Relationship of Capacity to Cause Decay to Other Physiological Traits in Isolates of Lenzites trabea

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ABSTRACT

Studies of variation among homokaryotic, dikaryotic, and mono-oidial isolates of *Lenzites trabea* showed that (i) there is no consistent relationship between the incompatibility factor and rate of linear growth of an isolate and its capacity to cause decay; (ii) homokaryons with low decay capacities may be mated to form dikaryons with a much greater capacity to cause decay; (iii) no evidence was found that cytoplasmic factors will account for variation in capacity to cause decay; (iv) arsenic appears to de-dikaryotize isolates by accelerating a natural tendency toward a preponderance of one of the two nuclear types of a dikaryon; (v) the ratios of the two nuclear types in a dikaryon may influence its capacity to cause decay and the frequency with which it produces clamp-connections. Phytopathology 60:955-960.

Lenzites trabea (Pers. ex Fries), a heterothallic, bipolar hymenomycete, is one of the principal fungi causing decay of wood products (11). Considerable variation in tolerance to preservatives and capacity to cause decay occurs among both homokaryotic and dikaryotic isolates of this species (1, 2, 3, 9, 15, 18). Similar variation has been demonstrated among isolates of other wood-destroying basidiomycetes in their relative capacity to produce cellulolytic enzymes (5, 6), polyacetylenes (7), and their rate of growth (8).

When dikaryotic isolates of *L. trabea* are grown on substrata containing arsenic or certain other wood preservatives, they become de-dikaryotized (hyphae lack clamp-connections), and either or both of the component homokaryons can be recovered. Only one of the component homokaryons is consistently recovered from dikaryons of *L. trabea* when they are de-dikaryotized using arsenic-containing media (1, 2, 15).

The objectives of this research were to determine (i) whether the capacity of an isolate to cause decay is linked to other physiological properties such as incompatibility factors and rate of growth, and (ii) the mechanism by which arsenic de-dikaryotizes isolates of *L. trabea*.

MATERIALS AND METHODS.—*Isolates.*—The test organisms used in these studies included eight wild-type dikaryons, primary homokaryons obtained from the dikaryons as monobasidiospore isolates, synthesized dikaryons formed by mating two compatible homokaryons, and chemically-induced homokaryons. Monobasidiospore isolates obtained from primary homokaryons which produced basidiocarps in culture were termed secondary homokaryons, and those obtained from secondary homokaryons were termed tertiary homokaryons. Chemically-induced homokaryons were isolates that lacked clamp connections obtained by growing synthesized dikaryons in a defined medium containing sodium arsenate.

The wild-type dikaryotic isolates were: 1 (Madison 5031); 19 [Madison 5060(2)]; 16 (Madison 5123a); 32 (Madison 617); 3 (Boat 182); 10 (22630); A3 (TA3); and 5A (TA5A). These isolates were provided by Plant Research Institute, Canada Department of Agriculture, Ottawa; Forest Disease Laboratory, Forest Service, USDA, Laurel, Maryland; and field collections by the author.

Decay tests.—Capacity of the various isolates to cause decay of loblolly pine sapwood (*Pinus taeda* L.) was determined by the soil-block or agar-block decay test methods. Soil-block chambers were prepared, and the decay tests conducted, according to the ASTM standard (4) with modifications as described by Amburgey (3).

Agar-block decay tests were conducted using 8-oz French square bottles as decay chambers. Each chamber contained 2% malt-extract agar. Two-mm glass v-supports were used to elevate the test blocks above the medium. Test blocks were placed in the chambers 1 week after the test fungi were introduced. These chambers were incubated under the same conditions as the soil-block chambers.

Linear growth.—Dam tubes (17) containing 2% malt-extract agar were used to compare the rates of linear growth of homokaryotic and dikaryotic isolates with their decay capacities when grown in agar-block decay chambers. The growth tubes were inoculated at the end distal to the cotton plug and incubated for 15 days in continuous light at 30 C and the linear growth rates of the isolates determined.

Cytoplasmic factors.—To determine whether cytoplasmic factors affect the control of decay processes, synthesized dikaryon 19-1 + 1-2 was resolved into its two component nuclear types by isolating mono-oidia as described by Amburgey (3). All mono-oidial isolates were paired to isolates 19-1 and 1-2 to determine the

incompatibility factor which each carried. By comparing the decay capacities of the mono-oidial isolates obtained from dikaryons with that of the primary homokaryon having the same incompatibility factor, one may determine whether the capacity of a given nuclear type to control decay processes is affected by changing the cytoplasm.

Arsenic treatment.—Chemically-induced (c-i) homokaryons were derived from synthesized dikaryons by growing them in 500-ml flasks with 100 ml of a defined liquid medium containing 0.064 M sodium arsenate. The cultures were shaken at room temp for 2-3 weeks. This was done in a hood to dissipate the arsine gas produced by their growth.

The chemically-defined medium was adapted from Jennison (13): 80 g glucose, 4 g KH_2PO_4, 2.1 g glutamic acid, 1 g $MgSO_4 \cdot 7H_2O$, 4 mg thiamine hydrochloride, 0.57 mg H_3BO_3, 0.31 mg $ZnSO_4 \cdot 7H_2O$, 0.18 mg $Fe_2(SO_4)_3$, 0.04 mg $CuSO_4 \cdot 5H_2O$, 0.03 mg $NaMoO_4 \cdot 2H_2O$, 0.03 mg $MnSO_4 \cdot H_2O$, and distilled water to make 1 liter. The initial pH was 4.4.

Mycelial pellets were removed from the flasks and placed in petri dishes containing 2% malt-extract agar. As hyphae began to grow from the pellets, hyphal tips were removed and transferred to fresh dishes of 2% malt-extract agar. Those cultures without clamps were termed "c-i homokaryons"; those with clamps were discarded. All of the c-i homokaryons derived from a given synthesized dikaryon were compatible with only one of the parental homokaryons. Hyphae growing from the mycelial pellets at first lacked clamp connections but as the mycelial mat increased in size, hyphae with clamp connections were produced. Thus, both nuclear types survived the arsenic treatment but one type consistently predominated.

In decay tests, the average wt loss of five wood blocks was used as the index of decay capacity for each isolate. The least significant difference (LSD) between averages was calculated for 95% probability limits. The components of variation due to genetic influences among related isolates (s_g) and to environmental influences and error (s_e) were calculated. The genetic component of variation (s_g) was expressed as a standard deviation for each group of isolates obtained from a given parent isolate.

Results and Discussion.—In interpreting the significance of differences among isolates in terms of their capacity to cause decay, it should be recognized that decay capacity (wt loss of wood blocks due to decay in a standard time) is the end result of a complex series of enzymatic reactions. Differences in decay capacity (DC.) among isolates indicate that the isolates probably differ in the amount of any one or possibly several enzymes or cofactors needed to catalyze the various enzymatic steps in the decay process.

Decay capacity vs. incompatibility factors.—The DC.'s of primary homokaryons obtained from a given dikaryon were highly variable (Fig. 1). At least some primary homokaryons showed greater capacity to cause decay than their parent dikaryon. No relationship was apparent between the sexual incompatibility factor of

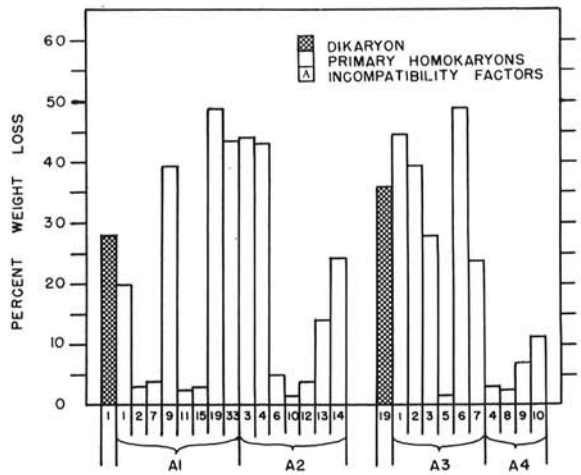

Fig. 1. Per cent wt loss due to decay by primary homokaryons obtained from two wildtype dikaryons. Each bar represents the average for five replicate blocks exposed to each isolate.

a homokaryon and its capacity to cause decay. These observations are consistent with results obtained in previous studies with this organism (1, 2, 3, 9). Thus, these two phenotypic traits appear to be inherited independently. Similar lack of correlation has been found between sexual incompatibility factors and various physiological traits including cellulolytic enzyme production by *Collybia velutipes* (5) and *Polyporus betulinus* (6) and production of polyacetylenes by an unknown tetrapolar basidiomycete (7).

Decay capacity of dikaryons vs. their component homokaryons.—Twelve of 15 synthesized dikaryons had greater capacities to cause decay than either of their component homokaryons (Fig. 2). Of the remaining four synthesized dikaryons, two had DC.'s equal to that of the component homokaryon with the lower DC., one had a DC. equal to that of the component homokaryon with the higher DC., and the DC. of the remaining synthesized dikaryon was intermediate to those of its component homokaryons. DaCosta & Kerruish (9) also observed that synthesized dikaryons of *L. trabea* usually had a greater capacity to cause decay than their component homokaryons. They found the reverse to be the case with synthesized dikaryons of *Poria vaillantii.* Aschan & Norkrans (5) found that synthesized dikaryons of *C. velutipes* produced less cellulase than either of their component homokaryons.

There was no consistent relationship between the DC.'s of homokaryons comprising a synthesized dikaryon and the DC. of the resulting synthesized dikaryon. In one case, two homokaryons with very low DC.'s (10-8 and 3-3) produced a synthesized dikaryon with a very low DC., whereas in another case two such homokaryons (1-2 and 3-3) produced a synthesized dikaryon with a very high DC. In no case did a pairing of a homokaryon with a high DC. and one with a low DC. result in the production of a synthesized dikaryon with a low DC. The above test was repeated by de-

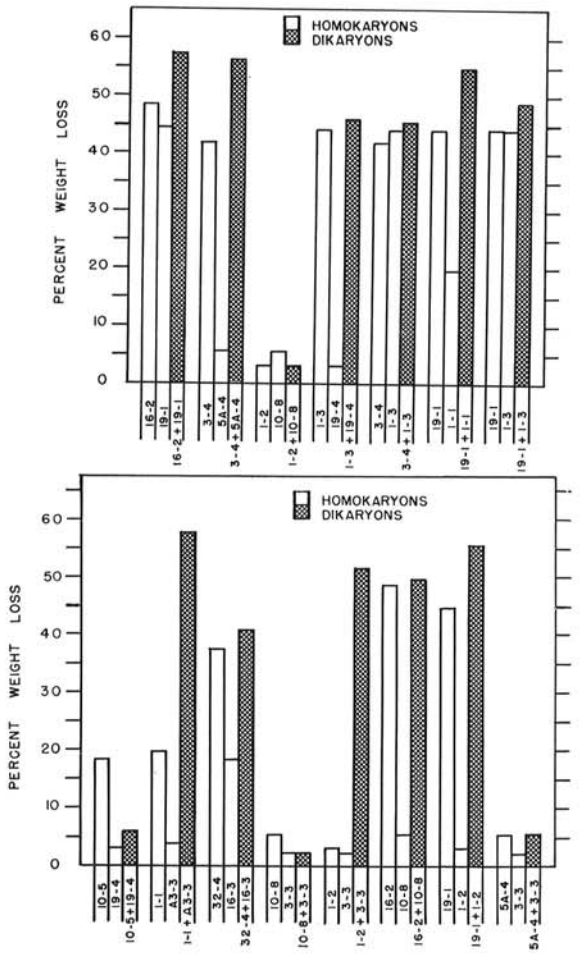

Fig. 2. Per cent wt loss due to decay by synthesized dikaryons and their component homokaryons. Each bar represents the average for five replicate blocks exposed to each isolate.

termining the DC.'s of the same 14 homokaryons and 18 synthesized dikaryons formed by pairing the compatible homokaryons in various combinations. As was evident in the tests discussed above, no consistent relationship existed between the DC. of a synthesized dikaryon and those of the homokaryons mated to form the dikaryon.

A further study indicated that not all of the isolates with low capacities to cause decay had deficiencies at the same locus. Of the six synthesized dikaryons formed by mating homokaryons 1-2, 3-3, 10-8, and 5A-4 in all combinations, only 1-2 + 3-3 had a high capacity to cause decay (Fig. 3). Based on this observation, it appears that homokaryons 1-2 and 3-3 had deficiencies at different loci, whereas the other two homokaryons (10-8 and 5A-4) had deficiencies at the same locus or loci and that these mutant sites overlap the mutant sites of both 1-2 and 3-3; noncomplementing mutations have been shown to occur at very closely linked sites (12). One could tentatively draw a complementation map based on the above data which would be as follows:

1-2	3-3
10-8	
5A-4	

Decay capacity vs. rate of linear growth.—No consistent relationship was shown between the rate of linear growth of homokaryotic and dikaryotic isolates and their capacities to cause decay (Fig. 4). Although the three fastest-growing isolates were dikaryotic and also had the highest DC.'s, some of the homokaryons grew faster than some of the synthesized dikaryons,

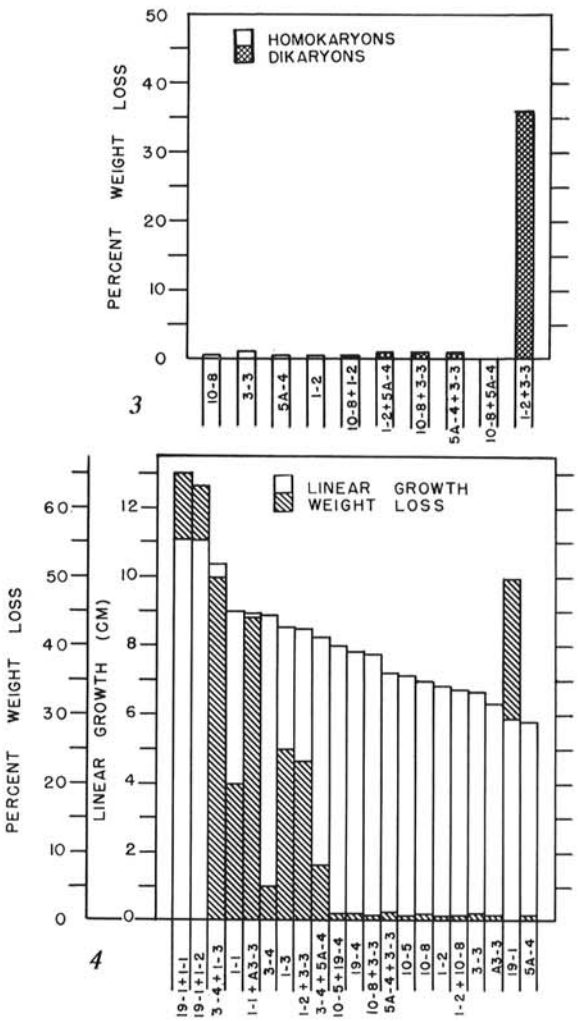

Fig. 3-4. **3)** Per cent wt loss due to decay by four homokaryons and the synthesized dikaryons formed by mating them in all combinations. Each bar represents the average for five replicate blocks exposed to each isolate. **4)** A comparison of the per cent weight loss of test blocks and the rate of linear growth by synthesized dikaryons and their component homokaryons. Each bar represents the average for five replicate blocks exposed to each isolate or the average rate of growth of two replicates per isolate.

and one of the slowest growing homokaryons had a high capacity to cause decay. The rate of linear growth of 7 of the 10 synthesized dikaryons was either greater than that of either of their two component homokaryons or equal to that of the homokaryon with the highest growth rate.

Influence of cytoplasmic factors on decay capacity.—A study comparing the DC.'s of the synthesized dikaryon 19-1 + 1-2, its component homokaryons, and mono-oidial isolates obtained from the dikaryon (Fig. 5) indicated that cytoplasmic factors had little effect on the capacity of a given nuclear type to control decay processes. If cytoplasmic factors influenced decay processes, the primary homokaryon and the oidial isolates with the same incompatibility factor would be expected to differ in capacity to cause decay. All five of the mono-oidial isolates from 19-1 + 1-2 that had the same incompatibility factor as 1-2 also had decay capacities similar to 1-2. Of the three mono-oidial isolates that had the same incompatibility factor as 19-1, two had DC.'s similar to 19-1. One mono-oidial isolate (0-4) with the same incompatibility factor as 19-1, however, had a greater capacity to cause decay than 19-1 (Fig. 5). Upon re-examination, the hyphae of this isolate were found to have a very few clamp-connections. It was therefore assumed that this isolate was heterokaryotic. Since the hyphae of the parent dikaryon 19-1 + 1-2 had an abundance of clamp-connections, and since mono-oidial isolate 0-4 had a greater capacity to cause decay than the parent dikaryon, it was assumed that these two isolates differed from one another in some way. It is possible that the differences observed between these isolates are a consequence of differences in the ratios of the two nuclear types present in their mycelia. In other fungi it has been observed that changes in various phenotypic traits may accompany changes in the nuclear ratios in a given heterokaryon (10, 16, 19).

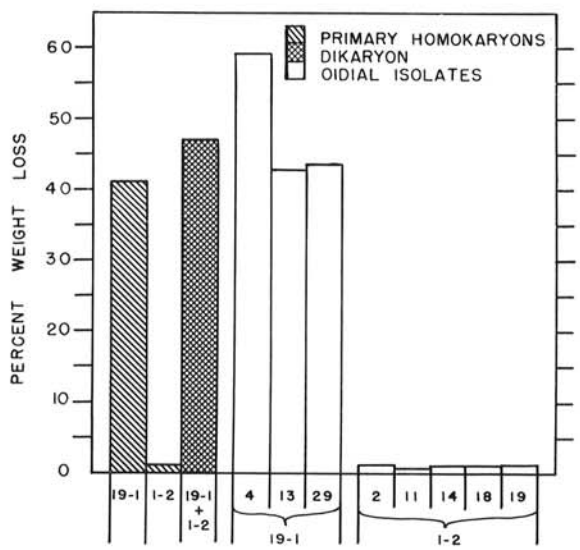

Fig. 5. Per cent wt loss due to decay by synthesized dikaryon 19-1 × 1-2, its two component homokaryons, and mono-oidial isolates obtained from the dikaryon. Each bar represents the average for five replicate blocks exposed to each isolate.

Assuming that the cytoplasms of isolates 19-1 and 1-2 differ from one another as well as from that of the dikaryon 19-1 + 1-2, these results indicated that the cytoplasm had little effect on the capacity of a given nuclear type to control decay processes. If cytoplasmic effects were influencing the capacity of a given nuclear type to produce enzymes that were involved in the decay process, or if cytoplasmic factors had a direct influence on the capacity of an isolate to cause decay, the amount of variation in DC. among the mono-oidial isolates with a given incompatibility factor should have been much greater.

Influence of arsenic on isolates.—In decay tests of 10 recently isolated c-i homokaryons derived from 19-1 + 1-2, seven had DC.'s like 1-2, while three had much greater DC.'s (Fig. 6-A). All 10 had the incompatibility factor of 1-2. At the conclusion of the decay tests, the three with high DC.'s (A, C, J) had abundant clamp-connections. Thus, all of the c-i homokaryons whose hyphae remained without clamp-connections had the same capacity to cause decay as 1-2, the parent homokaryon with which they share a common incompatibility factor. When these isolates were backcrossed to their compatible parent homokaryon 19-1, the resulting dikaryons showed considerable variability (Sg = 4.2%) with respect to DC.

Decay tests of 10 c-i homokaryons derived from another dikaryon, 19-1 + 1-1, showed more variation (Sg = 7.3%) among the c-i homokaryons with respect to DC. (Fig. 6-B). Each had the incompatibility factor of 1-1. Eight had higher and two lower DC.'s than 1-1. A very few clamplike structures (hook cells that did not fuse with the hyphae) were present in isolates B, E, G, and J (Fig. 6-B). It is not certain whether this should be interpreted as evidence of heterokaryosis. While the reason for the variation among the c-i homokaryons from 19-1 + 1-1 is not clear, earlier tests of 1-1 indicated that it may contain more than one nuclear type (3). Thus, the variation in DC. among the c-i homokaryons with the same incompatibility factor as 1-1 may be due to differences in the ratios of the nuclear types originally present. The dikaryons formed by backcrossing the c-i homokaryons from 19-1 + 1-1 to the compatible parent homokaryons varied considerably (Sg = 8.4%) in their capacities to cause decay (Fig. 6-B).

The variation in decay among the dikaryons formed by backcrossing (Fig. 6) suggests that (i) the ratios of the two nuclear types in a dikaryon may influence its DC., or (ii) the cytoplasm in which nuclei are situated may influence their capacity to control the decay processes. Either or both hypotheses could explain the observations of Kaufert & Schmitz (14) that isolates of *L. trabea* caused greater decay of red pine (*Pinus resinosa*) sawdust when it contained arsenic than when it was untreated. Earlier tests with synthetic dikaryon 19-1 + 1-2 indicated that the cytoplasm had

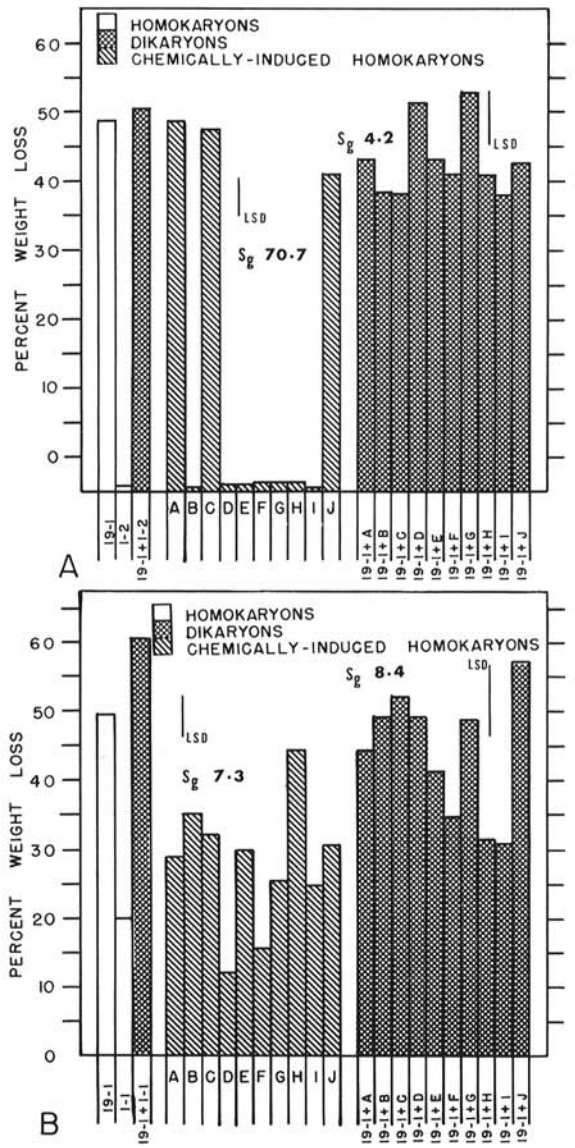

Fig. 6. Per cent wt loss due to decay by synthesized dikaryons, their component homokaryons, chemically-induced homokaryons derived from the dikaryons, and dikaryons formed by backcrossing the chemically-induced homokaryons with the parent homokaryon with which they are compatible. Each bar represents the average of five replicate blocks exposed to each isolate.

little effect on the capacity of a given nuclear type to control decay processes.

The fact that the hyphae of some of the c-i presumed homokaryons contained only a few clamp-connections whereas the hyphae of their parent dikaryon contained abundant clamps may indicate that arsenic treatment affects the ratio of the two nuclear types within a dikaryon.

If clamp-connection formation required the heterozygous condition of a given gene or group of genes, a lower percentage of one of the nuclear types (lower gene dose) may decrease the number of clamps formed. Finally, a point may be reached where both nuclear types are still present but no clamps are formed. Thus, differences in nuclear ratios may be responsible for some of the variation in capacity to cause decay observed among the c-i homokaryons tested above.

Of 34 mono-oidial isolates obtained from isolate 19-1 + 1-2, 16 (47%) were dikaryotic, 16 (47%) were compatible with 1-2, and 2 (6%) with 19-1. Thus, the incompatibility factor recovered in the c-i homokaryons from 19-1 × 1-2 (Fig. 6-A) is the most frequently recovered in the mono-oidial isolates. Perhaps 1-2 nuclei divide more frequently than 19-1 nuclei in dikaryon 19-1 + 1-2, or perhaps the nuclear ratio established in the dikaryon is such that there are many more 1-2 nuclei than 19-1 nuclei.

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