilbertson (1979) accepted P. arctostaphyli, P. igniarius, and P. tremulae as separate species.

In a recent monograph, Bondartseva and Herrera (1980) established eight sections in the genus Phellinus. P. igniarius and P. tremulae occur in the same section and subsection along with species in the P. robustus group. However, P. laevigatus, considered by others to be closely allied to P. igniarius (Niemelä, 1972), appears in a separate section. P. arctostaphyli is not mentioned in this monograph.

These three species share similarities with other Phellinus spp.: brown, xanthocroic context; clampless, dimitic hyphal system; perennial basidiocarp; and ability to degrade lignin. The features that unify the P. igniarius group include: a hard pseudocrust of the basidiocarp; woody context from thick-walled skeletal hyphae; short setae and no setal hyphae in the hymenium; white mycelium in older tube layers; hyaline, subglobose, indextrinoid basidiospores; and parasitism on angiosperms. Niemelä views P. arctostaphyli to be outside of the P. igniarius group owing to its longer

more slender setae and cyanophilaus basidiospores. However, its close relationship in all other respects should be recognized.

Because of so many similarities, P. arctostaphyli, P. igniarius, and P. tremulae should be viewed as closely related species. However, the existence of numerous differences warrant species segregation (Table 1). The orientation of basidiocarp tramal hyphae was of particular interest to Niemelä, who viewed this feature as a major taxonomic property delimitating two groups of P. igniarius and its closely related species.

The objectives of the present study of these three Phellinus spp. were:

1) to distinguish homokaryotic isolates from heterokaryotic isolates
2) to describe vegetative and sexual incompatibility reactions
3) to describe the nuclear condition through their life cycles
4) to determine the validity of each species

Three avenues were investigated to attain these objectives. First, cultural characteristics of homokaryons and heterokaryons were examined for differences. Second, the presence or absence of antagonism between paired isolated was observed. Finally, three nuclear stains were employed for observations of the number and distribution of nuclei in various stages of the life cycles of these fungi.

Table 1. Taxonomic characteristics of P. arctostaphyli, P. igniarius and P. tremulae.

Characteristics	Species		
	P. arctostaphyli	P. igniarius	P. tremulae
basidiocarp size	small	large	large
pore surface orientation	± horizontal	± horizontal	45 degree angle with horizontal
tramal hyphae	parallel	intermingled	parallel
setae	infrequent 30-50 μm length[a]	few to abundant[a] 14-17 μm length	few to abundant[a] 12-30 μm length
basidiospores	cyanophilous; germination in H_2O agar	acyanophilous; germination in H_2O agar	acyanophilous; poor germination in H_2O agar
host range	Arctostaphylus spp.[a] and Cercocarpus sp.	many angiosperms	Populus tremuloides and allied species

[a]setae measurements and host from Gilbertson, 1979.

MATERIALS AND METHODS

P. arctostaphyli, P. igniarius, and P. tremulae basidiocarps were identified according to characteristics in Table 1 and the key to western North American Phellinus spp. (Gilbertson, 1979). Heterokaryotic cultures were isolated from the context of field-collected basidiocarps. Single spore isolates were gathered as follows: square sections (e.g., 3mm X 3mm) from the underside of field-collected basidiocarps were attached to the lids of petri plates and allowed to sporulate for several hours. Basidiospore deposits were spread with a sterile loop. Basidiospores of P. arctostaphyli and P. igniarius germinated on water agar; P. tremulae required a medium richer in sugars, 8% malt extract agar was used (Good and Spanis, 1958). Following several days of incubation at room temperature, distantly-spaced germinating basidiospores were located using a dissecting microscope (72x) with bottom illumination. Individual germlings were then transferred to separate petri plates by removing a small square of medium with a sharpened insect pin. Malt extract agar (1.5% Difco malt extract; 2% Bacto agar) containing 2ppm benomyl (for reduced contamination) was utilized as the standard medium, 20 ml pipetted into 9 cm petri plates.

Cultural Characteristics

Forty-six heterokaryotic and 200 homokaryotic isolates (Table 2) of the three Phellinus spp. were examined for gross

Table 2. Origin of heterokaryotic and derived single spore isolates of P. arctostaphyli, P. igniarius, and P. tremulae. het = heterokaryotic isolates, s = homokaryotic (single spore) isolates. ⌐ = basidiocarps collected from the same host tree.

Isolate	Host	Date	Location
P. arctostaphyli			
⌐ PAR 01-d het s01-07	Arctostaphylus patula	3-15-80	Douglas Co., OR
⌙ PAR 01-e het s01-07	A. patula	3-15-80	Douglas Co., OR
PAR 03 het s01-09	A. patula	3-15-80	Douglas Co., OR
PAR 04 het s01-07	A. columbiana	3-16-80	Josephine Co., OR
PAR 05 het s01	A. columbiana	3-16-80	Josephine Co., OR
PAR 06 het s01-04	A. columbiana	3-16-80	Jackson Co., OR
PAR 07 het s01-09	A. columbiana	3-16-80	Jackson Co., OR
PAR 08 het s01-05	A. columbiana	3-16-80	Jackson Co., OR
PAR 09 het s01-07	A. patula	3-17-80	Deschutes Co., OR

Table 2 continued.

Isolate	Host	Date	Location
P. arctostaphyli continued			
PAR 30 het	*Arctostaphylus patula*	5-11-80	Deschutes Co., OR
PAR 84 het s01-28	*A. patula*	5-11-80	Deschutes Co., OR
P. igniarius			
PIG 01 het	*Salix sp.*	7-1-79	Lane Co., OR
PIG 37 het	*S. scouleriana*	3-26-80	Benton Co., OR
PIG 40 het	*S. scouleriana*	3-26-80	Benton Co., OR
PIG 51 het s01-36	*S. scouleriana*	2-14-80	Benton Co., OR
PIG 55 het s01-13	*S. scouleriana*	2-14-80	Benton Co., OR
PIG 96-a het	*Amelanchier alnifolia*	7-22-80	Deschutes Co., OR
PIG 96-b het	*A. alnifolia*	7-22-80	Deschutes Co., OR
PIG 80 het	*Populus trichocarpa*	9-25-80	Polk Co., OR

Table 2 continued.

Isolate	Host	Date	Location
P. tremulae			
PTA 03 het	*Populus tremuloides*	11-15-79	Deschutes Co., OR
PTA 14 het	*P. tremuloides*	9-4-79	Bryce Canyon, UT
PTA 16 het	*P. tremuloides*	9-4-79	Grover, UT
PTA 30 het	*P. tremuloides*	3-17-80	Deschutes Co., OR
PTA 31 het	*P. tremuloides*	3-17-80	Deschutes Co., OR
PTA 32 het	*P. tremuloides*	3-17-80	Deschutes Co., OR
PTA 33 het	*P. tremuloides*	3-17-80	Deschutes Co., OR
PTA 40 het	*P. tremuloides*	6-4-80	Eagle River, WI[a]
PTA 41 het	*P. tremuloides*	6-4-80	Eagle River, WI[a]
PTA 44 het	*P. tremuloides*	6-5-80	Hayward, WI[a]
PTA 47 het	*P. tremuloides*	6-5-80	Bayfield City, WI[a]
PTA 48 het	*P. tremuloides*	6-5-80	Bayfield City, WI[a]
PTA 49-a het	*P. tremuloides*	6-5-80	Bayfield City, WI[a]
PTA 49-b het	*P. tremuloides*	6-5-80	Bayfield City, WI[a]

Table 2 continued.

Isolates	Host	Date	Location
P. tremulae continued			
PTA 60 het s01-04	*Populus tremuloides*	5-11-80	Deschutes Co., OR
PTA 61 het	*P. tremuloides*	5-11-80	Deschutes Co., OR
PTA 62 het	*P. tremuloides*	5-11-80	Deschutes Co., OR
PTA 63 het	*P. tremuloides*	5-11-80	Deschutes Co., OR
PTA 64 het	*P. tremuloides*	5-11-80	Deschutes Co., OR
PTA 65 het s01-36	*P. tremuloides*	5-11-80	Deschutes Co., OR
PTA 66 het	*P. tremuloides*	5-11-80	Deschutes Co., OR
PTA 67-a het	*P. tremuloides*	5-11-80	Deschutes Co., OR
PTA 67-b het	*P. tremuloides*	5-11-80	Deschutes Co., OR
PTA 68 het s01-11	*P. tremuloides*	5-11-80	Deschutes Co., OR
PTA 69 het s01-07	*P. tremuloides*	5-11-80	Deschutes Co., OR
PTA 70 het	*P. tremuloides*	5-11-80	Deschutes Co., OR

Table 2 continued.

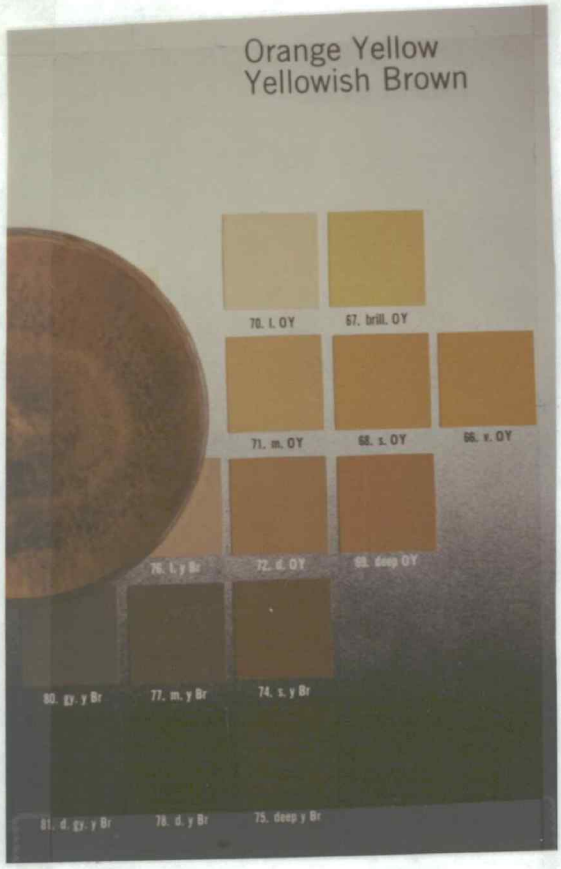
Isolate	Host	Date	Location
P. tremulae continued			
PTA 71 het s01-09	*P. tremuloides*	5-11-80	Deschutes Co., OR

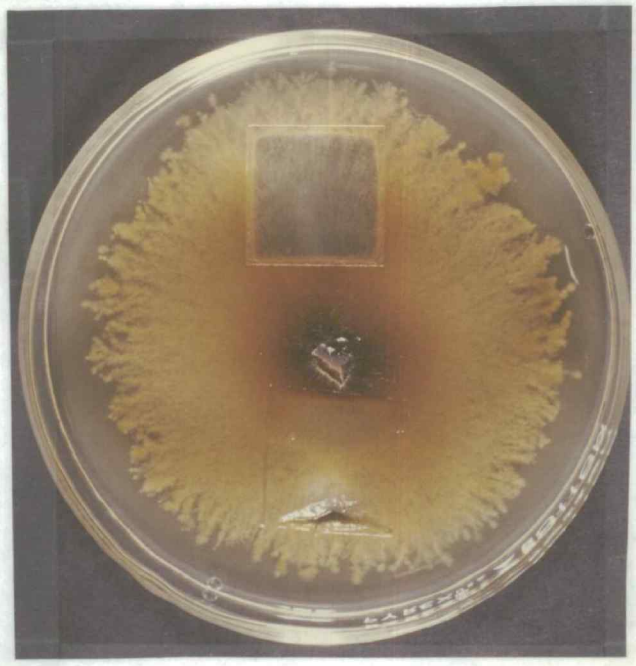
[a]basidiocarp collected by R. F. Patton, U. Wisconsin, Madison

cultural characteristics. A 7 mm diameter plug from the margin of an actively growing culture was transferred, mycelium side down, to the center of each plate. To maintain unbiased measurements, all plates were coded with random numbers and placed in a 25 C dark incubator and allowed to grow for six weeks. Each culture was observed weekly for the following characteristics: radial growth (two radial measurements 90 degrees apart), regular or irregular form of culture margin (Fig. 3a), aerialness of culture center and margin (Fig. 3b), pigmentation color (Fig. 3c) (Kelly and Judd, 1965), zonation, and reverse stain (described by Nobles, 1948) (Fig. 3a). Radial growth rates were approximately linear if initial lag periods and slowed growth at the plates' edge were accounted for. Consequently, radial measurements began as the culture radius reached 5 mm and ceased after it surpassed 35 mm.

Pairing

To study the interaction of different isolates, two isolates were transferred to the same petri plate and allowed to grow together. For each of 220 pairings, two transfers were positioned, mycelium side down, 10 mm apart and 15 mm from the plate's edge. All plates were incubated at 25 C in the dark and examined weekly. Five classes of paired isolates were established to examine sexual and vegetative incompatibility:





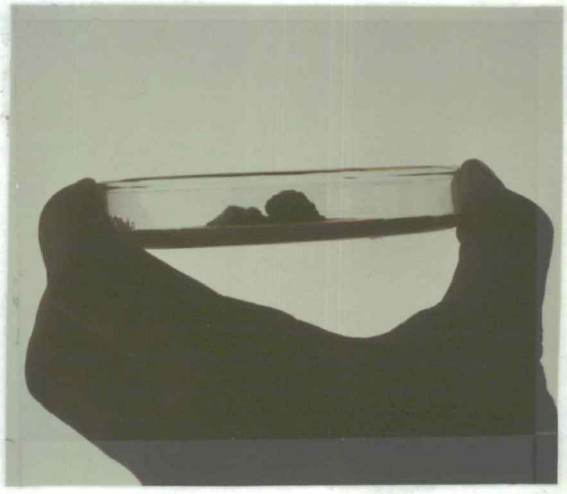


Fig. 3a. An isolate exhibiting an irregular margin and the method of gathering mycelium on cover slips for nuclear staining. Notice the lack of reverse stain under the cover slip. (upper right)

Fig. 3b. Aerial growth habit of some isolates. (lower right)

Fig. 3c. ISCC-NBS method of determining pigmentation colors of isolates (Kelly and Judd, 1965). (left)

sexual incompatibility

I. homokaryon X homokaryon, from the same basidiocarp (isolates also self-paired)
72 plates

II. homokaryon X homokaryon, from different basidiocarps of the same species
54 plates

III. homokaryon X homokaryon, from different species (hybridization)
27 plates

vegetative incompatibility

IV. heterokaryon X heterokaryon, from different basidiocarps of the same species (isolates also self-paired)
41 plates

V. heterokaryon X heterokaryon, from different species
26 plates

Antagonism was evidenced by the formation in the zone of interaction of reverse stain, pigmented, aerial, or appressed hyphae, a submerged band of hyphae, or an uncolonized area. Antagonism was concluded only if the change occurred in greater magnitude in the zone of interaction between the two paired isolates than in other areas of the isolate. For example, an isolate inherently producing reverse stain might react antagonistically to another isolate by secreting an especially dark stain into the medium in the area of interaction. Also, if pigmentation color of the mycelium interacting with its pair was darker than

in other areas of the plate, then the reaction would be accepted as antagonism. Particularly raised aerial hyphae or especially prostrate-appressed reactions between paired isolates provide further examples of antagonism. Dense hyphal bands occurred as unpigmented submerged mycelia beneath the surface of the media between pairs. Avoidance distance was measured between two isolates never making contact.

In addition, the radial growth rate of both isolates of a pair was recorded following contact. Radial growth rate parallel with the line of interaction was measured for each isolate.

Nuclear Staining

The nuclei of basidiospores, germinating basidiospores, and cultured homokaryons and heterokaryons were all stained using the giemsa nuclear stain. The staining series followed that of CMI (1968) except that a hot HCl wash was not used, three buffer changes were used before staining, and the staining time was 45 min. Basidiospores were collected on glass slides or cover slips beneath sporulating field-collected basidiocarps. The slides were air dried, fixed in 3:1 absolute ethanol:glacial acetic acid and stained. Slides were fixed immediately after spore cast or were incubated in moist chambers at room temperature for several days then fixed and stained.

Fifteen homokaryotic and fifteen heterokaryotic isolates of each species were transferred to petri plates containing

sterilized cover slips on the surface of the media. Following mycelial growth over the cover slips (Fig. 3a), they were removed, air dried, fixed and stained. The number of nuclei from twenty randomly located hyphal cells (the volume between adjacent septa) was counted for each isolate. Nuclei were considered to be paired if the distance separating them was less than two hyphal diameters. All observations of giemsa-stained nuclei were conducted using a compound microscope (1000x) with bright field illumination.

Small pieces of sporulating, field-collected basidiocarps were fixed, imbedded in paraffin, sectioned at 7 μm or 10 μm and stained using Delafield's hematoxylin (Johansen, 1940). The staining series followed that of Jensen (1962), except 4% ferric chloride (20 min.) functioned as a mordant and 2% ferric chloride (15 sec.) served as a destainer. Slides were mounted in Kleermount (Carolina Biological Supply) and observed under bright field illumination at 640x with a green filter.

An additional nuclear stain, DAPI (4', 6-diamidino-2-phenylindole, Sigma Chemical Co.), was employed to confirm results of the nuclear condition of homokaryons, heterokaryons, basidia, and basidiospores. Mycelia of two homokaryotic and two heterokaryotic isolates of P. arctostaphyli growing across cover slips were air-dried, fixed in 3:1 absolute ethanol glacial acetic acid, and stained. Pieces of sporulating P. igniarius basidiocarps were hand-sectioned and stained without fixation. Hand-sections (but not mycelia on cover slips) were treated with RNase (0.5 mg/ml in dis-

tilled (d) H_2O) for 2 hr. All material was rinsed in dH_2O and stained in 0.5 μg/ml DAPI in dH_2O for 10 min. Slides were rinsed twice in cold (4 C) dH_2O and mounted in dH_2O or glycerol. Observations were made with a Zeiss photomicroscope equipped with a 50 W mercury lamp providing epi-illumination and a Zeiss 48 77 02 filter combination for fluorescence microscopy.

RESULTS

Cultural Characteristics

P. arctostaphyli, P. igniarius, and P. tremulae each have distinctive colony appearances in culture. However, homokaryons and heterokaryons cannot be reliably separated on appearance (Table 3).

Phellinus arctostaphyli

Isolates produced moderately fast growing aerial colonies with smooth, regular margins. Reverse stain diffusing into the medium was uncommon, but when present, often occurred as faint radiating streaks. The colony became darkly pigmented closely behind the advancing margin. Although homokaryons and heterokaryons were generally indistinguishable, the latter were more often zonate and their aerial mycelium tended to be fluffier. Colonies were zonate in concentric, raised, pigmented rings, always equal to the number of weekly observations.

Phellinus igniarius

Isolates of P. igniarius were characterized by a relatively fast growing, pale, appressed mycelium. At the edge of the plate, isolates often produced aerial mycelium reaching to the lid. Again, homokaryotic and heterokaryotic isolates were difficult to differentiate, but heterokaryons more often displayed irregular margins and a darker color. Only three (6%) homokaryotic and no heterokaryotic isolates exhibited zonation.

Table 3. Cultural characteristics of homokaryons (hom) and heterokaryons (het) of P. arctostaphyli, P. igniarius, and P. tremulae

	P. arctostaphyli		P. igniarius		P. tremulae	
	hom	het	hom	het	hom	het
Number of isolates	83	11	49	9	66	27
%[a] zonate	44	82	6	22	0	0
%[a] irregular margin	1	0	36	80	57	67
%[a] center aerial	78	100	48	22	96	67
%[a] margin aerial	61	100	6	0	71	42
%[a] reverse stain	15	9	40	22	78	100
Most common color[b]	69 74	69 74	73 86	71 72	74 72	74 69

[a] % of isolates

[b] ISCC-NBS method of designating colors (Kelly and Judd, 1965)

69 = deep orange yellow
71 = moderate orange yellow
72 = dark orange yellow
73 = pale orange yellow
74 = strong yellowish brown
86 = light yellow

Phellinus tremulae

Isolates of P. tremulae grew much slower than the other two species, and were never zonate in the manner of P. arctostaphyli. Two isolates (PTA 65 s03 and PTA 65 s11) exhibited a form of zonation, but the number of tightly-formed concentric rings approximated the number of days since their initial transfer. This behavior seems curious, as they were maintained in a dark incubator free of any obvious daily environmental fluxuation. Homokaryotic and heterokaryotic isolates of P. tremulae ordinarily produced a reverse stain, uncommon in the other two species.

Cultural dimorphism (isolates switching between two distinct culture types during transfers) was infrequent. Hopp (1936) first recognized two culture types in P. tremulae, subsequently called Staining type and Bleaching type (Hiorth, 1965). In the present study, the faster growing Bleaching type was more common. Typically, all of the isolates from one basidiocarp were of one type or the other. Some isolates displayed a unique combination of characteristics rather than conforming to two distinct patterns.

Only P. tremulae isolates produced a strong wintergreen odor (Collins and Halim, 1972). P. tremulae isolates from Wisconsin and Utah did not differ appreciably in culture from Oregon isolates.

Growth Rate

Homokaryotic and heterokaryotic isolates of the same species differ in growth rate. Heterokaryons grew significantly ($P=0.05$) more quickly (29%, P. arctostaphyli; 30%,

P. igniarius; 26%, P. tremulae) than homokaryons of the same species (Fig. 4). The growth rates of homokaryotic isolates, all of which were taken from the same basidiocarp, are compared to the parent heterokaryon (Figs. 5 - 7). The heterokaryon consistently has a faster growth rate than the mean of its homokaryons (Figs. 5 - 7). These figures also illustrate the wide variation of growth rates among isolates taken from different basidiocarps. Isolates from basidiocarps growing on the same host tree (PAR 01-d and PAR 01-e, Fig. 5) had nearly identical growth rates.

Incompatibility

The primary goal of this particular study was to demonstrate a homothallic or heterothallic mating system of these three species. Class I and II homokaryotic pairings used a few isolates from several basidiocarps for each species. Class III pairings, crossing homokaryotic isolates from the different species, attempted hybridization. Heterokaryotic isolates from the same species (Class IV) and from different species (Class V) were paired to test for and demonstrate vegetative incompatibility.

Characteristics of antagonism included: reverse stain, darker color, raised aerial mycelium, submerged hyphal band, appressed hyphae, and avoidance distance. In pairings of all classes, where antagonism occurred it ranged from one (reverse stain was the most common) to a combination of four characteristics. One feature, darker pigmentation color in the zone of interaction, always was accompanied

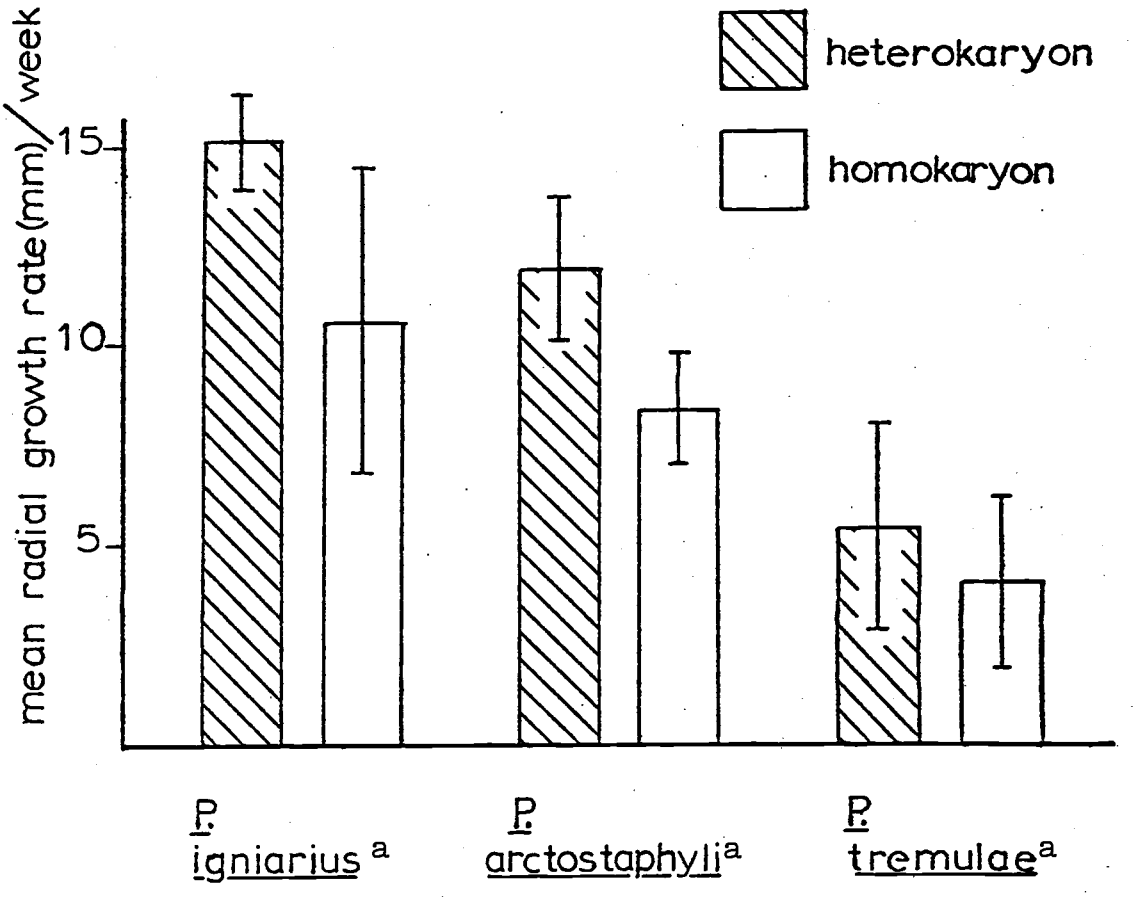


Fig.4 Average radial growth rates of heterokaryons and homokaryons of P. igniarius, P. arctostaphyli, and P. tremulae. Variation bars= ± one standard deviation.

[a]significant difference (P = 0.05) between the growth rate of heterokaryons and homokaryons.

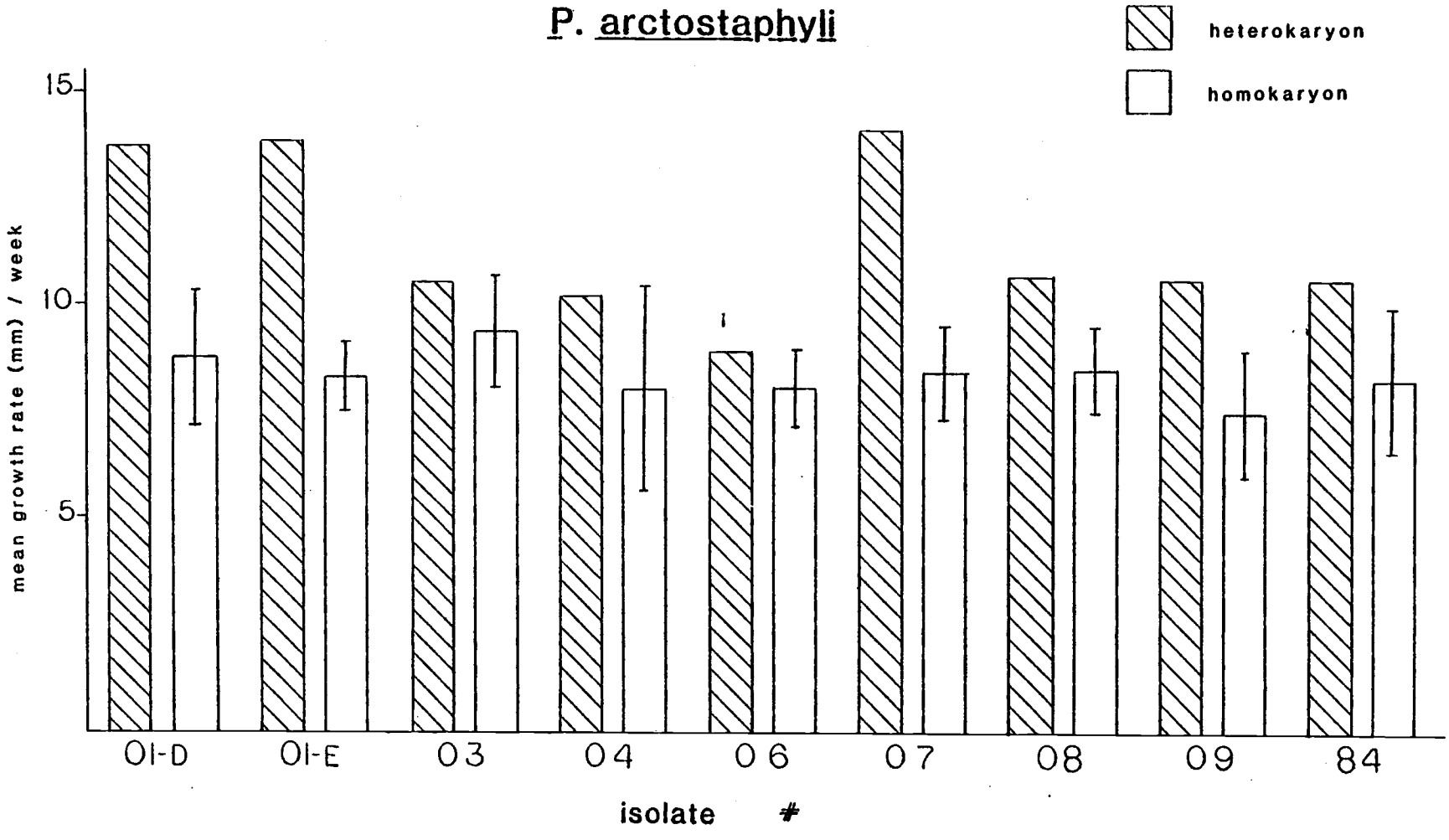


Fig. 5 Radial growth rate of P. arctostaphyli parent heterokaryon as compared to the mean radial growth rate of homokaryons isolated from the same basidiocarp. Variation bars = ± one standard deviation. 01-D and 01-E from the same host tree.

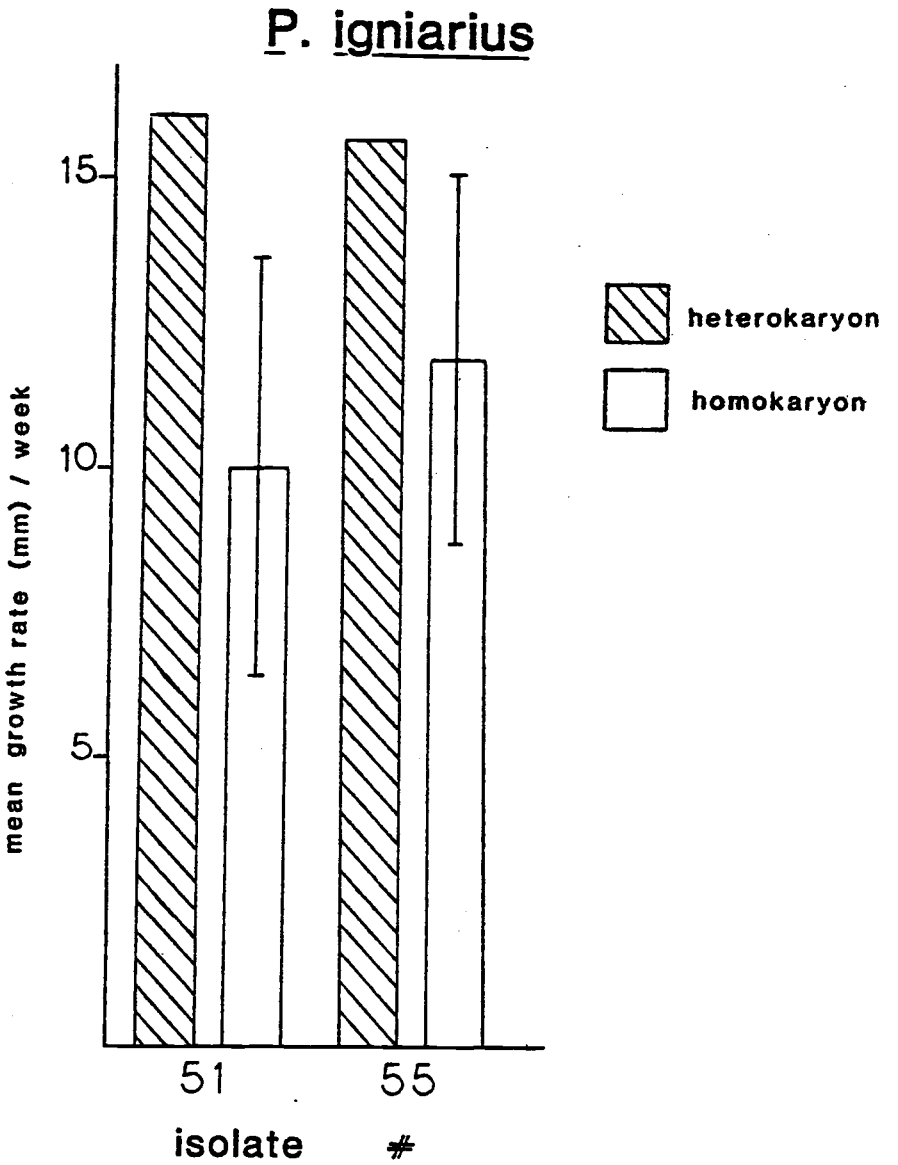


Fig.6 Radial growth rate of P. igniarius parent heterokaryon as compared to the mean radial growth rates of homokaryons isolated from the same basidiocarp.

P. tremulae

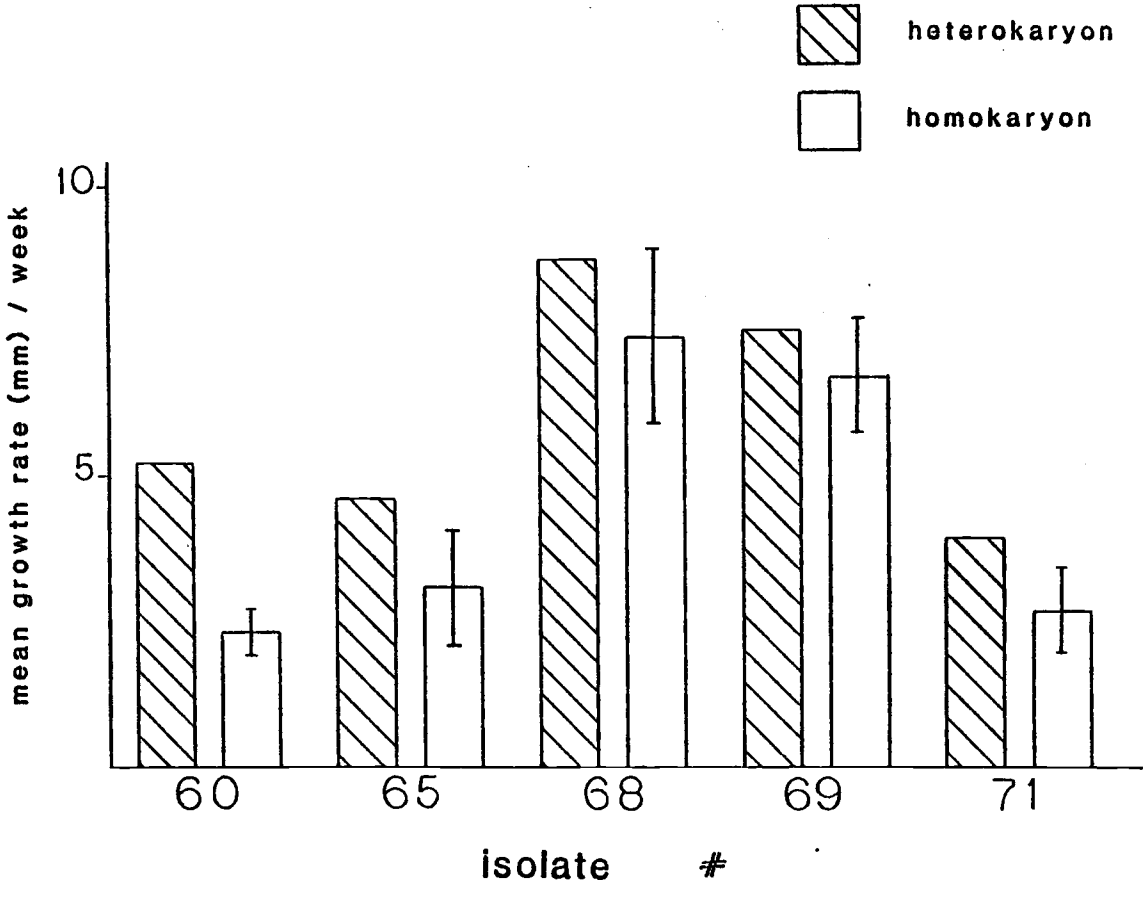


Fig.7 Radial growth rate of P. tremulae parent heterokaryon as compared to the mean radial growth rate of homokaryons isolated from the same basidiocarp. Variation bars = ± one standard deviation.

by reverse stain. Both sexual (Classes I - III) and vegetative (Classes IV and V) incompatibility reactions had a similar appearance (Table 4).

Sexual Incompatibility

For all Class I pairings, three homokaryons from a single basidiocarp were crossed in all combinations. Homokaryons from two basidiocarps of P. igniarius and three each of P. arctostaphyli and P. tremulae were used. In these Class I pairings both compatible and incompatible matings were observed as evidenced by the respective absence or presence of antagonism (Fig. 8). Upon contact of paired compatible isolates, the subsequent growth appeared as one colony without noticeable differences on either side and a lack of antagonism in the area of initial mycelial contact. The initial interaction between some compatible matings resulted in appressed mycelium and mild reverse stain in the zone of interaction giving way to a more complete intermingling as the cultures aged. An isolate paired with itself always yielded a non-antagonistic reaction, but some of these pairings initially produced a line of appressed mycelium before achieving a more homogeneous mat. Incompatible pairings produced various combinations of antagonistic responses (Table 4) and each isolate of the pairings maintained distinctive cultural characteristics.

In pairings testing compatibility of two single spore isolates from the same species, but different basidiocarps (Class II), only non-antagonistic reactions occurred.

Fig. 8. Antagonistic, sexually incompatible Class I pairing (left); non-antagonistic, sexually compatible Class I (middle) and Class II (right) pairings of P. arctostaphyli.

Table 4. Frequencies of occurrence of different expressions of antagonism among incompatible pairings in different pairing classes. Class II pairings were compatible.

	Pairing Classes														
	P. arctostaphyli					*P. igniarius*					*P. tremulae*				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
reverse stain	.71	-	1.0	.93	1.0	.33	-	.80	.75	.60	.69	-	.85	.63	.56
pigmentation color	.21	-	.11	.69	.67	.33	-	.33	.88	.20	.13	-	.07	.29	.22
aerial mycelium	.28	-	.06	.19	.67	.11	-	.07	.75	.10	.18	-	.07	.14	.11
submerged hyphal band	.21	-	.06	0	0	0	-	.07	-	0	0	-	0	0	0
appressed mycelium	.07	-	.39	.13	0	.44	-	.13	-	0	.26	-	.38	.15	.22
avoidance	0	-	0	0	0	.44	-	0	.30	0	0	-	.16	.15	0

P. arctostaphyli Class II pairings intermingled without any signs of antagonism. Generally, P. igniarius pairings appeared compatible, but some plates showed initial antagonism that disappeared as the cultures aged. Weak levels of antagonism, especially reverse stain, sometimes remained in older pairings of P. tremulae, but mycelium on both sides of the pairing acquired a similar appearance.

Growth Rates of Sexually Compatible Pairings

Since growth rates of heterokaryons were comparatively faster than homokaryons (Fig. 4), heterokaryons resulting from a compatible pairing (Class I and II) should grow faster than the respective homokaryons. This hypothesis was tested in two ways. First, the growth rates of isolates after a compatible Class I or II pairing were compared to the same isolates' growth rate when self-paired (control). Homokaryotic isolates with relatively slow or moderate growth rates invariably grew faster following a compatible mating. However, homokaryotic isolates with a fast growth rate did not always grow faster. Significantly ($P=.05$) faster average growth rates were found in these non-antagonistic pairings only for P. arctostaphyli. Class I pairings judged to be incompatible, based on antagonism, showed no significant change in growth rate.

In the second test, the average of the growth rates of the isolates when self-paired was compared to the growth rate following pairings of those isolates. In compatible pairings, the growth after interaction was significantly

Table 5. Average of the growth rates of two isolates (A & B) when each is self-paired as compared to the growth rates of those same two isolates in compatible Class I or Class II pairings (growth rate values of compatible pairings usually based on an average of two plates)

Growth rates (mm / week)											
P. arctostaphyli[a]				P. igniarius[a]				P. tremulae[a]			
A	B	$\bar{x}$ of A&B	A X B pairing	A	B	$\bar{x}$ of A&B	A X B pairing	A	B	$\bar{x}$ of A&B	A X B pairing
3.4	3.0	3.2	3.6	4.4	10.3	7.4	10.9	1.3	4.6	3.0	4.2
7.8	7.4	7.6	7.8	4.4	4.3	4.4	5.1	2.5	0.8	1.7	3.1
3.9	6.5	5.2	7.0	3.5	4.3	3.9	3.7	1.1	0.8	1.0	1.4
6.6	6.5	6.6	7.1	7.2	10.3	8.8	11.1	1.3	3.2	2.3	3.5
3.9	6.6	5.3	5.1	3.5	10.3	6.9	9.9	2.2	3.2	2.7	3.7
3.4	7.8	5.6	8.4	7.2	5.5	6.4	5.4	1.3	2.2	1.8	1.4
4.4	7.8	6.1	7.9	3.5	5.5	4.5	4.7	2.5	1.1	1.8	2.8
3.4	4.4	3.9	5.6	4.4	5.5	5.0	7.4	2.4	4.6	3.5	3.3
3.0	7.4	5.2	6.7	7.2	4.3	5.8	9.8	0.8	4.6	2.7	5.0
3.0	3.8	3.4	5.4	4.3	3.9	4.1	4.6	1.1	3.6	2.4	2.4
3.8	7.4	5.6	8.1	3.5	4.4	4.0	4.6				

[a]Significant difference (P=0.05, using paired t test) between mean of growth rates of A & B and the growth rate of the compatible (class I or II) pairing

Table 6. Average of the growth rates of two isolates (A & B) when each is self-paired as compared to the growth rates of those same two isolates in incompatible Class I pairings (grwoth rate values of incompatible pairings usually based on an average of two plates)

Growth rates (mm / week)

P. arctostaphyli				P. igniarius				P. tremulae			
A	B	$\bar{x}$ of A&B	A X B pairing	A	B	$\bar{x}$ of A&B	A X B pairing	A	B	$\bar{x}$ of A&B	A X B pairing
3.9	3.4	3.7	3.5	5.5	10.3	7.9	8.5	1.7	2.9	2.3	1.4
3.0	3.9	3.5	3.2	4.3	10.3	7.3	3.5	2.0	1.7	1.9	2.2
6.5	7.8	7.2	5.7	10.3	7.6	8.8	9.1	5.3	6.2	5.8	5.3
6.5	7.4	7.0	5.6	7.3	3.5	5.4	6.6	5.3	4.1	4.7	3.9
4.4	3.8	4.1	3.2	3.5	8.2	5.9	9.3	4.1	6.2	5.2	5.9
4.6	3.8	4.2	6.2	4.4	7.2	5.8	4.1	3.1	3.3	3.2	1.8
4.6	4.4	4.5	5.4					3.3	2.6	3.0	3.1
								2.6	3.1	2.9	3.4

greater (P=0.05) than the average growth of the self-paired isolates for all three species (Table 5). These sexually compatible matings acquired the faster growth characteristics of context-isolated heterokaryons.

In contrast, the growth of incompatible pairings was not significantly changed from the average growth of the self-paired isolates (Table 6).

Hybridization

All but two attempts at single spore hybridization (Class III) among these three species resulted in incompatible reactions exhibiting antagonism (e.g., Fig. 9). Even the two questionable crosses (PAR 01-d s02 X PTA 65 s18 and PAR 03 s05 X PIG 51 s33) showed weak antagonism and the two paired isolates did not come to resemble one another.

Vegetative Incompatibility

Most paired heterokaryotic isolates reacted antagonistically (Class IV and V). The only exceptions were: 1) self-paired heterokaryons (Fig. 10) and 2) the three cases where two heterokaryons isolated from basidiocarps occurring on the same host tree were paired.

Nuclear Condition

Basidiospores

Of the 2,553 giemsa-stained basidiospores examined, the uninucleate condition was the most common (P. arctostaphyli = 94.5%, P. igniarius - 98.1%, P. tremulae = 98.3%. The remaining basidiospores were binucleate. Nuclei appeared

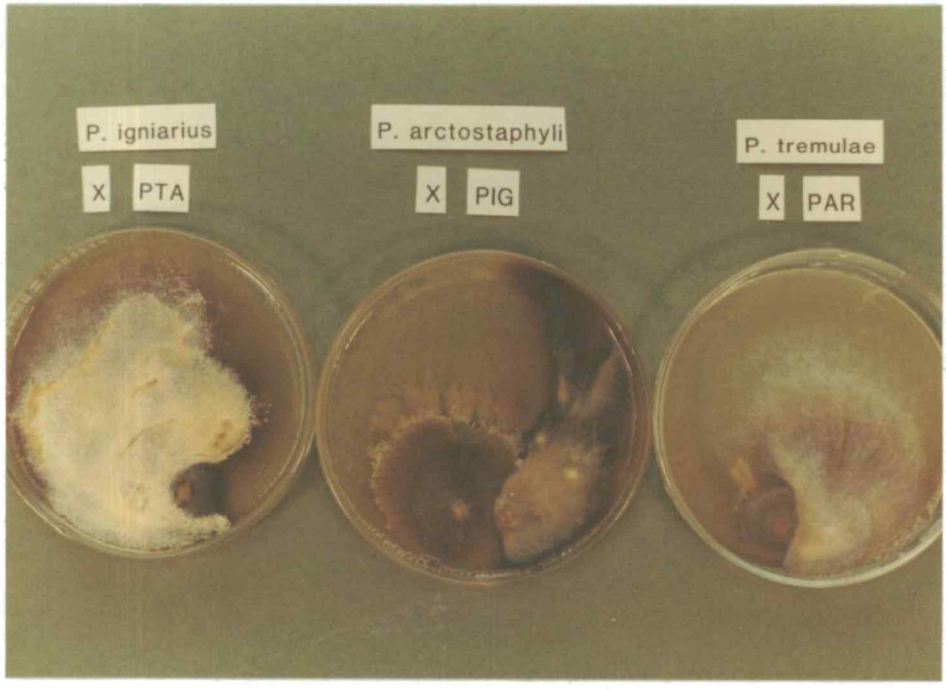


Fig. 9. Antagonistic reactions in sexually incompatible Class III single spore hybridization pairings.

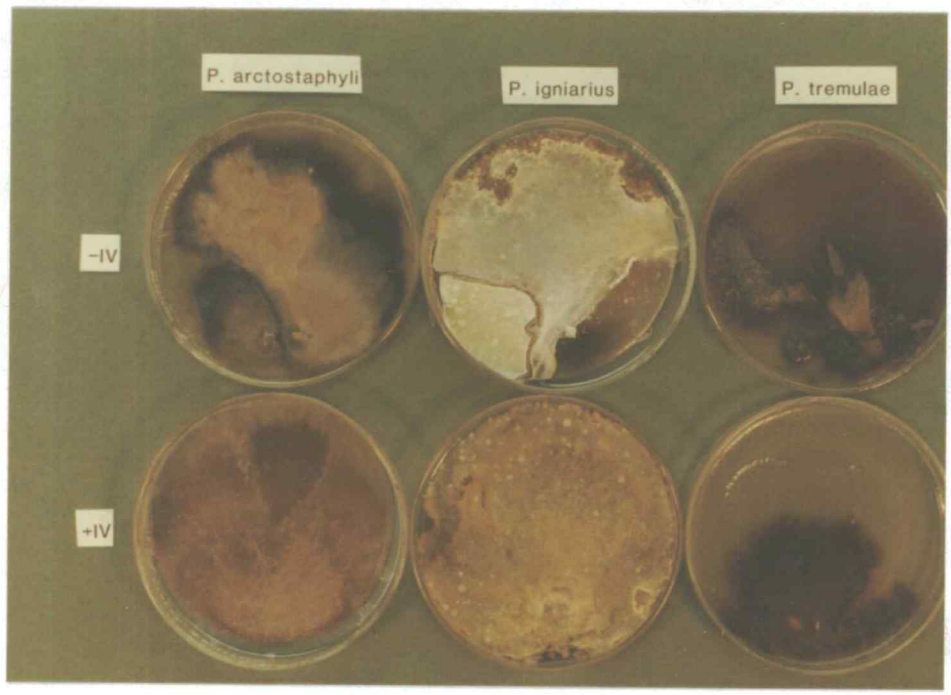


Fig. 10. Antagonistic reactions in vegetatively incompatible Class IV pairings and non-antagonistic vegetatively compatible Class IV pairings.

as round, reddish-staining bodies (Fig. 11a).

Germinating Basidiospores

In dense clusters of basidiospores, germination was reduced or inhibited altogether and spores normally remained uninucleate. Most commonly, basidiospores became binucleate prior to germination. Eighty-three percent of newly germinated basidiospores (i.e., germ tube $\leq 5\ \mu m$) contained two or more nuclei in the spore and germ tube. As the germ tube lengthened and produced septa, dumbbell-shaped stained-bodies interpreted to be mitotic figures were observed in the basidiospore as well as in the germ tube. In over 99% of the germlings inspected, a nucleus remained in the basidiospore. Seventy-seven percent of germlings of all sizes with septate germ tubes had one nucleus per cell. Most distal cells were also uninucleate. Often, there were no nuclei between the basidiospore and the first apparent septum as if there was no septum at the germ tube-spore interface, and the nucleus in the basidiospore was functioning for that proximal portion of the germ tube. Several times, an elongated nucleus was observed extending from the basidiospore to the germ tube, perhaps in migration.

Vegetative Hyphae

The number of nuclei per cell of homokaryotic and heterokaryotic hyphae varied widely (Figs. 12 - 14). The number of nuclei per cell was not significantly different when contrasting homokaryons to heterokaryons or with interspecific comparisons. Furthermore, the nuclei of

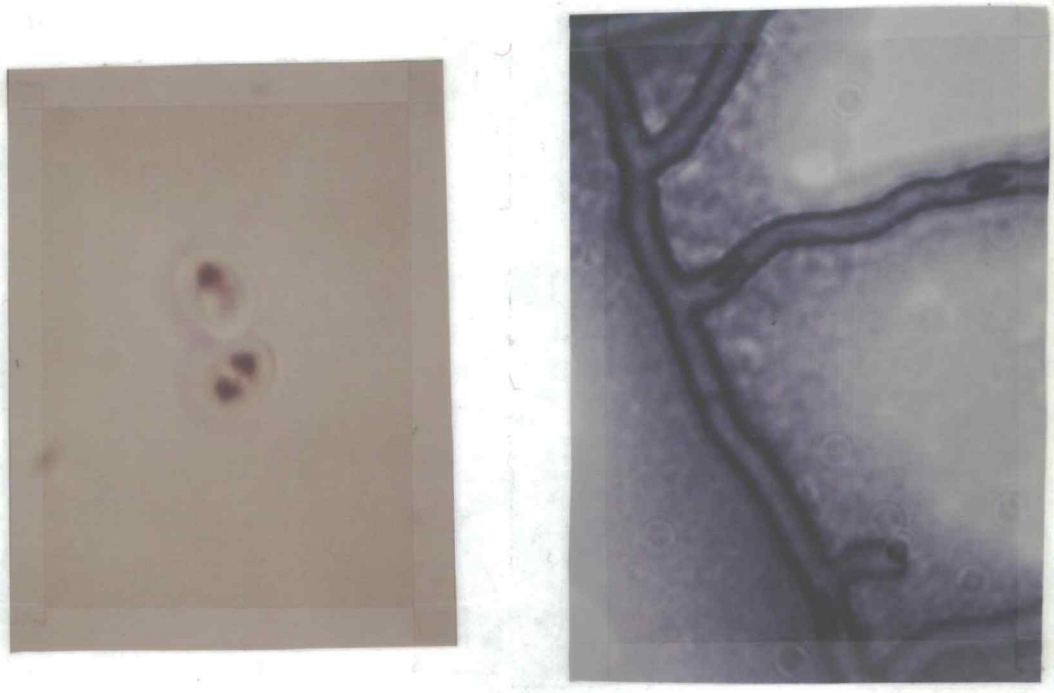


Fig. 11a. Uninucleate and binucleate giemsa-stained basidiospores of P. arctostaphyli (left).

Fig. 11b. Unpaired nuclear condition of a heterokaryotic P. arctostaphyli isolate (right).

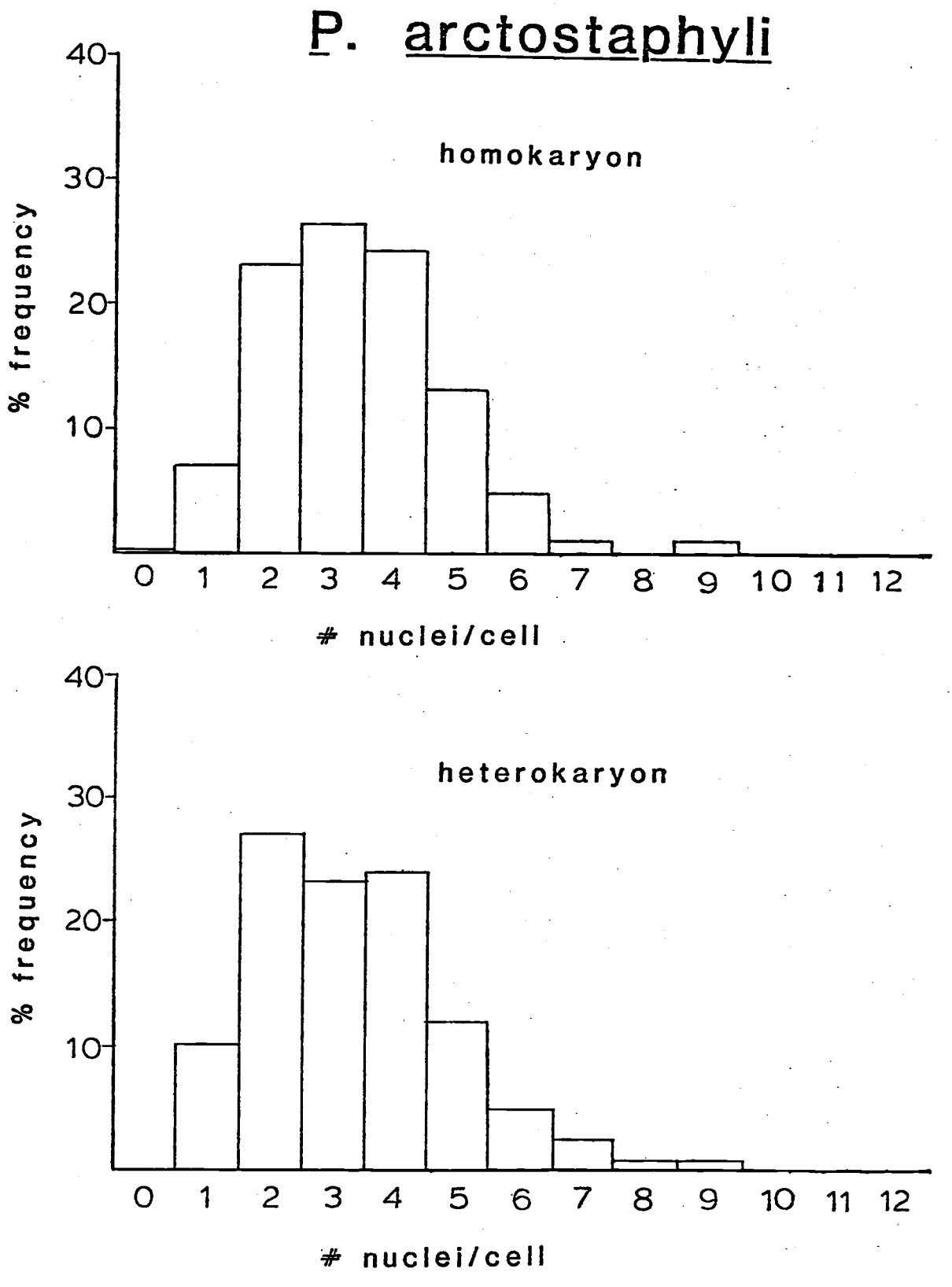


Fig.12. Percent frequency of the number of nuclei per cell in 20 hyphal cells of 15 homokaryotic and 15 heterokaryotic isolates of P. arctostaphyli.

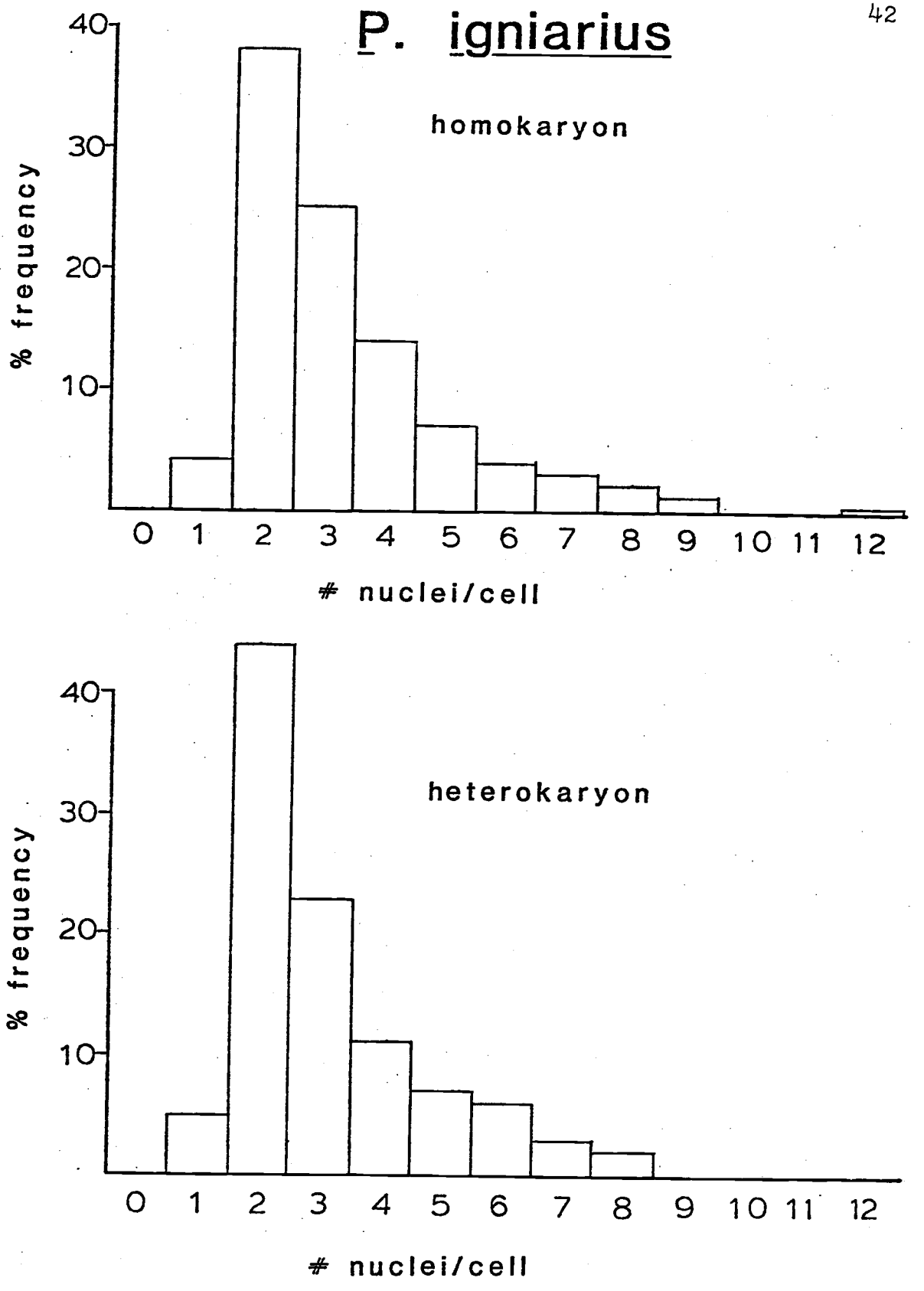


Fig.13. Percent frequency of the number of nuclei per cell in 20 hyphal cells of 15 homokaryotic and 15 heterokaryotic isolates of P. igniarius.

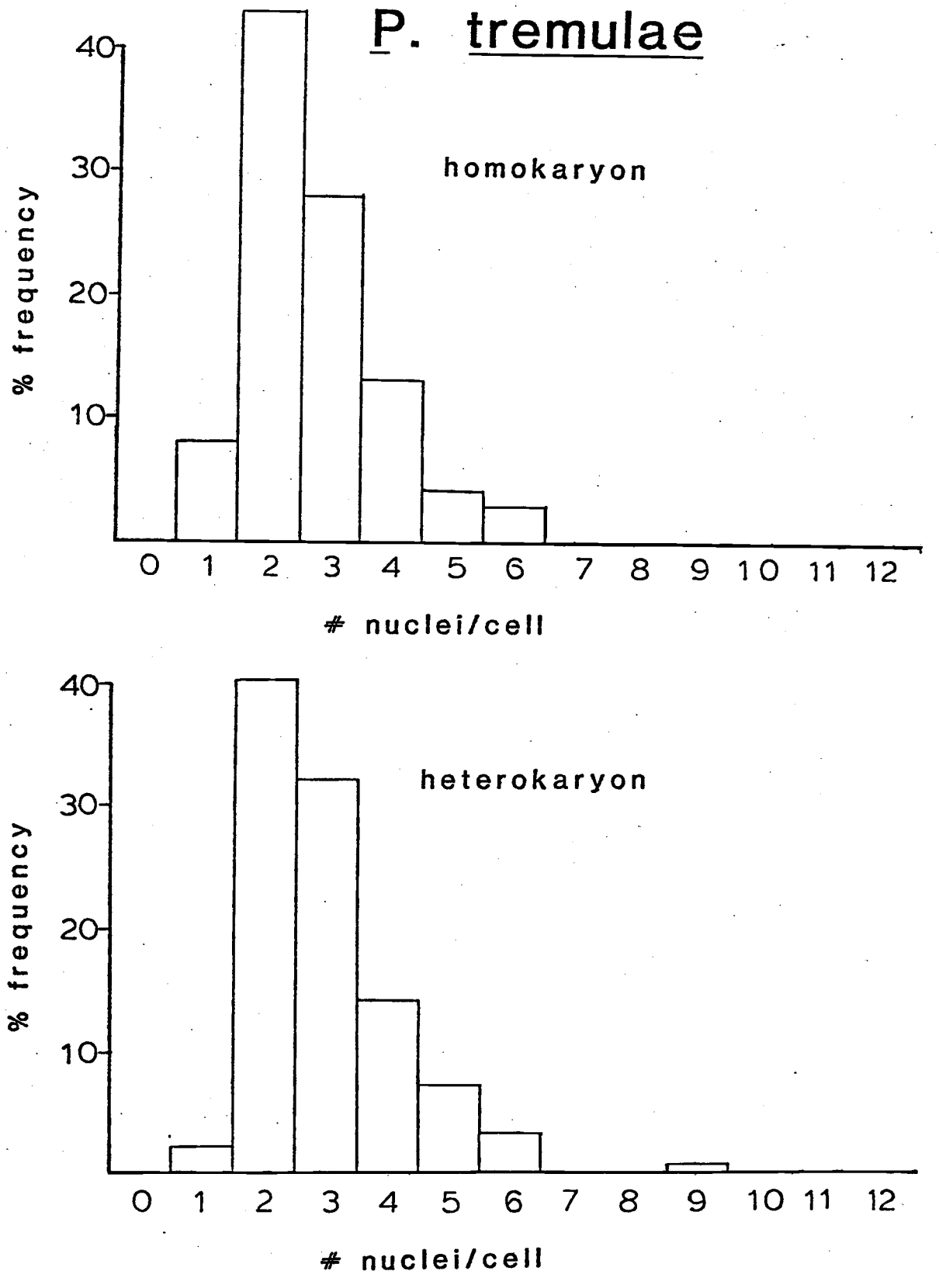


Fig.14. Percent frequency of the number of nuclei per cell in 20 hyphal cells of 15 homokaryotic and 15 heterokaryotic isolates of P. tremulae.

heterokaryons did not form consistent pairs. For example, in P. arctostaphyli, only 13.5% of heterokaryotic hyphal cells contained paired nuclei while 10.8% of homokaryotic hyphal cells had paired nuclei. Also, the nuclei in heterokaryotic hyphal cells were not consistently present in even numbers as they would be if they occurred as multiple pairs. Typically, nuclei appeared to occur equidistantly spaced in hyphal cells of homokaryons and heterokaryons of each species. The thick walled darkly pigmented fiber hyphae of P. tremulae, when not vacuolated, possessed the nuclear distribution of other hyphae. The same was true for the tightly-coiled hyphae present in both P. igniarius and P. tremulae isolates.

Basidiocarp Tramal Hyphae and Subhymenium

Paired nuclei were not observed in the advancing tramal hyphae from the underside of P. igniarius basidiocarps stained with DAPI. Similarly, nuclei were unpaired in the white mycelium growing through the old tube layers in these basidiocarps. Nuclei in the tramal hyphae directly adjacent to the hymenium were difficult to detect due to dark pigmentation of skeletal hyphae and the density of all hyphae in this region. When nuclei in hyphae in this region were visible, however, they were usually closely paired. Nuclei in this area could be distinguished when stained with DAPI but not hematoxylin.

Basidia

The number of hematoxylin-stained nuclei observed in 271 basidia of each species ranged from zero to four (two basidia appeared to have five nuclei). Either one, or more frequently two, nuclei were found in the smaller, less developed basidia (Fig. 15). Basidia with four nuclei (three were occasionally counted) and basidia from which the nuclei had already migrated to sterigmata or basidia tended to be wider (Fig. 16). Only slight differences in the sizes of basidia containing one or two nuclei were found. Following spore discharge, basidia contained no nuclei and began to collapse. Immature basidia of P. igniarius stained with DAPI were most frequently binucleate.

All 331 basidiospores found attached to sterigmata were uninucleate. In addition, all basidiospores seen in the tubes of sectioned basidiocarps were uninucleate.

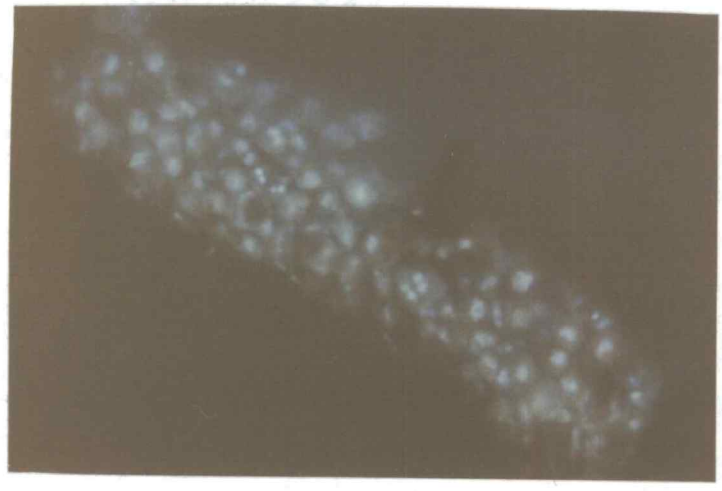


Fig. 15a. The predominance of binucleate DAPI-stained basidia (arrows) as seen by viewing through the tops of basidia. (top)

Fig. 15b. DAPI-stained uninucleate basidiospore still attached to sterigma (basidium is out of focus), binucleate subhymenial cells, and a binucleate basidium. (bottom)

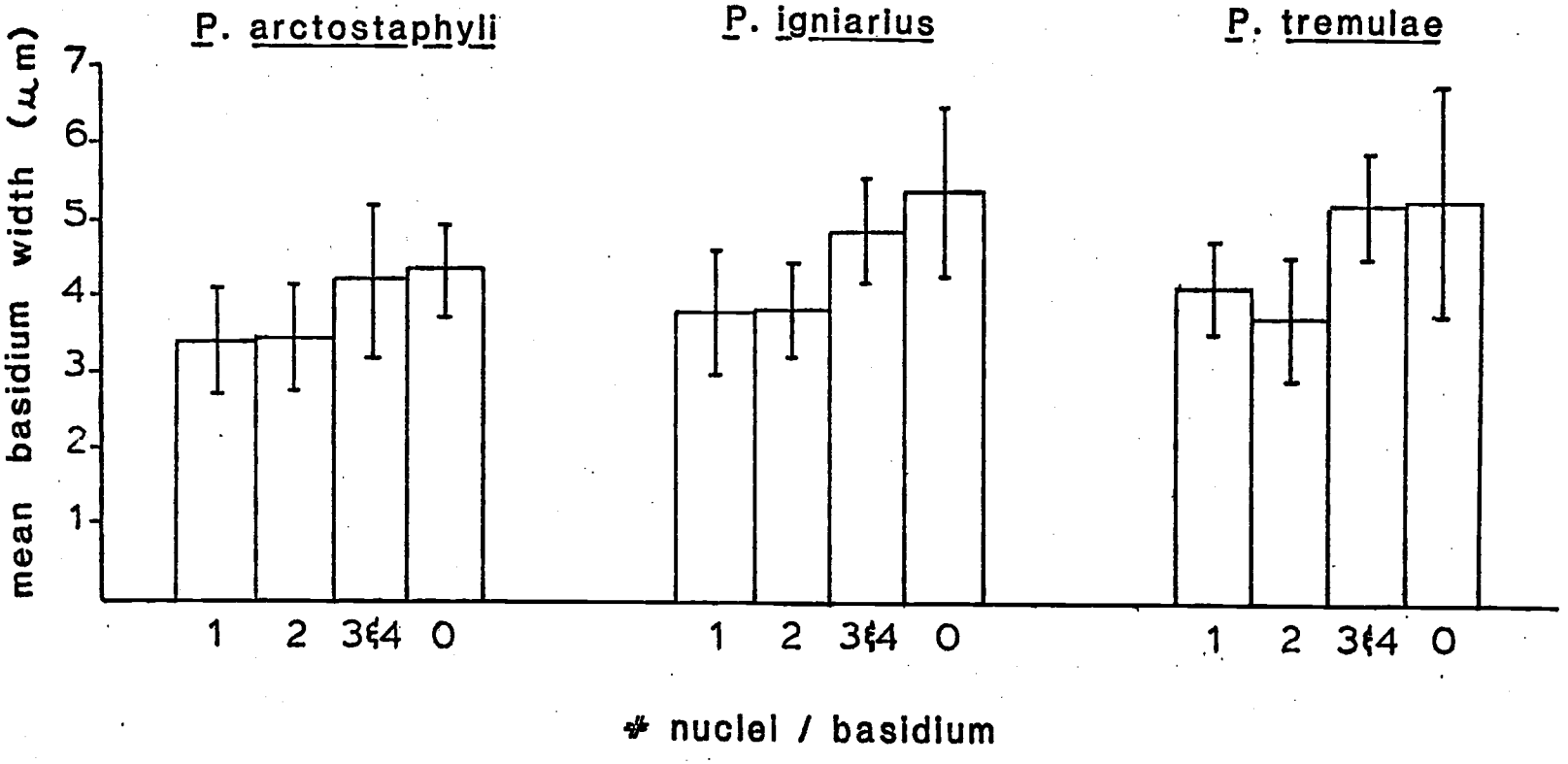


Fig.16. Width of basidia containing 1, 2, 3 Or 4, and 0 nuclei. Basidia containing no nuclei were measured only if nuclei were present in sterigmata or basidiospores.

DISCUSSION

Sexual Incompatibility

Mating studies of Phellinus species cannot follow traditional avenues of research. In most studies concerning the sexual incompatibility systems of Agaricales and Aphyllophorales, one of several indicators of successfully compatible matings between paired homokaryotic isolates is commonly utilized: formation of clamp connections, fruiting in culture, paired nuclei, or altered cultural characteristics.

Since the earliest works on the sexuality of basidiomycetes (Bensaude, 1918; Kniep, 1920), the presence of the clamp connection has remained the most frequently used test of sexual compatibility. Indeed, species bearing clamp connections have been preferentially selected for mating studies. Surveys of the taxonomic distribution of different mating systems in the basidiomycetes (e.g., Whitehouse, 1949), are biased by ignoring the many species which lack clamps (Furtado, 1966). Members of the Hymenochaetaceae, all of which lack clamp connections, represent a large number of species that may not follow the mating patterns of the species studied to date. Since clamp connections are controlled by alleles at the mating loci of at least some species (Raper, 1966), the lack of clamp connections of Phellinus spp. may further suggest a departure from typical basidiomycete mating systems.

The formation of basidiocarps in heterokaryotic cultures served as an indicator to demonstrate the heterothallism of

P. weirii (Gillette, 1975; Hansen, 1979b). However, fruiting occurred in none of the three species investigated in the present study nor has in vitro fruiting been reported in the literature.

Closely paired nuclei were only infrequently encountered in the hyphae of these three Phellinus spp. The lack of differences in the distribution of the nuclei between homokaryons and heterokaryons makes this characteristic unsuitable for indicating compatible matings.

While subtle differences in cultural appearances may exist between homokaryotic and heterokaryotic isolates, these differences are neither pronounced nor consistent enough for use in a mating study. Commonly in hymenomycetes, context isolates differ sufficiently from single spore isolates for utilization as an indicator of sexual compatibility. For example, Hintikka (1973) found that Armillaria mellea single spore isolates, with cotton-like aerial mycelium, differed in their appearance from basidiocarp isolates with flat crustose mycelium. Actually, there is no compelling genetic reason why homokaryons and heterokaryons should exhibit cultural differences, especially in species where heterokaryons lack rigid nuclear organization (i.e., the dikaryon).

In this study, two criteria have been developed for judging the sexual compatibility of paired homokaryons. First, heterokaryons, whether isolated from basidiocarp context or resulting from paired homokaryons, grow more rapidly than homokaryons. Second, the lack of antagonistic response between paired homokaryons serves as an indicator

of sexual compatibility. These two criteria, especially when used in conjunction, can be used to establish whether the mating systems in these three Phellinus spp. are homothallic or heterothallic. Based on these criteria, P. arctostaphyli, P. igniarius, and P. tremulae all possess a heterothallic sexual incompatibility system. When homokaryons from the same basidiocarp are paired, both antagonistic reactions with no increased growth rate, and non-antagonistic reactions with increased growth rate occur, representing sexually incompatible and compatible reactions, respectively. Sexually compatible matings have acquired the faster growth rate of heterokaryons and sexually incompatible pairs have retained the slower growth rate of homokaryons.

Further evidence of heterothallism in each of these species is supplied by the compatiblity of homokaryons from different basidiocarps. If these homokaryotic isolates had the properties of heterokaryotic isolates, as they would in a homothallic system, then these pairings would exhibit vegetative incompatibility. Instead, these pairings are compatible, resulting in non-antagonism and a significantly faster growth rate.

Compatibility of homokaryons from different basidiocarps has been used to determine the number of alleles at the mating locus (or loci) occurring in the population (Burnett, 1965). The genetic interaction of heterothallic hymenomycetes is governed by a homogenic incompatibility system (Esser and Raper, 1965). Two paired homokaryons possessing the same allele at a mating locus will be

incompatible. The incompatible reaction of two homokaryons from the same basidiocarp is the result of their possessing common alleles at a mating locus. However, the consistent compatibility between homokaryons from different basidiocarps indicates that many potential alleles can occur at mating loci. Consequently, this demonstrates the multiallelism of the heterothallic system in these species.

Verrall (1937) came to the same conclusion regarding the heterothallism of P. igniarius and P. tremulae (he did not investigate P. arctostaphyli). His claim of two mating loci (tetrapolarity) will be neither supported nor refuted by the data of this study.

To properly identify the number of sexual incompatibility loci, at least fifteen homokaryons from the same basidiocarp should be paired in all combinations. Compatibility could be judged utilizing the criteria developed in this study, that is, growth rates and antagonism.

The sexual incompatibility system of these fungi replaces morphological differentiation of sexual structures common in other organisms (Raper and Esser, 1964). It has ecological significance by favoring outbreeding, increasing genetic recombination. The three Phellinus spp. investigated in this study join the 90% of hymenomycetes that have been shown to be heterothallic (Esser, 1967).

Vegetative Incompatibility

Some of the antagonistic responses in pairings in the present study are interpreted to result from vegetative

incompatibility. Vegetative incompatibility functions heterogenically (Esser and Blaich, 1973); isolates interacting with different genetic make-up are incompatible. The complete nature of this system is not well understood. In the present study, heterokaryotic isolates, when self-paired or sometimes when paired with another heterokaryotic isolate from the same host tree, react non-antagonistically. All other pairings between heterokaryons result in antagonism. A homokaryotic isolate paired with itself shows a non-antagonistic reaction yet lacks the faster growth rate of a sexually compatible response. Therefore, these pairings are vegetatively compatible but not sexually compatible.

Vegetative compatibility seems to serve a self-recognition function. Day (1968) suggested that it may function to prevent or limit the risk of infection by cytoplasmic determinants. Hortl (et.al., 1975) put forth a different theory; hyphal fusions, if unchecked, would lead to less adaptive nuclei dividing at the expense of nuclei better adapted to the local environment. This possibility exists in all three Phellinus spp. where multiple infections would make contact in the heartwood of their living hosts. Whatever the selective advantage, vegetative incompatibility functions as a genetic isolating mechanism (Esser, 1962).

Nuclear Condition

The behavior of nuclei throughout the life cycle of these three species has been elucidated. Basidiospores are produced uninucleate, one of the four tetrad nuclei from the

basidium passing through each sterigma into one of the four basidiospores. Following spore discharge, mitotic divisions of nuclei occur yielding binucleate spores that can be detected several hours following spore discharge. Basidiospores are commonly binucleate prior to germination. Both homokaryons and heterokaryons contain a variable number of unpaired nuclei between septa.

The lack of paired nuclei in the heterokaryons of these species contradicts the implication of Verrall's (1937) statement that "sexual fusions are determined definitely only by nuclear stains." The multinucleate condition of heterokaryons has been recognized previously; Boidin (1971) included P. igniarius in the 26% of Aphyllophorales to possess this nuclear condition. Unpaired nuclei appear to be the rule rather than the exception in the Phellinus genus (Kühner, 1950). Without the presence of paired nuclei in heterokaryons (context isolates), the location of the dikaryon and karyogamy in the life cycle of these fungi is open to question.

At least two models could explain the unpaired, variable number of nuclei found in hyphal cells. First, it is conceivable that karyogamy occurs upon initial anastamosis of two sexually compatible homokaryons. Diploid nuclei would increase in number and distribution via mitosis and nuclear migration. Clamp connections would be unnecessary and thus not expected. Young basidia would contain one diploid nucleus, followed by two (first meiotic division), and then four (second meiotic division) haploid nuclei. The ploidy

of fungi is difficult to determine directly because chromosomes are so small, and counts are impractical. Bolland (personal communication) has investigated the ploidy of the nuclei of P. noxious mycelia and asexual spores. Based primarily on nuclear volume, he has concluded that nuclei exist in a polyploid state, except for the haploid nuclei of homokaryotic hyphae and asexual spores.

The second model proposes the delay of the dikaryophase and karyogamy until formation of the hymenium. Nuclei in vegetative hyphae remain haploid. Nuclei in young basidia would initially be paired and haploid, followed in turn by one diploid nucleus (via karyogamy), then two nuclei (first meiotic division), and four haploid nuclei (second meiotic division).

Therefore, the critical phase of development to differentiate between these models is the young basidium. Our data support the latter model. Unpaired nuclei in most basidiocarp tissue indicates that the dikaryon is formed only in hyphae near the basidia. Also, most young (small) basidia contained two nuclei. Two nuclei would be encountered only infrequently just following the first meiotic division in the former model. This stage is brief in Schizophyllum commune (Stamberg, 1978). Furthermore, the presence of paired nuclei in subhymenial hyphae and young basidia indicates their probable haploid state.

The delaying of the dikaryophase may produce an increased versatility in these species. In nature, the heterokaryon may contain more than two genetically distinct

nuclei. If the selection for the nuclei for the dikaryotic pair occurs in the hymenium during basidium formation, then it is conceivable that dikaryotic pairs may be composed of different nuclei in adjacent young basidia. Or, one pair of nuclei may be selected to serve in all basidia in the current tube layer of a basidiocarp. A new pair of nuclei could be selected from the perennial mycelium from year to year to meet changing environmental conditions. Burnett (1965) has found new mating alleles appearing in successive years in annual basidiocarps of Polyporus betulinus supported by perennial mycelia.

The unpaired condition of the nuclei in heterokaryons and the similarity of growth characteristics between homokaryons and heterokaryons may compliment each other. The dikaryons of Schizophyllum commune possess noticeably different cultural characteristics as compared to homokaryons (Raper, 1966). Also, the diploid mycelium of Armillaria mellea has a different appearance in culture than homokaryons (Hintikka, 1973). Perhaps, these different growth habits are controlled by genes activated by the initiation of the dikaryon or the diploid. Therefore, heterokaryons of these Phellinus spp. may resemble their homokaryons in culture because of the delay of the dikaryon.

Taxonomy

There should be little doubt regarding the close taxonomic affinities of P. arctostaphyli, P. igniarius, and P. tremulae. The behavior of these species was remark-

ably similar in all phases of this study. Although cultural differences have been established, general similarities should not be overlooked. The nuclear behavior of each of these species throughout the life cycle was nearly identical.

Morphological differences in basidiocarp structure have already been described (Table 1). Notable differences occur in the orientation of tramal hyphae, the frequency and length of setae, and staining properties and germination requirements of basidiospores.

Attempted hybridization studies provide further evidence of species segregation. These three species occur sympatrically, and the opportunity for hybridization in nature exists. Their differing host ranges may offer an isolating mechanism. Nevertheless, these three species failed to hybridize in culture, as evidenced by antagonistic reactions and lack of increased growth in interspecific pairings. Meanwhile, single spore isolates among basidiocarps from the same species were fully compatible.

This interspecific incompatibility should not be confused with the biological species phenomenon of Armillaria mellea (Ullrich and Anderson, 1978). Here, intersterility groups occur within morphologically similar populations of A. mellea causing some single spore isolate crosses to be sexually incompatible. The intersterility of the three Phellinus spp. in the present study accompanies a series of morphological dissimilarities.

The presence of two nuclei in most young basidia and the occurrence of paired nuclei in hyphae near the hymenium

indicates that nuclei in vegetative mycelium are probably haploid.

P. arctostaphyli, P. igniarius, and P. tremulae, and perhaps other clampless hymenomycetes, represent a departure from the nuclear behavior of better understood species (e.g., S. commune). The primitive basidiomycete was probably binucleate, clamped, and tetrapolar (Boidin, 1971; Raper and Flexer, 1971). In particular, these three Phellinus spp. differ significantly from "model" basidiomycetes in that the dikaryophase does not occupy a predominant role in the vegetative phase of their life cycle. This may enable increased genetic diversity in these long-lived mycelia. Since these species are neither binucleate (not until basidium formation) nor clamped, we should not expect them to be tetrapolar until proven so.

CONCLUSIONS

Phellinus arctostaphyli, *P. igniarius*, and *P. tremulae* are closely related, yet distinct species. Homokaryotic and heterokaryotic isolates of the species are similar with regard to most cultural characteristics, except for the faster growth rate of heterokaryons.

The three *Phellinus spp.* are similar in the variation of the nuclear condition throughout their life cycles. Basidiospores are produced with one nucleus, but are commonly binucleate prior to spore germination. Both homokaryotic and heterokaryotic hyphal cells contain a variable number of unpaired nuclei. Paired nuclei are present in the subhymenium and the hymenium of basidiocarps.

The presence or absence of antagonism between paired homokaryons and growth rate measurements provide evidence for judging sexual compatibility. Compatible matings acquire the faster growth rate of context-isolated heterokaryons. *P. arctostaphyli*, *P. igniarius*, and *P. tremulae* all possess a multi-allelic heterothallic mating system.

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APPENDIX

Antagonism and compatibility of pairings from different pairing classes

top symbol:

+ = no antagonism

o = weak antagonism

— = strong antagonism

bottom symbol:

+ = compatible (sexually compatible if in Pairing Classes I, II or III and vegetatively compatible if in Pairing Classes IV or V)

— = incompatible

Pairing Class	Isolates	Antagonism/ Compatibility
I	PAR 01-d	
	s02 X s02	$\frac{+}{-}$
	s02 X s03	$\frac{-}{-}$
	s02 X s03	$\frac{-}{-}$
	s02 X s08	$\frac{-}{-}$
	s02 X s08	$\frac{-}{-}$
	s03 X s03	$\frac{+}{-}$
	s03 X s08	$\frac{+}{-}$
	s03 X s08	$\frac{+}{+}$
	s08 X s08	$\frac{+}{-}$
I	PAR 03	
	s05 X s05	$\frac{+}{-}$
	s05 X s07	$\frac{-}{-}$
	s05 X s07	$\frac{-}{-}$
	s05 X s09	$\frac{-}{-}$
	s05 X s09	$\frac{-}{-}$
	s07 X s07	$\frac{+}{-}$
	s07 X s09	$\frac{o}{-}$
	s07 X s09	$\frac{o}{-}$
	s09 X s09	$\frac{+}{-}$

Appendix (cont'd)

Pairing Class	Isolates	Antagonism/ Compatibility
I	PAR 07	
	s03 X s03	±
	s03 X s05	−
	s03 X s05	−
	s03 X s06	−
	s03 X s06	−
	s05 X s05	±
	s05 X s06	‡
	s05 X s06	‡
	s06 X s06	±
I	PIG 51	
	s21 X s21	±
	s21 X s33	‡
	s21 X s33	‡
	s21 X s37	−
	s21 X s37	−
	s33 X s33	±
	s33 X s37	−
	s33 X s37	o
	s37 X s37	±
I	PIG 55	
	s04 X s04	±
	s04 X s09	−
	s04 X s09	−
	s04 X s22	o
	s04 X s22	−
	s09 X s09	±
	s09 X s22	‡
	s09 X s22	‡
	s22 X s22	±
	s17 X s19	−
	s17 X s20	o

Appendix (cont'd)

Pairing Class	Isolates			Antagonism/ Compatibility
I	PTA 60			
	s01	X	s01	±
	s01	X	s03	−
	s01	X	s03	−
	s01	X	s04	−
	s01	X	s04	−
	s03	X	s03	±
	s03	X	s04	o
	s03	X	s04	o
	s04	X	s04	±
I	PTA 65			
	s18	X	s18	±
	s18	X	s27	o
	s18	X	s27	o
	s18	X	s32	−
	s18	X	s32	−
	s27	X	s27	±
	s27	X	s32	−
	s27	X	s32	−
	s32	X	s32	±
I	PTA 69			
	s01	X	s01	±
	s01	X	s03	−
	s01	X	s03	o
	s01	X	s06	−
	s01	X	s06	−
	s03	X	s03	±
	s03	X	s06	‡
	s03	X	s06	‡
	s06	X	s06	±

Appendix (cont'd)

Pairing Class		Isolates	Antagonism/ Compatibility
II	PAR		
		01-d s02 X 03 s05	‡
		01-d s02 X 03 s05	♀
		01-d s02 X 07 s03	‡
		01-d s02 X 07 s03	‡
		01-d s03 X 03 s07	‡
		01-d s03 X 03 s07	‡
		01-d s03 X 07 s05	‡
		01-d s03 X 07 s05	‡
		01-d s08 X 03 s09	‡
		01-d s08 X 03 s09	‡
		01-d s08 X 07 s06	‡
		01-d s08 X 07 s06	‡
		03 s05 X 07 s03	‡
		03 s05 X 07 s03	‡
		03 s07 X 07 s05	‡
		03 s07 X 07 s05	‡
		03 s09 X 07 s06	‡
		03 s09 X 07 s06	‡
II	PIG		
		51 s21 X 55 s04	♀
		51 s21 X 55 s04	♀
		51 s21 X 55 s09	‡
		51 s21 X 55 s09	‡
		51 s21 X 55 s22	‡
		51 s21 X 55 s22	‡
		51 s33 X 55 s04	♀
		51 s33 X 55 s04	♀
		51 s33 X 55 s09	‡
		51 s33 X 55 s09	‡
		51 s33 X 55 s22	‡

Appendix (cont'd)

Pairing Class	Isolates	Antagonism/ Compatibility
II	PIG (cont'd)	
	51 s33 X 55 s22	‡
	51 s37 X 55 s04	♀
	51 s37 X 55 s04	♀
	51 s37 X 55 s09	‡
	51 s37 X 55 s09	‡
	51 s37 X 55 s22	‡
	51 s37 X 55 s22	♀
II	PTA	
	60 s01 X 65 s18	♀
	60 s01 X 65 s18	♀
	60 s01 X 69 s01	‡
	60 s01 X 69 s01	‡
	60 s03 X 65 s27	♀
	60 s03 X 65 s27	♀
	60 s03 X 69 s03	♀
	60 s03 X 69 s03	‡
	60 s04 X 65 s32	‡
	60 s04 X 65 s32	‡
	60 s04 X 69 s06	‡
	60 s04 X 69 s06	‡
	65 s18 X 69 s01	♀
	65 s18 X 69 s01	‡
	65 s27 X 69 s03	‡
	65 s27 X 69 s03	‡
	65 s32 X 69 s06	‡
	65 s32 X 69 s06	‡

Appendix (cont'd)

Pairing Class	Isolates	Antagonism/ Compatibility
III	PAR 01-d s02 X PIG 51 s33	=
	PAR 01-d s02 X PIG 51 s37	=
	PAR 01-d s02 X PIG 55 s22	=
	PAR 03 s05 X PIG 51 s33	o
	PAR 03 s05 X PIG 51 s37	=
	PAR 03 s05 X PIG 55 s22	=
	PAR 07 s03 X PIG 51 s33	=
	PAR 07 s03 X PIG 51 s37	=
	PAR 07 s03 X PIG 55 s22	=
	PAR 01-d s02 X PTA 60 s01	=
	PAR 01-d s02 X PTA 65 s18	o
	PAR 01-d s02 X PTA 69 s01	=
	PAR 03 s05 X PTA 60 s01	=
	PAR 03 s05 X PTA 65 s18	=
	PAR 03 s05 X PTA 69 s01	=
	PAR 07 s03 X PTA 60 s01	=
	PAR 07 s03 X PTA 65 s18	=
	PAR 07 s03 X PTA 69 s01	=
	PIG 51 s33 X PTA 60 s01	=
	PIG 51 s33 X PTA 65 s18	=
	PIG 51 s33 X PTA 69 s01	=
	PIG 51 s37 X PTA 60 s01	=
	PIG 51 s37 X PTA 65 s18	=
	PIG 51 s37 X PTA 69 s01	=
	PIG 55 s22 X PTA 60 s01	=
	PIG 55 s22 X PTA 65 s18	=
	PIG 55 s22 X PTA 69 s01	=

Appendix (cont'd)

Pairing Class		Isolates	Antagonism/ Compatibility
IV	PAR		
		01-d het X 01-e het	‡
		01-d het X 01-e het	‡
		01-d het X 04 het	–
		01-e het X 04 het	–
		04 het X 04 het	‡
		04 het X 30 het	–
		04 het X 30 het	–
		04 het X 84 het	–
		04 het X 84 het	–
		30 het X 30 het	‡
		30 het X 84 het	–
		30 het X 84 het	–
		84 het X 84 het	‡
IV	PIG		
		01 het X 01 het	+
		01 het X 37 het	–
		01 het X 37 het	–
		01 het X 80 het	o
		01 het X 80 het	o
		01 het X 80 het	–
		01 het X 96-b het	–
		37 het X 37 het	‡
		37 het X 80 het	–
		37 het X 80 het	–
		80 het X 80 het	‡
		80 het X 96-b het	–
		80 het X 96-b het	–
		96-a het X 96-b het	‡
		96-a het X 96-b het	‡

Appendix (cont'd)

Pairing Class	Isolates	Antagonism/ Compatibility
IV	PTA	
	16 het X 16 het	‡
	16 het X 33 het	=
	16 het X 33 het	=
	16 het X 65 het	o
	16 het X 65 het	o
	33 het X 33 het	‡
	33 het X 65 het	=
	33 het X 65 het	=
	49-a het X 49-b het	‡
	49-a het X 49-b het	‡
	65 het X 65 het	‡
	67-a het X 67-b het	‡
	67-a het X 67-b het	‡
V	PAR 04 het X PIG 01 het	=
	PAR 30 het X PIG 01 het	=
	PAR 04 het X PIG 51 het	=
	PAR 04 het X PIG 55 het	=
	PAR 04 het X PIG 80 het	=
	PAR 30 het X PIG 01 het	=
	PAR 84 het X PIG 01 het	=
	PAR 84 het X PIG 80 het	=
	PAR 84 het X PIG 96-b het	=
	PAR 04 het X PTA 16 het	=
	PAR 04 het X PTA 33 het	=
	PAR 04 het X PTA 65 het	=
	PAR 30 het X PTA 33 het	=
	PAR 84 het X PTA 33 het	=
	PAR 84 het X PTA 65 het	=
	PIG 01 het X PTA 16 het	=
	PIG 01 het X PTA 65 het	=

Appendix (cont'd)

Pairing Class	Isolates	Antagonism/ Compatibility
V (cont'd)	PIG 55 het X PTA 33 het	=
	PIG 80 het X PTA 33 het	=
	PIG 80 het X PTA 65 het	=
	PIG 96-b het X PTA 65 het	=
	PLT 01 het[a] X PIG 51 het	=
	PLT 01 het[a] X PIG 51 het	=
	PIG 51 het X PNG 01 het[b]	=
	PIG 51 het X PNG 01 het[b]	=

[a] Phellinus laevigatus

[b] Phellinus nigricans

both isolates from F. Lombard, Forest Products Lab, Madison, WI.