BIOLOGICAL STUDIES OF SHIITAKE LOGS AND ASSOCIATED MYCOFLORA IN THE VIRGINIA HIGHLANDS

by

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DEDICATION

I would like to dedicate this thesis to my father (the late) Félix Guevara Martínez, my mother Filadelfa Guerrero Vda. de Guevara, my wife and son Amy and Félix A. Guevara Wiley, and my brothers and sisters Francisco, Félix, Daniel, Josefina, and María.

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CHAPTER 1. IDENTIFICATION AND PHYSIOLOGY OF MAJOR FUNGAL COMPETITORS ("WEED FUNGI") IN SHIITAKE LOGS AND OF FUNGI IN LIVING OAK TREES

ABSTRACT

Shiitake growers in Virginia are experiencing considerable diminution of the fruiting life of oak logs, due primarily to Ascomycetous, competing, wood-rotting, contaminating "weed fungi" that either invade the logs after trees are felled or are present in tree tissues before felling. We surveyed several shiitake farms, and, although the fungal flora differed among them, the predominant colonizing fungi were identified as *Graphostroma platystoma* (also identified as *Diatrype stygma*), *Eutypa spinosa* and *Stereum* aff. *complicatum*. Other less frequently observed fungi were *Stereum ostrea*, *Schizophyllum commune*, *Hymenochaete* sp., *Poria* sp., *Hypoxylon atropunctatum* and *Hypoxylon punctulatum*. Site and means of invasion (colonization) of these "weed fungi" were studied by monitoring endophytic fungal populations in stressed and non-stressed oak trees in the Jefferson National Forest Montgomery County, Virginia. Twenty-six samples from Poverty Creek (non-stressed) and 23 from Brush Mountain (stressed) were studied. Fifty percent of the samples from the non-stressed were sterile, 23% yielded *Paecilomyces variotii*, and 27% were colonized by other fungi. On the other hand, 100% of the samples from stressed trees (Brush Mountain) were colonized by fungi; 74% yielded *P. variotii* and 26% by other fungi. Thirty samples from a pin oak (*Quercus palustris*) plot at Virginia Tech (non-stressed) were 74% sterile, 16% colonized by an unidentified yeast, and 10% colonized by other unknown fungi. The cardinal and optimum temperatures and pH growth profile on laboratory media were determined for *Lentinus edodes* (shiitake), *G. platystoma*, and *E. spinosa*. *G. platystoma* had

a minimum of 10°C, an optimum of 25-28°C, and a maximum of 32°C; the optimum pH range for growth was 6.5-7.0. *E. spinosa* had a minimum growth temperature of 15°C an optimum of 28-32°C, with an undetermined maximum temperature; the optimum pH for growth was 5-7. *L. edodes* had a minimum temperature of 5°C, an optimum of 25°C, and a maximum of 32°C; the optimum pH for growth of 3.5-4.5. These findings suggest that log inoculation with *L. edodes* would be better done during the late winter/early spring when temperatures are lower and more suitable for the growth of shiitake than for the growth of "weed fungi", and by using oak species with highly acidic sapwood (shiitake is a acidophilic fungus), and from non-stressed oak populations (tree selection).

INTRODUCTION

The shiitake mushroom (*Lentinus edodes* (Berk.) Sing.) is a white rot fungus or lignin decomposer (Oki et al. 1981; Rayner & Boddy, 1988; Diehle & Royse, 1986; Przybylowicz & Donoghue, 1988; Fisher et al. 1988), that is native to Asia (Singer, 1987) and belongs to the Basidiomycetes. Shiitake is the second most important edible mushroom after the button mushroom, *Agaricus bisporus* (San Antonio, 1981; Tan & Chang, 1989; Leatham, 1982; Chang & Miles, 1989). In 1986 shiitake production worldwide was over 314 thousand metric tons (MT), with almost 51% being produced in Japan. Other large producers are China, Taiwan and Korea (Breene, 1990; Chang & Miles, 1989).

Currently, the two basic methods for shiitake production are by inoculation of natural and synthetic logs (Royse, 1989). Most shiitake is grown outdoors on hardwood logs mainly from the oak family (Fagaceae), where cultivation is undertaken in an open, uncontrolled or semi-controlled environment. Understory shade that has both lower ambient temperatures and higher relative humidities seems to be a critical and essential

production site. More modern cultivation techniques (indoor) under fully-controlled conditions have been employed in farms in the USA (Tan & Chang, 1989; Royse et al. 1990). The major advantages of producing shiitake on synthetic logs compared with production on natural logs include a consistent market supply through year-round production, increased yields, and decreased time required to complete a crop cycle (Royse et al. 1990; Diehle & Royse, 1986). The steps for shiitake cultivation in logs include determining a production area, selecting trees, obtaining logs, choosing shiitake spawn and strains, inoculating, incubating, and controlling diseases and pests. Shiitake cultivation on sawdust is like cultivation on logs; the response of shiitake to the environment is similar. The fungus goes through the same stages of mushroom production: inoculation, incubation, induction, pinning, fruiting, and resting (Przybylowicz & Donoghue, 1988; Singer, 1987; Ho, 1989).

Shiitake has good nutritional values (Royse & Schisler, 1980). It contains moderate quantities of good quality protein and dietary fiber, vitamins C and D, and minerals. Lipid levels are low, but unsaturated to saturated fatty acids ratios are high. Cadmium has been reported in shiitake fruiting bodies. Furthermore, it contains medicinal compounds with antiviral, antitumor, anti-cholesterol and other properties (Breene, 1990; Leung, 1988; Yang & Jong, 1989; Mori & Takehara, 1989; Mori et al. 1989; Tokuda & Kaneda, 1978; Pettipher, 1988; Chihara,1978; Suzuki et al. 1989).

This research was prompted by a plea for help to Dr. R. J. Stipes, Professor of Plant Pathology and Dr. James R. Nichols, Dean of the College of Agriculture and Life Science, from the Appalachian Mushroom Growers who were experiencing no production as pronounced "short life" of fruiting in their shiitake logs. Instead, for example, of obtaining mushrooms for 5 or 6 yr (Dr. Linzey, personal communication to Dr. Stipes), some were

able to collect fruiting bodies for only 1 yr, and sometimes not even a single harvest was made.

Stipes (1961) and predecessors in oak wilt (*Ceratocystis fagacearum* (Bretz) Hunt) research reported the rapid colonization by Ascomycetous white rotting fungi of oak trees in West Virginia; the predominant invaders included *Hypoxylon punctulatum* (Berk. & Rav.) Cke., *H. atropunctatum* (Schw. ex Fr.) Cke., *H. mediterraneum* (de Not.) J. H. Miller, *Diatrype stigma* (Hoffm.: Fr.) Fr. (possibly *Graphostroma platystoma* (Schw.: Fr.) Pirozynski), and others. With this background, when we examined mushrooms growers' logs, we were able to identify most of the fungi present, and to form a working hypothesis in attacking and resolving the problem (Guevara et al. 1989). In the Virginia highlands and piedmont physiographic provinces we identified the following genera on shiitake production logs: the Ascomycetes *Graphostroma*, *Eutypa*, and *Hypoxylon* and the Basidiomycetes *Stereum*, *Schizophyllum*, *Hymenochaete*, and *Poria*. We call these "weed fungi" since they, as unwanted higher plants in the field crops for example, likewise compete with the planted crop for nutrients and depress yields. Royse (1989) also reported the occurrence of "weed fungi" and suggested that, in addition to competition for nutrients in production logs, they may also produce metabolites injurious to *L. edodes*. Virginia growers have found that chestnut oak (*Quercus prinus* L.) and white oak (*Quercus alba* L.) are the best oak species for resisting invasion by the "weed fungi" because of their bark structure while red/black oak group species are rapidly invaded.

The shiitake mushroom industry was begun in Virginia in 1981 by some Korean nationals in Buckingham County with the now-defunct Elix Corporation, a very large one-time producer. In spite of the "weed fungi" interference in mushroom production in Virginia, interest has escalated greatly in shiitake farming as a profitable, if not lucrative,

alternative agricultural project which is needed in response to the decline of tobacco culture and other traditional farming systems in Virginia and other Appalachian Mountain regions. Approximately 80% of Virginia farmers have small farms and therefore those who have woodlots on their acreages could exploit this alternative crop and practice. Approximately 65% of Virginia is forested (of this about 40% is mixed oak), therefore providing the shiitake industry the potential of a positive impact on local economies in rural areas where most of the land is combined with rugged rocky sites occupied by hardwood forest, which is the main source of logs for shiitake production (Cotter & Lamphear-Cook, 1988). Currently, there are about 60 known major shiitake growers in Virginia (Andy Hankins, personal communication to R.J. Stipes, November, 1991).

There were three major objectives of this thesis: (1) Identify and study basic endophytic and epiphytic fungal populations and physiological growth parameters of the "weed fungi," and *L. edodes* (Berk.)Sing.; (2) To evaluate the suppressive potential of fungicides to the "weed fungi," *L. edodes*, and to evaluate competitive potential of these; and (3) To compare the sapwood rotting potential of the "weed fungi" and *L. edodes*.

MATERIALS AND METHODS

1. Identification of "weed fungi"

In June of 1988, Dr. Stipes, Dr. Linzey, and I surveyed 5 shiitake farms in Virginia (including the largest one which had 0.5 million shiitake logs at that time) for "weed fungi". We carefully examined their operations and observed their logs for fungal fruiting bodies. The most commonly used trees were white oak (*Quercus alba* L.), chestnut oak (*Q. prinus* L.), black oak (*Q. velutina* Lom.), and some hickory (*Carya* spp.). Growers were not in agreement on which trees provided the best substrate for mushroom production, and used

trees from different sites, age groups, and varying bole sizes. Some used dominant trees in wood lots, while the majority of them used understory, suppressed and slow-growing trees.

The most common fungi occurring in shiitake logs were collected in plastic bags and labeled for later determination in the laboratory. Determination of the main "weed fungi" was performed by using specialized literature and monographs of the fungi in question such as von Arx (1981), Glawe & Rogers (1972, 1982, 1986), Miller (1961), Barnett & Hunter (1987), Wehmeyer, (1975), Pirozynski (1974), and Stipes (1961). Dr. Stipes made initial tentative identifications after which we sent companion specimens to other scientists specializing in Ascomycetes: Dr. Jack D. Rogers (Washington State University), Dr. Dean A. Glawe (University of Illinois), and Dr. Lois D. Tiffany (Iowa State University).

Once in the laboratory, perfect stage fruiting bodies (stromata) were studied dissected and described macro-and microscopically. Descriptions included the size of stroma, surface, texture, color, presence or absence of ectostroma, presence or absence of perithecia (one or more layers), perithecia shape, ostiole shape and ornamentation, habitat (e.g. on decorticated or corticated wood). Macro-and microstructures were drawn or photographed and other microscopic observations were made. Description of microscopic structures of the perfect stage (stroma) included the size and shape of the asci and ascospores and reaction to Melzer's reagent.

To describe the imperfect stages of these "weed fungi", either fresh specimens (stromata) were put in moisture chambers for 48 hr to induce imperfect fruiting body production on the surface of the stroma, or asci were collected by pressing the moistened stroma with a needle or scalpel to obtain pure culture from multi-ascospores. A whitish gelatinous exudate (collapsed hymenium) containing asci and ascospores was collected from the pressed stromata, placed in sterile distilled water, and vortexed for about 10 min to

allow the release of the ascospores from asci. Ascospores (single or multiple) were streaked onto potato-dextrose agar amended with 200 µg/ml each of streptomycin and chloramphenicol to obtain pure cultures. A less reliable method was done by soaking the stromata in water for 3 hr, sticking them on petri plates lids, and allowing the ascospores to be discharged onto the agar just described. The cultures were maintained on Difco potato-dextrose agar (DPDA); its pH was 5.5 after cooling.

The macroscopic appearance of the imperfect stage of these "weed fungi" cultured on DPDA was observed. Agar coloration on the obverse and reverse sides of the petri dish, mycelial pigmentation, zonation, texture, and odor, at different periods of time during incubation were observed. Microscopic observations of the imperfect stage including size, color, shape and ornamentation of the conidia, conidiophores, and condiogenous cells were carefully made. Observations of the Basidiomycetous wood decay fungi were also made. For wood decaying fungi size, shape, texture, margin, spore print, color, odor, chemical reaction to 5% KOH, and Melzer's reagent, type of surface of the pileus, hymenium, and stipe (if present); and on synthetic medium (DPDA) clamp connections and encrusted pigments were observed and recorded.

To obtain pure cultures of "weed Basidiomycetes," small pieces of fresh fruiting bodies including hymenium were likewise attached to the top of a petri dish with Vaseline inverted over a thin layer of DPDA to which in some cases, antibiotics were added. Basidiospores were discharged on the medium after 24 hr and germination took place about 48 hr after discharge at 25°C. Isolations were obtained from multi-single basidiospores, and maintained on DPDA at 4°to 5°C in darkness and re-cultured every 5 to 6 mo. The tissue isolation technique was also used, consisting of excised tissue from the context of the pileus placed on agar plates. Dikaryotic (secondary) mycelium was produced after several days of

incubation at 25°C in darkness. This technique has been used in shiitake strains to avoid spontaneous mutation (degeneration).

2. Endophytic fungal populations in scarlet and pin oaks growing on stressed and/or non-stressed sites

After "weed fungi" were identified, and based on our observations from the shiitake farms (on which most shiitake growers were using understory, suppressed and weakened trees), Dr. Stipes and I hypothesized that these main "weed fungi" might have colonized living xylem tissues before felling. Drs. Jack D. Rogers and Patrick Fenn (personal communication to Dr. Stipes) also suggested that a considerable number of these wood rotters might be asymptomatically infecting living, putatively healthy oak trees. To test this hypothesis, we examined xylem (outer sapwood) tissues from living, standing oak trees on three sites for resident endophytic fungi occurring in stressed and non-stressed scarlet oak (*Quercus coccinea*) trees on Brush and Gap Mountains (Poverty Creek) in the Blacksburg Ranger District in Montgomery County, Jefferson National Forest, Virginia. Healthy rapidly growing 20-yr-old pin oak (*Quercus palustris*) trees from Dr. Stipes' research plantations on the Virginia Polytechnic Institute and State University (Virginia Tech) campus were examined as non-stressed trees. Selection of the scarlet oak mountain sites was kindly made by Dr. David Wm. Smith, Department of Forestry, Virginia Tech. Brush Mountain is characterized as a water-stressed site. Growth rings were obtained to corroborate this versus non-stressed sites. Growth rings were counted in the first and second inch from the outer part of the core samples. For the non-stressed site 14 growth rings (average) from scarlet oak were counted in the first and second inch of the outer part of the core sample. For the water-stressed sites 22 growth rings (average) were counted in

the outer inch of the core sample and 18 growth rings in the second inch.

In October, 1990, core samples were collected from individual, random scarlet oak trees; 26 xylem core samples at Poverty Creek, 23 from Brush Mountain and 30 from a pin oaks research plot at VPI & SU. At the biopsy site, the bark was surface sterilized with 70% EtOH and the tree bark removed with a sterile tree scribe. A sterile increment borer was then used to aseptically remove the sample that was placed into dry, sterile sample tubes in transit back to the laboratory where they were placed onto CSPDA (PDA with 200 µm/ml each of streptomycin and chloramphenicol) and incubated at 25°C for 8 days to await emergence of fungi.

3. Temperature and pH growth optima of "weed fungi" and *L. edodes*

Defining and comparing biological growth parameters *in vitro* were viewed as helpful in understanding competition phenomena *in situ*. To determine the temperature and pH optima for growth *in vitro* of these fungi, radial mycelial growth of the colonies were measured in mm at 2-day interval and at incubation temperatures of 5, 10, 15, 20, 25, 28, 32 and 40°C in darkness. Isolates of *G. platystoma*, *E. spinosa*, *H. punctulatum*, (Ascomycetes), *P. ostreatus* and *T. versicolor* versus *L. edodes* (strain VT17 = 1S, originating in Korea via Elix Corporation, Arvonia, VA) were tested. More emphasis was given to *Graphostroma*, *Eutypa*, and *Lentinus*. The synthetic medium was glucose-yeast extract agar (GYEA) consisting of D-glucose (5 g), yeast extract (1 g), microelements (2 ml), agar (20 g), and distilled water to 1000 ml. Preparation of the cultures and incubation were performed simultaneously in the same way and manner for all of the fungi to avoid variation. Three replicates for each fungus at each temperature were made. To avoid the loss of water from the media by evaporation, labeled petri dishes (cultures) were placed into

transparent plastic bags.

The pH assay was done by measuring the biomass production during 1 mo of incubation by the main "weed fungi" at pHs 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, and 7.0 at the incubation temperature of 25°C. The fungi tested were *G. platystoma*, *E. spinosa*, and *L. edodes* VT17. Glucose-yeast extract wood decoction liquid medium was used in this test consisting 10 g of D-glucose, 2 g of yeast extract and 2 ml of microelements. Hydrochloric acid (36%) and sodium hydroxide (50%) were added to obtain the desired pH. The filtered wood extract was made from bark, sapwood and heartwood blocks of *Q. palustris*: 400 g of air dry wood blocks were boiled for 1 hr in 1000 ml of distilled water. These solutions were then mixed, autoclaved, and cooled. Three mycelial replicates for each fungus for each treatment were incubated at 25°C in darkness for about 1 mo in stationary cultures on 100 ml of the medium in 250 ml Erlenmeyer flasks. After the incubation period, the mycelium was collected from each culture by pouring the liquid medium onto a previously weighed (initial weight) filter paper in a Buchner funnel, which then was placed in a side-arm suction flask and connected to a vacuum pump. Once the mycelium was collected, the filters papers were oven-dried at 67°C for 24 hr, cooled and again weighed. Dry weight production was calculated in g by subtracting the initial dry weight from the final dry weight of the filter paper less 0.01 g, the weight of the original inoculum plug used to start the test.

RESULTS

1. Identification of "weed fungi"

The most commonly occurring "weed fungi" in shiitake logs were: (1) *G. platystoma*, considered by some mycologists to be *D. stigma*, a common Ascomycete on oak in the Appalachians (Stipes, 1961), (2) *E. spinosa*, considered by some to be *E. lata*, and (3)

Stereum aff. *complicatum*. Other fungi observed fruiting in lesser frequencies were *Stereum ostrea*, *Schizophyllum commune*, *Hymenochaete* sp., *Poria* sp., *Lenzites* sp., *Phellinus* sp., and the ubiquitous *Hypoxylon atropunctatum* and *Hypoxylon punctulatum* (Guevara et al. 1989, 1991; Guevara, & Stipes, 1990). The stroma of the latter in a moisture chamber emits a strong aroma of cucumber fruit. We shall now address the three most commonly occurring fungi in shiitake logs in some detail.

A. *Graphostroma platystoma* (Schw.:Fr.)Pirozynski (Figs. 1-4, & 13)

= *Sphaeria afflata* Schw., Schrift. Nat.-Forsch. Ges. 1:34, 1822.

= *S. subaffixa* Schw., Trans. Am. Phil. Soc. IIA, 4:191, 1832.

= *Diatrype discostoma* Cooke, Grevillea, 6:144, 1878.

= *D. tenuissima* Cooke, Grevillea, 6:144, 1878.

= *Eutypa micropuncta* Cooke, Grevillea, 6:144, 1878.

On PDA and GYEA, the reverse of the petri dish was white to cream, then reddish pink to brown, and finally reddish black in color. The fungus covered the petri dish in about 12 days (inoculated at the edge of the 90 mm petri dish) when cultured at 25°C in continuous darkness. The mycelial surface was appressed to more or less lannose, white to gray and finally grayish black after 30 days of incubation. On GYEA, formation of "pseudosclerotia" of less than 1 mm long on the surface were seen as well as conidiophores (mainly when wood chips were used in the medium). A walnut fruit (*Juglans nigra*) odor, as in *Hypoxylon atropunctatum*, was noted in petri plates of actively growing cultures. Conidiophores were 100-250 µm long, hyaline to brownish or brown, smooth or verrucose with secondary branches. Conidiogenous cells with apices were more or less inflated and irregular giving the appearance of scares or teeth. Conidia were 4-8 x 4-6 µm, hyaline in KOH 5%, smooth, ovoid to more or less ellipsoid. Dark hyphae intermixed were seen

beneath the conidiogenous mycelial layer on the surface of wood chips and medium.

The stromata were erumpent from between the inner to the outer bark, widely spread (up to 2 m along dead trees), cortical (attached to the bark), grayish to black. The fragile papyraceous ectostroma was seen to separate from the entostroma with time (deciduous). Entostroma was formed to some extent by black hyphae of pseudoparenchymatous tissue. The entostroma was extensively cracked, mostly transversely. Perithecia occurred in one layer and were globose, pyriform to obpyriform. Ostioles were versiform, most of them donut-like, dark, even, slightly apiculated, or depressed in the center, not sulcated. Asci were 25-40 x 4-6 µm, short-stalked or claviform, eight-spored, apex with a minute ring that turns blue in Melzer's reagent, thin walled; paraphyses were not seen. Ascospores were 6-12 x 1.5-2.0 µm, suballantoid or allantoid, thin-walled.

B. *Eutypa spinosa* (Pers.: Fr.)Tul. (Figs. 5-8, & 14)

Cultures on DPDA covered the plates after 15-21 days of incubation at 25°C in darkness. The fungus grew slowly and formed aerial to appressed mycelium, first white then becoming grayish black. The reverse of the petri dish was cream to dark brown and finally brownish black to black. Conidiophores were not abundant, erect branching, smooth then becoming verrucose due to conidiospore production giving a appearance of denticulation or scars, hyaline to dark brown. Conidiogenous cells were cylindrical with proliferation sympodial, apices becoming distorted by sporogenesis. Conidia were 15-21.5 x 1.5-2.5 µm, smooth, more or less cylindrical to slightly curved, hyaline in KOH 5%, with thin-walls.

The stroma on the bark surface was black (entostroma) and rough, with irregular margins, widely sprayed up to 2 m in length along dead trees (mainly oak). Stromata were 0.5-1 mm thick and tough. Ectostroma was more or less deciduous; no odor was detected. Perithecia were versiform, occurring in one layer, more or less globose to irregularly

globose; ostioles were sulcated, dark, and versiform. Asci were 35-52 x 5-7 µm, claviform, more or less stalked. Ascospores were 4-9 x 1.5-2 µm, allantoid and pale in KOH 5%. Imperfect state of this fungus is very similar to that described for the genus *Selenosporela* Arnaud ex MacGarvie. Habitat, on dead pin oak branches (*Quercus palustris*).

C. *Stereum* aff. *complicatum* (Figs. 9-12)

Colonies on PDA covered the 90 mm plate in 3 wk at 25°C; upper surface was velvety to wooly, white to orange with an orange reverse and fragrant odor. Clamp connections were observed usually two or more per septum. Fruiting bodies were 5-20 mm, fruiting individually or united. Pilei were coriaceous, thin, effuse-reflexed, with the reflexed portion consisting of small umbonated pilei which were divided into lobes many times; they also were tomentose to fibrous toward the base and more or less glabrous on the margin, with a cinnamon orange color and more or less zonated. The hymenium was even, smooth and glabrous, cream orange to yellow orange; the context was 1 mm wide, cream colored, odorless; the spore print was white. Spores were 7-8 x 2-3 µm, cylindric, smooth, more or less with thick-walled, hyaline in KOH 5%, and amyloid in Melzer's reagent. The basidia were 29-39 x 4-5 µm and claviform. Pleurocystidia were 18-25 x 5-6 µm, and more or less claviform, thick-walled.

D. Other less frequently observed fungi were *Stereum ostrea*, *Schizophyllum commune*, *Hymenochaete* sp., *Poria* sp., *Phellinus* sp., *Lenzites* sp., *Hypoxylon* sp., *H. atropunctatum*, *H. punctulatum*.

2. Endophytic fungal populations in scarlet and pin oaks growing on stressed and non-stressed sites.

Results of the survey for endophytic fungi in living, standing, stressed and

non-stressed scarlet oak trees are given in Table 1. This assay showed that 50% of 26 samples from the non-stressed trees (Poverty Creek) were sterile: 23% yielded *Paecilomyces* aff. *variotii*, and 27% were colonized by numerous other fungi including unidentified Basidiomycetes. Furthermore, 100% of the samples from stressed trees (Brush Mountain) were colonized by fungi in which 74% yielded *Paecilomyces* aff. *variotii* and 26% other fungi including unidentified Basidiomycetes (Guevara et al. 1991).

Samples from Dr. Stipes' non-stressed pin oak (*Q. palustris*) plot at Virginia Tech were 74% sterile; 16% were colonized by an unidentified yeast, and 10% by other unknown fungi. *Paecilomyces* aff. *variotii* was not seen (Table 2).

Attempts to find *Graphostroma platystoma* and *Eutypa spinosa* from non-stressed or stressed, standing trees were unsuccessful.

3. Temperature and pH growth optima of "weed fungi" and *L. edodes*

The results of the temperature and pH growth optima test of *Lentinus edodes* versus major shiitake log "weed fungi" are given in Figs. 15 and 16. *Graphostroma platystoma* has cardinal temperatures of 10°C (minimum), 25-28°C (optimum), and 32°C (maximum). For *Eutypa spinosa* they were 15°C (minimum), 28-32°C (optimum), and ?°C (maximum). Two *Lentinus edodes* strains VT17=S1 and U8 had the same cardinal temperatures: 5°C (minimum), 25°C (optimum), and 32°C (maximum). Those for *L. edodes* strain WG were 5°C (minimum), 25-28°C (optimum), 32°C (maximum) and for strain W4, 15°C (minimum), 25°C (optimum), and 28°C (maximum).

Cardinal temperatures for growth of *Trametes versicolor* were 5°C (minimum), 25-32°C (optimum) ?°C (maximum); for *Pleurotus ostreatus* 5°C (minimum), 20-28°C (optimum), 32°C (maximum); and for *Hypoxylon punctulatum* were 10°C (minimum),

25-28°C (optimum), and 32°C (maximum) (Guevara & Stipes, 1991).

No fungi grew at 40°C. None of the Ascomycetes and only the W4 strain of shiitake was able to grow at 5°C. Generally speaking the other Basidiomycetes were able to grow very slowly at 5°C.

The optimum pH ranges, respectively for growth of *G. platystoma*, *E. spinosa* and *L. edodes* strains VT17, U8, and WG were 6.5-7.0, 5.0-7.0, 3.5-4.5, and 4.5-5.0, but for strain WG too much variation was seen therefore a pH was not determined (Guevara & Stipes, 1991) (Fig. 16). The final pH of all culture filtrates in the treatments was measured after the incubation period, and in all cases there were decreases.

DISCUSSION

G. platystoma was found to be the major "weed fungus" in shiitake logs in Virginia (Guevara & Stipes, 1990). This fungus strongly resembles *Diatrype stigma* and was (and is currently) believed by some mycologists and plant pathologists to be *D. stigma*. It also resembles macroscopically *Hypoxylon* spp., but the latter differs in ascospore shape. *G. platystoma* is a member of the family Xylariaceae in the Ascomycetes. Its anamorphic state belongs to the genus *Nodulisporium* Preuss (some *Hypoxylon* spp. have the same imperfect state)(Pirozynski, 1974). Typical conidiophores of *Nodulisporium* are difficult to see, but sapwood samples from pin oak logs presenting stromata of *G. platystoma* and cultured on GYEA, developed abundant conidiophores on the surface of the wood. This fungus has been found on dead trunks and branches of *Acer*, *Quercus*, *Hamamelis*, *Ostrya*, *Persea*, *Tilia*, and possibly *Fagus*, *Lithocarpus*, *Populus*, and *Pyrus* in temperate North America east of the Rockies (Pirozynski, 1974).

E. spinosa was found to be the second most prevalent (important) "weed fungus" in

shiitake logs. Macroscopically, the fungus differs from *G. platystoma* in the morphology of the entostroma which is rougher in the first case and smooth in the second.

Stereum aff. *complicatum* was another common "weed fungus", and the role of this wood decomposer along with other fungi observed in lesser frequencies, was not studied, e.g. *Stereum*, *Schizophyllum*, *Hymenochaete*, *Poria*, *Phellinus*, *Lenzites*, and *Hypoxylon*. These wood-decomposing fungi might be a serious problem if stringent cultural practices (sanitation) are not implemented.

Some of the species reported to cause serious losses to shiitake growers in Japan include *Hypoxylon coccineum*, *H. truncatum*, *Fusicoccum quercinum*, *Trametes sanguinea*, *Schizophyllum commune*, *Poria vaporia* and many other wood decomposers (Royse, et al. 1985; Ishikawa, et al. 1980; Abe, 1989; Kawamura, et al. 1980).

Infection of living oak trees by white-rot and brown-rot fungi was studied by Berry and Lombard (1978). The most frequently isolated species reported by them in order of frequency were *Stereum frustulatum*, *Inonotus andersonii*, *Polyporus compactus*, *S. gausapatum*, *Laetiporus sulphureus*, *Phlebia chrysocrea*, *Spongipellis pachyodon*, *Poria oleracea*, *Hericium erinaceus*, *P. cocos*, and *Tyromyces spraguei*. In scarlet oak they reported infections by the white-rot fungi, *S. frustulatum*, *Inonotus andersonii*, *Polyporus compactus*, *S. gausapatum*, *Phlebia chrysocrea*, *Hymenochaete rubiginosa*, *S. subpileatum*, *C. versicolor*, among others.

With few exceptions, oak infection requires a wound for the entry of decay fungi. Oaks are relatively intolerant of shade, and when they grow with beech, large branches may be overshadowed and killed by the under the canopy. Lignicolous fungi can colonize these dead branches, and some can enter the stem from the branch (Murray, 1974).

Reports by Fenn (1988) and Bassett & Fenn (1984), have shown that *Hypoxylon*

atropunctatum, which is a competitor of shiitake in logs, asymptomatically infects living oaks and have isolated the fungus in high percentages from living green leaves, sapwood and/or bark tissue of putatively healthy trees. When these oaks become drought stressed, *H. atropunctatum* appears to become highly aggressive, colonizing even more tissue and finally fruits in extensive stromata on the bark tissue. The life cycle of the fungus in oak is poorly if at all understood, and its portal of entrance into the living oak system is unknown precisely.

Stipes (1961) found several species of *Hypoxylon* on oaks dying from oak wilt in West Virginia but did not attempt to isolate them from healthy trees. Dr. Jack D. Rogers (personal communication to Dr. Stipes) suggested that a considerable number of these rotters might asymptomatically infect living, putatively healthy, oak trees. His suggestions encouraged us to survey without success for the shiitake log "weed fungi" living endophytically in living stressed trees.

On synthetic logs, the major "weed molds" belong to the genus *Trichoderma* (Royse *et al.* 1985; Yoshimichi, 1982; Doi, 1982; Huang, 1988; Badham, 1991; Tokimoto, 1979). Other less common molds on synthetic shiitake logs are *Mycelophthora*, *Rhizopus*, *Penicillium*, *Monilia*, *Aspergillus*, (*Aspergillus flavus*) *Botrytis cinerea*, *Fusarium graminearum* = *Gibberella zeae* (Huang et al. 1988; Brodziak, 1980).

About 150 species of problem fungi can affect the log, mycelium or mushroom during shiitake cultivation (Longhou, 1981 in Przybylowicz & Donoghue 1988). They divided these problem fungi into 3 categories based on the degree of damage inflicted:

1. Disease fungi. They are antagonistic to shiitake mycelium, by secretion of antifungal compounds, which inhibit growth, and can parasitize and kill shiitake hyphae, e.g. *Trichoderma*, *Gliocladium*, *Hypoxylon truncatum*, *Diatrype stigma*, *Cephalosporium* spp.,

Phialophora lignicola, and *Stemonitis* spp. Miller & Baldwin (1990) reported *Trichoderma* sp., *Mycelia*, *Sterilia*, *Botrytis* sp., *Mucor* sp. and *Chrysosporium* sp. on shiitake fruiting bodies in Virginia.

2. Competitor fungi. Most are Basidiomycetes and do not parasitize shiitake hyphae but merely occupy space in the logs. They reduce the amount of wood that can be used to produce mushrooms, e.g. *Polyporus versicolor* (= *Trametes versicolor*), *Stereum* spp. *Schizophyllum commune*, *Lenzites betulina*, *Poria* spp., *Phlebia*, *Phellinus*, *Polystictus*, and others.

3. Weed fungi. These include Ascomycetes and Basidiomycetes. They are rarely antagonistic and do not adversely affect shiitake mycelium. These fungi usually are on the logs before bringing them in from the woods, e.g. *Bulgaria inguinans* (Ascomycete), and *Mycena* spp. (Basidiomycete) (Przybylowicz & Donoghue, 1988).

The assay for resident endophytic fungal populations in stressed and non-stressed scarlet oak strongly supports our hypothesis that cull (stressed), understory trees or those on poor soils in wood lots commonly used by shiitake growers are poor choices for shiitake production in logs because they may contain aggressive competitors present inside the tree before cutting. Although the main "weed fungi" in shiitake logs were not found inside living trees, other wood decomposers (*Paecilomyces variotii* mainly) were found in this assay, and because "metabiotic succession" normally occurs in trees, that is "migratory waves" of various taxonomic groups during the decomposing process, we strongly suspect that the fungi we found were "precursors" or "forerunners" of the "weed fungi". This, of course, is yet to be proven.

The mode of establishment of most heart-rot fungi must be through wounds. About 40% of decay in oak is caused by fire injury infection (Rayner & Boddy, 1988).

Paecilomyces variotii has been reported in trunks and rots from white oak and red oak after mechanical and fire wounding (Shigo, 1972). It is a heart rot fungus present in standing trees (Rayner & Boddy, 1988).

A scheme was proposed by Rayner (1986) (in Rayner & Boddy, 1988) based on the concept that distribution of water and its reciprocal relation with aeration are primary determinants of colonizing patterns either directly or through their effect on living wood cells. According to this scheme, distinctive patterns are recognized; heart rot, active pathogenesis, specialized opportunism, and desiccation tolerance; these four patterns involve a kind of tolerance or overcoming of selective stress conditions including unfavorable aeration, nutrient deficiency, allelopathic chemicals; and the remaining category, unspecialized opportunism, depends on enrichment disturbance and consequent alleviation of stress conditions.

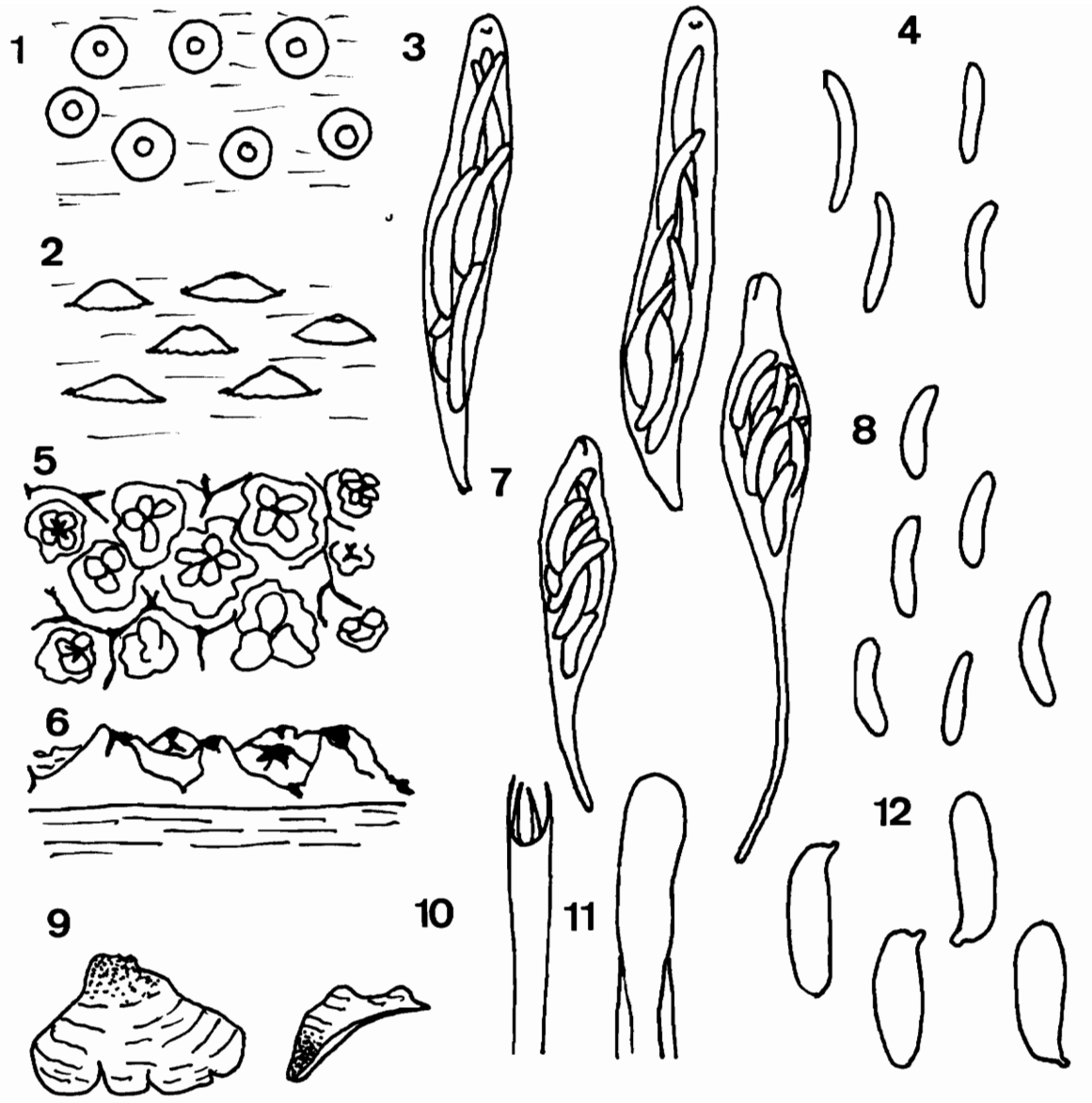
In other studies (Chapter 2) we found that the main "weed fungi" were present on the bark and were probably waiting for good conditions, e.g. drought stressed in tree to enter the tree and complete their cycle.

From these data we conclude that tree selection (from non-stressed communities) might improve shiitake production by preventing or minimizing colonization by wood decaying fungi, so that *L. edodes* would have minimal competition during its colonization period.

The temperature and pH optima for *in vitro* growth of these "weed fungi" indicated that these decomposers do not grow at low temperatures (at 5 °C) or grow very little (at 10 to 15 ° C), whereas *L. edodes* grows better at the same temperatures. Some strains of shiitake have been reported to grow better at 35 °C; a pH of 3.5; glucose, mannose, maltose, and starch as the best carbon sources; and yeast, meat extracts, peptone, and maize

flour as the best sources of nitrogen (Brodziak, 1980; Royse et al. 1990).

The data indicated that shiitake inoculation would be better done during late winter or early spring when the temperatures are neither too low nor too high, more suitable for growth of shiitake, and less suitable for growth of many "weed fungi". Also, the bark is secured more tightly on the trees at this time. The pH *in vitro* test indicated that these main "weed fungi" grow better at high pH (6.5-7.0 for *G. platystoma* and 5.0-7.0 for *E. spinosa*), while *L. edodes* grows better in lower pH medium (3.5-4.5). These findings suggest that shiitake is an acidophilic fungus and therefore likely grow better in oak species with highly acidic sapwood. In addition to this, tree selection (non-stressed versus stressed trees) would improve shiitake production outdoors. Methods for determination of pH in wood are given by Kubinsky & Ifju (1973), Stamm (1961), and McNamar et al. 1970).



Figures. 1-12. Morphology of *Graphostroma platystoma, Eutypa spinosa* and *Stereum complicatum*. Figs. 1-4. *Graphostroma platystoma*. Perithecial ostioles on stroma surface, 2. Side view of protruding necks with ostioles, 3. Asci, and 4. Ascospores. Figs. 5-8. *Eutypa spinosa*. 5. Perithecial ostioles on stromal surface, 6. Side view of protruding perithecial necks with ostioles, 7. Asci, and Ascospores. Figs. 9-12. *Stereum complicatum*. 9. Basidiocarp, 10. Basidium, 11. Pleurocystidium, and 12. Basidiospores.

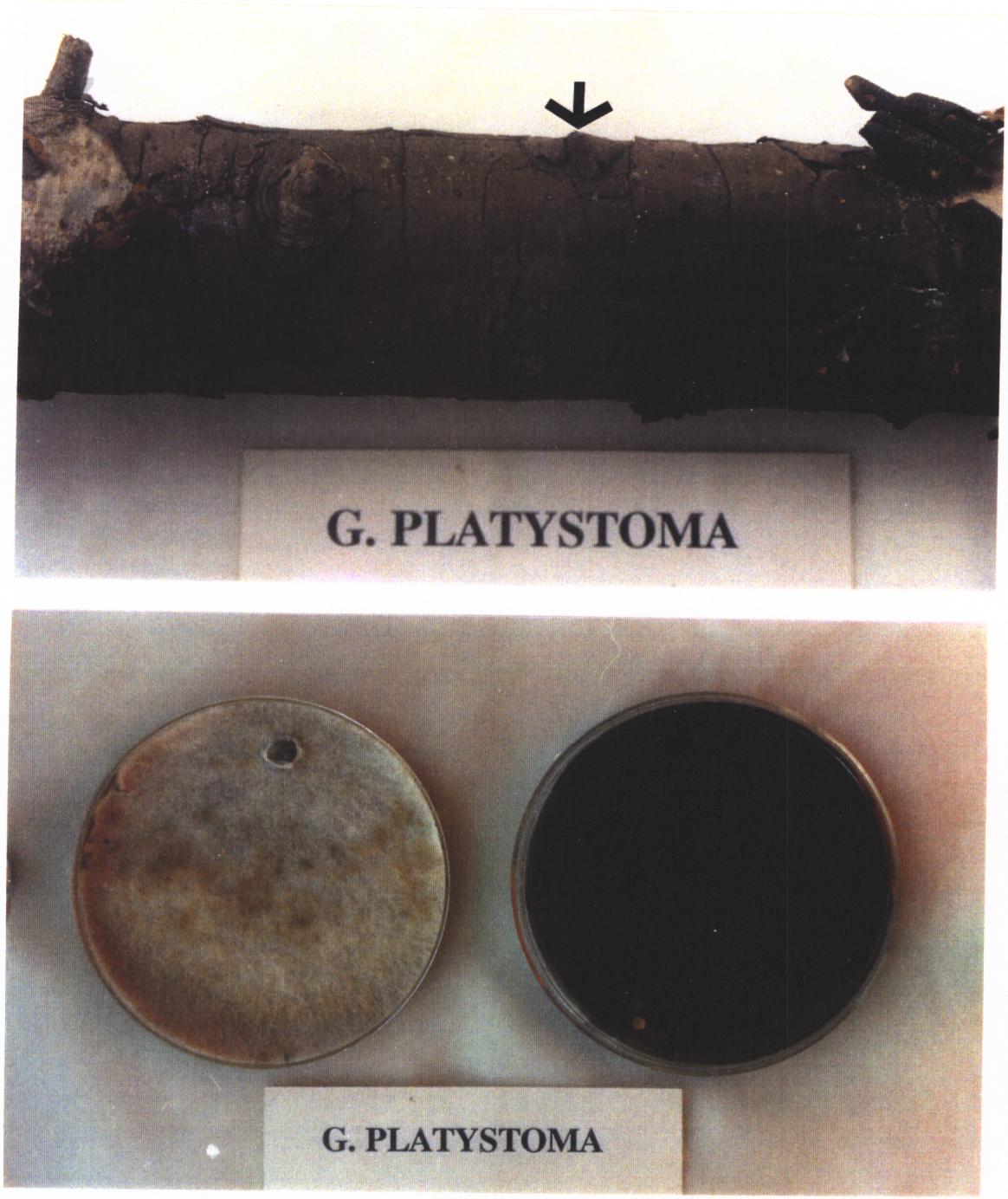


Figure 13. *Graphostroma platystoma*. Upper picture shows stroma, deciduous ectostroma (arrow), and entostroma cracked transversely. Lower picture shows the obverse and reverse of pure culture growing on PDA.

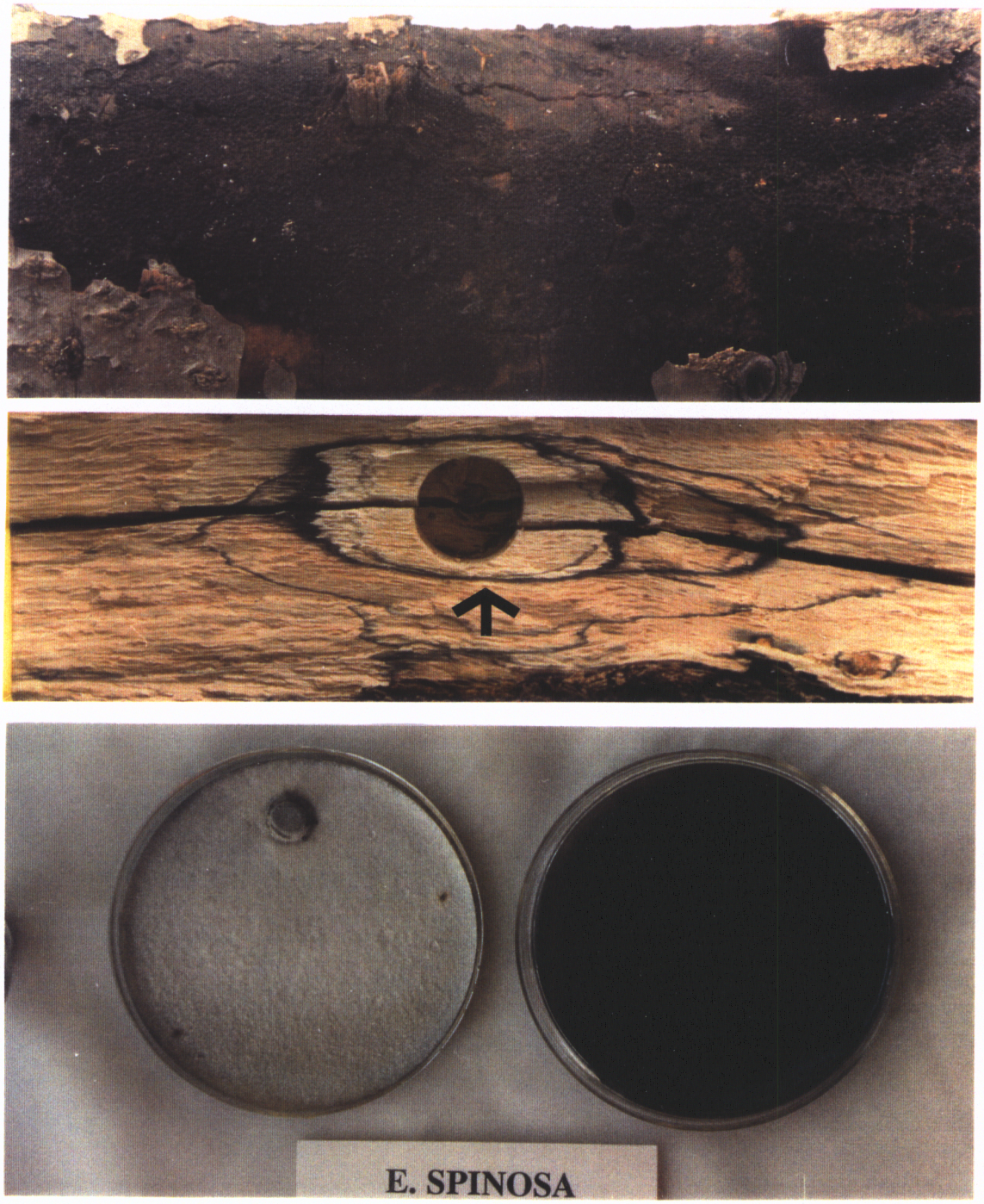


Figure 14. *Eutypa spinosa*. Upper picture shows rough stroma. Middle picture shows "pseudosclerotia" formation (arrow) around shiitake hole. Lower picture shows the obverse and reverse of pure culture growing on PDA.

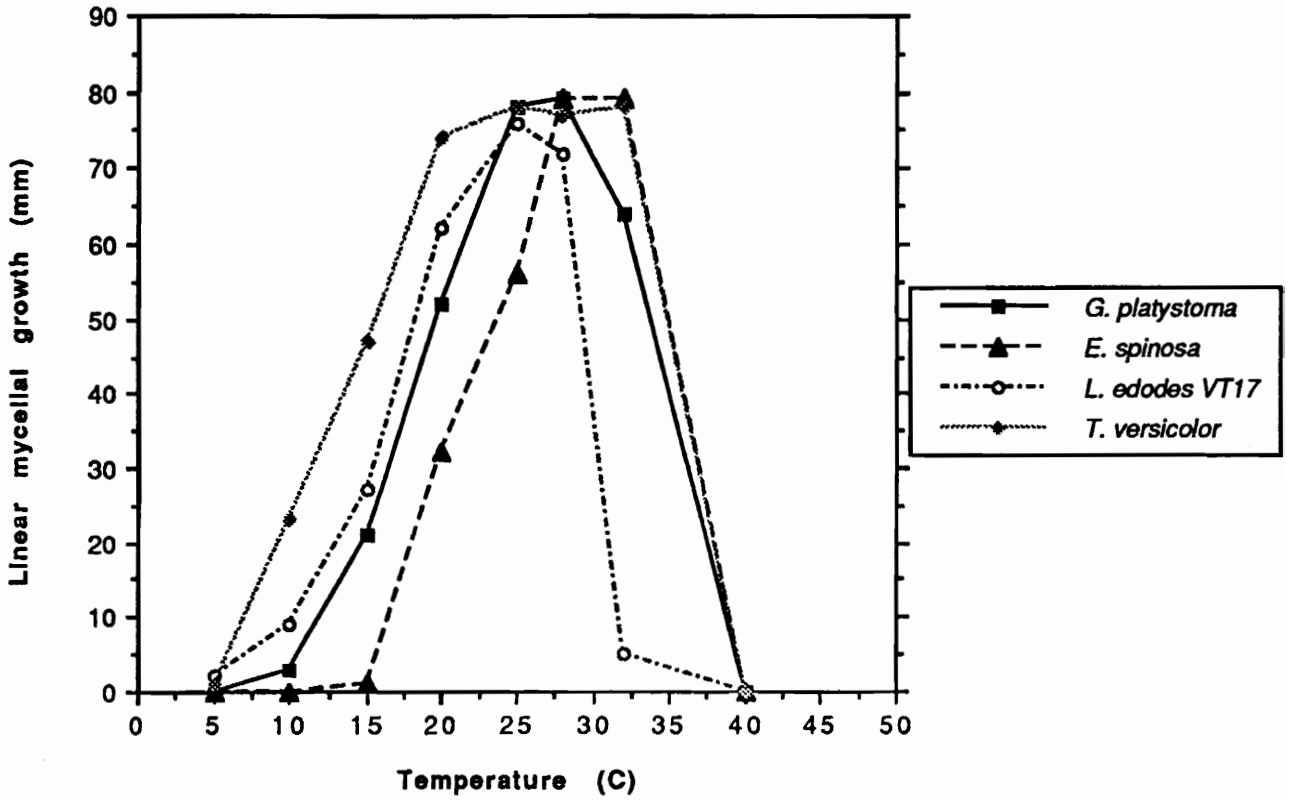


FIG. 15. Effect of temperature on mycelial growth of *G. platystoma*, *E. spinosa*, *L. edodes* VT17, and *T. versicolor*.

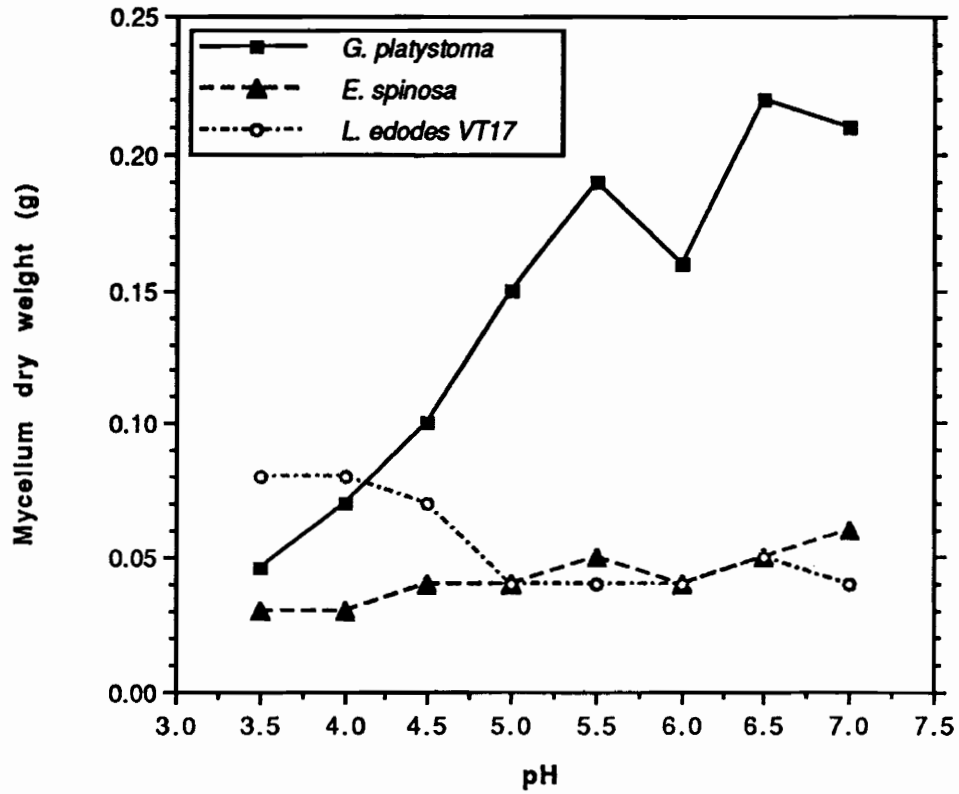


FIG. 16. Effect of pH on mycelial growth of *Graphostroma platystoma*, *Eutypa spinosa*, *Lentinus edodes* VT17 in glucose-yeast extract wood decoction medium.

TABLE 1. Resident endophytic fungal populations in stressed and non-stressed scarlet oak (*Quercus coccinea*) in the Jefferson National Forest at Blacksburg, Virginia.

Location	No. Samples	Sterile (%)	Colonized by fungi (%)
Brush Mountain (stressed)	23	0	100*
Poverty Creek (non-stressed)	26	50	50**

* 74% yielded *Paecilomyces variotii* and 26% by other fungi.

** 23% yielded *Paecilomyces variotti* and 27% by other unknown fungi.

TABLE 2. Resident endophytic fungal populations in non-stressed, 17-yr-old pin oak (*Quercus palustris*) at Virginia Polytechnic Institute & State University.

Location	No. Samples	Sterile (%)	Colonized by fungi (%)
Pin oak plot at VPI & SU	30	74	26*

* 16% yielded an unknown yeast, and 10% by other fungi including Basidiomycetes.
Paecilomyces variotii was not seen in this survey.

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CHAPTER 2. SUPPRESSIVE POTENTIAL OF FUNGICIDES TO THE "WEED FUNGI" (*Graphostroma platystoma, Eutypa spinosa*) AND *Lentinus edodes*, AND COMPETITIVE POTENTIAL OF "WEED FUNGI" TO *L. edodes* IN INOCULATED OAK LOGS

ABSTRACT

Shiitake growers in Virginia are experiencing considerable losses of the fruiting life of oak logs primarily due to Ascomycetous "weed fungi" present in tree tissues before felling. Several shiitake farms were surveyed and the predominant colonizing fungi were identified as *Graphostroma platystoma* and *Eutypa spinosa*. The fungitoxicity tests showed that these "weed fungi" are sensitive to Arbotect 20S and Lignasan BLP fungicides at acceptably low concentrations while *L. edodes* (shiitake) strain VT17 was not. *G. platystoma* was inhibited at 10-1000 µg/ml by both fungicides, and *E. spinosa* was inhibited at 1.0-100-µg/ml by both fungicides while *L. edodes* strain VT17 was not. Sensitivity was determined by mycelial growth inhibition *in vitro* and *in situ* (in logs). These findings suggest that shiitake farmers might be able to control "weed fungi" by soaking their logs in these fungicides previous to inoculation with shiitake spawn while doing no harm to *L. edodes*. If these fungicides are to be labeled for this use, residue analysis on mushrooms from treated logs must be done to establish tolerance limits.

INTRODUCTION

Graphostroma platystoma (Schw.: Fr.), and *Eutypa spinosa* (Pers.: Fr.)Tul. are the major wood decaying fungi ("weed fungi") responsible for economic losses in the production of shiitake in natural logs in Virginia (Guevara et al. 1989; Guevara & Stipes 1991).

After visiting, observing, and collecting mycological material at several shiitake farms, at least 3 fungitoxicity tests were performed *in vitro* against these "weed fungi". This was done to determine a good fungicide and concentration that could be used to treat the logs before inoculation with shiitake spawn. Among other fungicides, Lignasan BLP (benomyl derivative) and Arbotect 20S (thiabendazole) were used in this experiment.

In previous studies, methyl bromide fumigation prior to inoculation has been shown to increase the colonization rate of natural logs by shiitake. The rate of growth was affected by elimination of competitive organisms or by modification of the substrate (e.g. cellulose methylation) that appeared to be more effective in northern red (*Quercus rubra* L.) than bur oak (*Quercus macrocarpa* Michx.) logs (Schmidt, 1984 in Royse, 1989). Heat and chemical treatments may alter the chemical structure (lignin, cellulose and hemicellulose) of the wood (Royse, 1989). An increased mycelial growth rate was found in natural logs sterilized with ethylene oxide when compared with non-sterilized logs (Tokimoto & Komatsu 1978).

The use of Benlate 50 DF (benomyl fungicide) on synthetic logs has been reported by Delpech & Oliver (1990) and Przybylowicz & Donoghue (1988) for the control of *Trichoderma* spp. Benlate 50 DF is used to protect the substrate by first mixing and heating the sawdust, wheat bran, and calcium carbonate at 100°C for 24-48 hr and then mixing with water containing 300 mg of Benlate 50 DF per liter (active ingredient 50%)(Kalberer, 1989).

Determination of fungicide residues in shiitake fruiting bodies from synthetic logs has also been reported by Delpech & Oliver (1990) and Kalberer (1989). In the first case, the investigators reported about 0.05 mg of BCM (carbendazim)/kg (substrate treated with 75 µg/ml of benomyl). In the second case, 0.2 mg or less of benomyl/kg of fruiting bodies was found in cultures treated with 300 mg of Benlate C/l water.

The use of Bavistin 20% (carbendazim) and Topsin-M 70% (thiophanate-methyl) in the medium at a dosage of 0.1-0.5% significantly controlled incidence of *Trichoderma* (Huang et al. 1988). Contamination of sawdust by *Trichoderma* spp. was effectively controlled by Penmush (=Y101) and Tecto-60 (thiabendazole) at 200 μg/ml without affecting cultivation of *L. edodes* (Liao, 1985). Benomyl 50 DF has also been used to control competitor molds in the production of the edible mushrooms *Pleurotus sajor-caju* and *Agaricus bisporus* (Peak, 1972 in Royse & Schisler 1987; Jhune et al. 1990). The fungicide, thiophanate-methyl (TPM), is known to exibit strong antifungal activity against many plant pathogenic fungi after conversion to methyl 2-benzimidazole carbamate (MBC). The addition of TPM before steam-sterilization into a shiitake sawdust medium prevents mycoparasitic infection; TPM was found to be completely converted into MBC by heating it in water for 1 hr (Tabata et al. 1982). In earlier studies, Tabata & Kondo (1977a) reported the use of 2-phenylbenzimidazole, methyl 2-benzimidazole carbamate hydrochloride (MBC.HCL), and TPM for control of parasites on shiitake mushrooms. MBC.HCL showed significant activity.

In another study, Tabata & Kondo (1977b) studied the relationship between chemical structure and selective antifungal activity by synthesizing several substituted benzimidazole derivatives, finding that unsubstituted MBC exhibited a strong inhibitory activity against shiitake parasites while 5-nitro-substituted MBC showed entirely no activity. Among others, 2-aryl-and 2-alky-substituted benzimidazole types, 2-(2-furyl)-benzimidazole and 2-(0-chlorophenyl) benzimidazole, exhibited significant selective antifungal activity. When the aryl group was substituted by phenyl, compounds having an o-methyl, o-carboxyl, o-amino, and o-hydroxyl group, in comparison with an o-chloro substituent, the compound showed a marked decrease in the antifungal activity on mycoparasites. Antifungal activity

disappeared completely when the benzimidazole ring of the phenylbenzimidazole was changed to benzoxazole or benzthiazole rings. S-octylisothiouronium chloride and S-alkyl chain length of S-alkyllisothiouronium salt were found to be effective against shiitake parasites (*Trichoderma* spp., perfect stage: *Hypocrea* spp.) Tabata & Kondo (1976a) (1976b). Mycelial growth of *Trichoderma harzianum* (a common pathogen of shiitake) was reduced greatly by using bark extract from oak (Yoshimoto et al. 1984).

Preliminary results for a chemical control of the main "weed fungi", *G. platystoma* and *E. spinosa*, in shiitake natural logs in Virginia were reported by Guevara et al. (1989) and Guevara & Stipes (1991).

MATERIALS AND METHODS

Several fungitoxicity tests were performed *in vitro* to determine the sensitivity of previously isolated and identified "weed fungi" G. *platystoma*, *E. spinosa*, *S. complicatum* verses *L. edodes* strain VT17=S1 (from Korea via Elix Corporation, Arvonia, VA). Some of the fungicides used were Arbotect 20S (thiabendazole hypophosphite 20% a.i. w/v) and Lignasan BLP (methyl 2-benzimidazolecarbamate phosphate, 0.7% a.i. w/v) at decreasing log concentrations. Linear growth of mycelium was measured at appropriate time intervals at the incubation temperature of 28°C.

In vitro standard agar amendment techniques were used at known concentrations into Difco potato-dextrose agar (DPDA). In May 1988, the first fungitoxicity test was performed against *G. platystoma* at 10,000, 1000, 100, 10, 1.0, 0.01, 0.001 μg/ml.

Three replicate petri dishes with medium were used. Small pieces of agar with pure cultures of *G. platystoma* were transferred to the edge of agar in petri dishes, which were incubated at 26°C in darkness. Mycelial growth was recorded at 2-day intervals. This

procedure was also used for Arbotect 20S Lignasan BLP, copper sulfate, Borax, and sodium chloride for control of *E. spinosa*, *G. platystoma*, *S. complicatum* versus *L. edodes*.

The fungitoxicity tests *in situ* (in logs) were begun after fungitoxicity tests *in vitro* were complete. On March 6, 1991, 100 pin oak logs (*Q. palustris*) about 40 cm long and 4.5-10 cm in diam. were selected for these experiments, the objectives were (1) to monitor the competitive potential *in situ* of "weed fungi" versus *L. edodes* strain VT17 (=S1), and (2) to evaluate the effectiveness of the fungicide Arbotect 20S (1000 µg/ml) in preventing colonization by "weed fungi". The presence of fungal structures (e.g. stromata, pseudosclerotia) in wood and bark of treated logs were noted after six mo of incubation. The main three treatments were a) dipping cut ends of logs only, b) immersing the entire log in fungicide, and c) no fungicide treatment as control. On March 8, 1991, the following nine experiments were set up in the laboratory.

Experiment 1. A 10-log set was inoculated at one end of each log with *G. platystoma* alone. Experiment 2. A 10-log set was inoculated with *G. platystoma* at one end and *L. edodes* at the other cut end of each log. Experiment 3. A 10-log set was inoculated at the end of each log with *L. edodes* only. Experiment 4. A 10-log set was inoculated with *L. edodes* at one end, and *E. spinosa* at the other end of each log. Experiment 5. A 10-log set was inoculated at one end of each log with *E. spinosa* only. Experiment 6. A 10-log set in which both cut ends were soaked for 3 min in a concentration of 1000 µg/ml of the fungicide Arbotect 20S. Treated logs were not inoculated with "weed fungi" and shiitake. Experiment 7. A 10-log set was immersed for 3 min in Arbotect 20S at 1000 µg/ml. Treated logs were not inoculated with "weed fungi" and shiitake. Experiment 8. A 10-log set was immersed completely in the Arbotect 20S solution and then inoculated with *L. edodes* to observe mushroom production (not shown in tables). The soaking time was 3 min

in a concentration of 1000 µg/ml. Experiment 9. A 10-log set (control logs) was neither treated with fungicide solution nor inoculated with "weed fungi" and shiitake. Control logs were placed in moisture chamber for 6 mo.

Logs were inoculated by making about 5-9 holes at the ends of the logs with a high speed drill. A small piece of spawn was pressed into each hole by hand and covered with parafilm or plugged with foam plugs. All of the logs were numerically labeled. Diameter of logs was recorded as well. Holes for the "weed fungi" were just deep enough to reach the cambial tissue: for shiitake they were just deep enough to reach the sapwood. After inoculation logs were put in a moisture chamber and incubated at 25° to 29°C in darkness.

After 6 mo, samples from sapwood of the logs of all the treatments were removed aseptically and placed on synthetic medium for fungal growth in glucose yeast-extract agar (GYEA). The petri plates were incubated at 25°C in darkness and observed every 3 days for 1 mo.

RESULTS

The *in vitro* fungitoxicity tests showed that the two major "weed fungi", *Graphostroma platystoma* and *Eutypa spinosa*, were sensitive to Arbotect 20 S and Lignasan BLP at relatively low concentrations, while *L. edodes* (shiitake) strain VT17 was not inhibited at any concentrations with either fungicide (Figs. 1-4, & Tables 1-5). Statistical analysis (ANOVA, $\alpha = 0.05$) showed that the effects of these fungicides differed significantly among treatments and species of fungi. Media with Arbotect 20S and Lignasan BLP at 10,000 µg/ml failed to gel, so it could be used.

Data in Table 1 shows that after 16 days of incubation, *G. platystoma* was inhibited (zero growth) by both fungicides at 1000, 100, and 10 µg/ml. Mycelium in control plates

completely covered the petri dish as well as dishes at concentrations 0.01 and 0.001 µg/ml in both fungicides. At 0.1 µg/ml, this fungus developed about 57 mm on both fungicides. *G. platystoma* grew up about 52 mm (average) in Arbotect 20 S at 1 µg/ml, and about 57 mm (average) at the same concentration in Lignasan BLP. Copper sulfate essentially stopped growth of *G. platystoma* at 1000 µg/ml, and borax at possibly some point between 1000 and 10,000 µg/ml. Sodium chloride was not effective even at 10,000 µg/ml.

Based upon *in vitro* tests, the fungicide Arbotect 20S (and concentration 1000 µg/ml) was selected to observe the effectiveness of this fungitoxicant *in situ*. On the other hand, *E. spinosa* was completely inhibited by both fungicides at 1000, 100, 10, and 1.0 µg/ml and covered petri dishes at concentrations 0.01 and 0.001 µg/ml. This fungus grew about 54 mm in Arbotect at 0.1 µg/ml, and 67 cm in Lignasan BLP at the same concentration. The fungus covered the control plates (Table 2).

Basidiomycete isolates were very tolerant of both fungicides *in vitro*. *L. edodes* (shiitake) strain VT17 was not inhibited at any concentration but grew slowly at high concentrations compared with the control plates and the other concentrations. Table 3 shows the results of this test after 16 days of incubation. Shiitake grew about 2 mm at 1000 µg/ml in Arbotect 20S and about 29 mm at the same concentration in Lignasan BLP. In contrast, the control dishes were completely covered. At 100 µg/ml, shiitake grew about 41 mm in Arbotect 20S, and 51 mm in Lignasan at same concentration. At 10, 1.0, 0.1, 0.01, 0.001, and 0.0 µg/ml concentrations, shiitake practically grew at the same rate. *Stereum* aff. *complicatum* was insensitive to Arbotect 20S, Lignasan BLP, copper sulfate, and borax at concentrations up to 10,000 µg/ml (Table 4).

Data in Table 5 show that many wood-inhabiting fungi can be controlled with Arbotect 20S *in situ*. The fungicide Arbotect C 20S in logs that had been dipped clearly

indicates that most "weed fungi" were controlled when compared with control logs and with logs inoculated with "weed fungi" and shiitake. However 100% of such logs were colonized by unknown Basidiomycetes after 6 mo of incubation when isolates were attempted from sapwood samples cultured in media agar. Logs dipped in fungicide solution and inoculated with shiitake, produced mushroom after 6 mo of incubation. *Trichoderma* spp. were seen on the bark but were displaced by *L. edodes*.

Sapwood samples cultured on media from logs inoculated with shiitake strain VT17, showed that 60% of the logs were colonized by *Graphostroma* and *Hypoxylon*, and 40% by *Gliocladium* spp. *Trichoderma* and *Sporothrix* were also seen growing along with these fungi about 80% and 10% respectively. The external appearance of these logs (the presence of fruiting bodies or other mycological structures on the bark) presented the formation of stromata in a 100% of the logs after 6 mo of incubation. The internal appearance of these logs (e.g. presence of pseudosclerotia, white or brown rot formation in the sapwood) showed the formation of pseudosclerotia in 100% of the logs. In many cases, these pseudosclerotia were seen surrounding the shiitake holes, therefore limiting its growth.

Treatment of cut ends of logs was less effective than dipping the logs but was better than no treatments. About 20% of isolations from these logs produced colonies of *Graphostroma* and *Hypoxylon*, 50% yielded Basidiomycetes, and 30% Deuteromycetes. *Gliocladium* was seen also in 10%, and *Helminthosporium* spp. in 20% of them. Surfaces of 30% of these treated logs produced stromata, and pseudosclerotia were seen in sapwood 10%. All sapwood from logs immersed in Arbotect 20 S produced colonies of Basidiomycetes, some colonies of *Penicillium* spp. also developed with these Basidiomycetes in 40% of the samples. About 20% of these logs showed some kind of stromata in the bark, and pseudosclerotia.

The results of the competitive potential in logs that had been inoculated with a "weed fungus" and shiitake after 6 mo of incubation are given in Table 6. About 20% of the wood samples from logs inoculated with *L. edodes* versus *E. spinosa* yielded *Graphostroma* and *Hypoxylon*, 70% produced *Trichoderma* spp., and 10% produced other Deuteromycetes. About 10% yielded *Gliocladium* spp. growing together with these fungi. About 10% of these logs produced stromata, and 70% of the logs showed the presence of pseudosclerotia in the sapwood. In most of the logs, these structures were seen growing around the shiitake holes.

Seventy of sapwood samples cultured from logs inoculated with *E. spinosa* yielded colonies of *Graphostroma* and *Hypoxylon*, 20% *Paecilomyces*, and 10% by other unknown Deuteromycetes. In addition, *Gliocladium* and *Trichoderma* were observed growing together with these fungi in 30% and 60% of the logs respectively. Formation of fruiting bodies (stromata) by *E. spinosa* on the bark was seen in 90% of the logs, and 100% of the logs showed the formation of pseudosclerotia (typical of *E. spinosa*) in the sapwood. Samples from logs inoculated with *G. platystoma* produced colonies of *Graphostroma* and *Hypoxylon* in 70% of the logs, *E. spinosa* in 10%, and other unknown Deuteromycetes in 20%. About 70% of the logs yielded *Trichoderma* spp., and 10% of the logs yield *Graphium* growing along with these fungi. About 90% of the logs yielded stromata, and 100% produced pseudoslerotia in the sapwood. About 30% from logs inoculated with *L. edodes* and *G. platystoma* yielded colonies of *Graphostroma* and *Hypoxylon*, 10% Basidiomycetes, and 60% *Trichoderma*. *Gliocladium* was also seen in 10%, and 40% of them yielded other fungi. Formation of stromata on the bark was seen in about 30%, and production of pseudosclerotia was seen in all of them. In most cases, pseudosclerotia were seen surrounding the shiitake holes.

Fifty of the sapwood chips cultured from control logs produced colonies of *Graphostroma* and *Hypoxylon*, 40% *Gliocladium*, and 10% other fungi. Furthermore, *Trichoderma* and *Graphium* were also seen in 20% and 10% of them respectively. Stromata were present in the bark in 80% of the logs and pseudosclerotia were seen in a 90% of them.

DISCUSSION

The systemic benzimidazole fungicides are known to be toxic to many Ascomycetes, Deuteromycetes, and some Basidiomycetes (Fry, 1982). These fungicides are mitotic synthesis inhibitors, And include benomyl, imazalil, thiabendazole, carbendazim, thiophanate-ethyl, and thiophanate-methyl (Fry, 1982; Hertel et al. 1980). Arbotect 20S and Lignasan BLP belong to this group of fungicides and were found to be toxic to the main Ascomycetous "weed fungi," *Graphostroma platystoma* and *Eutypa spinosa*, in shiitake logs (*in situ*) and *in vitro* and were nontoxic to shiitake.

The *in vitro* agar fungitoxicity test was performed at least 3 times and some variation in growth was seen when *G. platystoma* was tested against Lignasan BLP and Arbotect 20S at 10 µg/ml after 16 days incubation. Two factors might explain this variation: the fungus might have become resistant to the fungicides, or the fungicides might have broken down faster in the last test since older fungicides were used. Even if the fungus were able to grow at this concentration, it is very unlikely that it would produce infective structures such as ascospores.

During the first 16 days the effectiveness of the fungicides against "weed fungi" was very constant *in vitro*, but *L. edodes* covered the plate completely. Thus shiitake should have an advantage over these "weed fungi" to colonize and get established in the substrate. *E.*

spinosa did not show variation in all of the repetitions. Shiitake was not inhibited at any concentration although at the high concentrations of 1000 µg/ml, the fungus grew slower when compared with growth on control plates with no fungicide and the rest of the concentrations.

Copper is usually toxic to fungi, although species of some genera are remarkably tolerant, e.g., *Aspergillus*, *Penicillium*, *Scytalidium*, and several species of *Phialophora* (Henningsson, 1976). Copper sulfate essentially stopped the growth of *G. platystoma* (it grew 3 mm) at 1000 µg/ml. However it also inhibited shiitake at the same concentration as also did Borax. The growth of both this fungus and shiitake were stopped by NaCl at 100,000 µg/ml. Therefore these fungicides would be unsuitable in "weed fungi" control in shiitake logs.

In the *in situ* test (in logs), Arbotect 20 S killed most "weed fungi" except Basidiomycetes and a *Penicillium* sp. when the logs were soaked for 3 min at 1000 µg/ml. The isolated Basidiomycete showed more than one clamp-connection per septum. Because of this, it is very likely that this fungus belongs to the genus *Stereum* which is a wood-decomposing fungus. *Stereum* is not a natural antagonist of shiitake but will occupy space and reduce the amount of nutrients that can be used by shiitake to produce mushrooms in natural logs. Thus shiitake will compete to get established in the log. Inoculation of shiitake spawn after soaking with Arbotect 20S should help shiitake get established in the log.

Although no "weed fungi" were obtained in culture of sapwood chips from logs soaked in Arbotect 20S, stromata and pseudosclerotia of these weed fungi were seen in 20% of the logs. The same result was obtained when logs were immersed in this fungicide and inoculated with shiitake. About 20% of logs possessed pseudosclerotia after 6 mo of

incubation. This was probably due to the inability of the fungicide to reach or cover the bark completely due to air or bubbles in the bark as well as the soaking time.

In earlier studies, biopsies of standing living trees for "weed fungi" had not included the bark, and these fungi were not present. Logs treated with Arbotect 20S at the cut ends did not exclude these "weed fungi" which were present in the bark and got into the sapwood via bark.

Non-inoculated control logs showed the presence of these "weed fungi", and were not too different from those logs inoculated with *L. edodes*. Since control logs were not inoculated, this suggests that those "weed fungi" were present in the bark *G. platystoma* at least.

Culture of wood samples from logs inoculated with *E. spinosa* did not produce colonies of *E. spinosa* on synthetic medium; instead, *Graphostroma* and *Hypoxylon* were obtained. However, stromata (fruiting bodies) of *E. spinosa* were produced in the bark in 90% of the logs, and the typical pseudosclerotia of this fungus were produced in the sapwood of the all the logs. *E. spinosa* in pure culture grows and produces conidiophores very slowly on a synthetic medium. If this fungus were present with two or more competitors in the same sapwood samples, the most aggressive fungus will be established first, and a selective medium would be needed for *E. spinosa* to be reisolated from wood.

Graphostroma and *Hypoxylon* are more aggressive in colonizing logs than *E. spinosa*. Shiitake, however, competes vigorously against these two fungi.

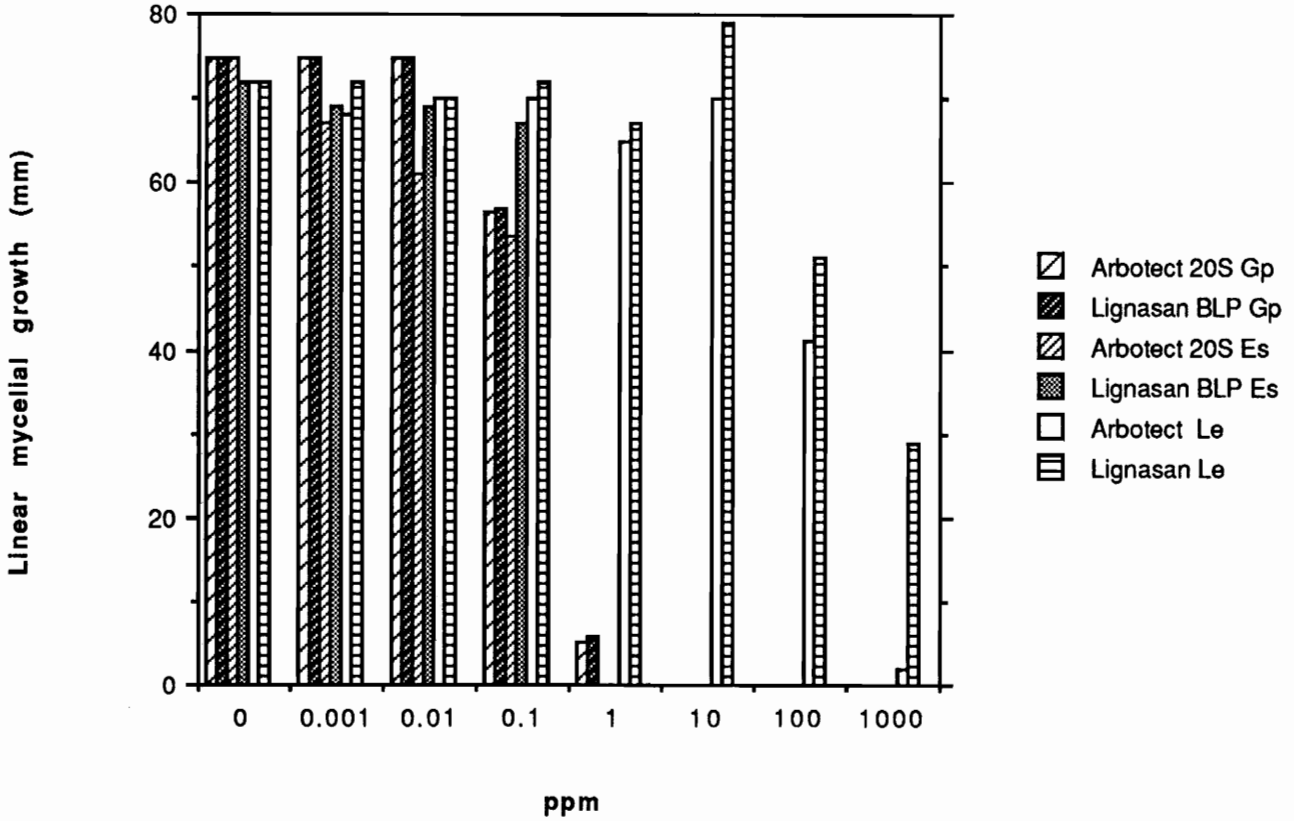

FIG. 1. Sensitivity of *Graphostroma platystoma* (Gp), *Eutypa spinosa* (Es), and *Lentinus edodes* (Le) to Arbotect 20S and Lignasan BLP *in vitro*.

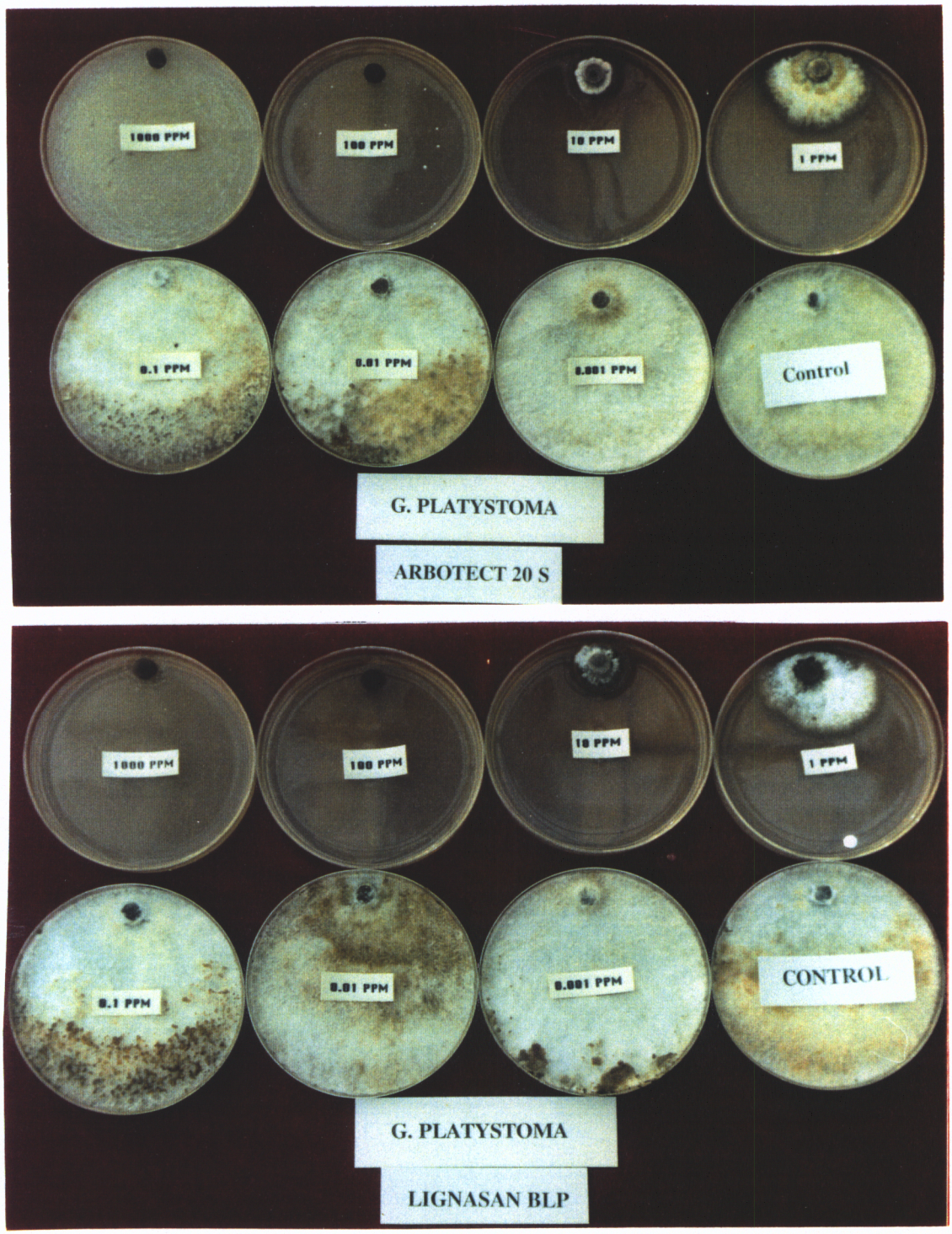

Figure 2. Growth responses of *Graphostroma platystoma* to the fungicides Arbotect 20S (upper picture) and Lignasan BLP (lower picture).

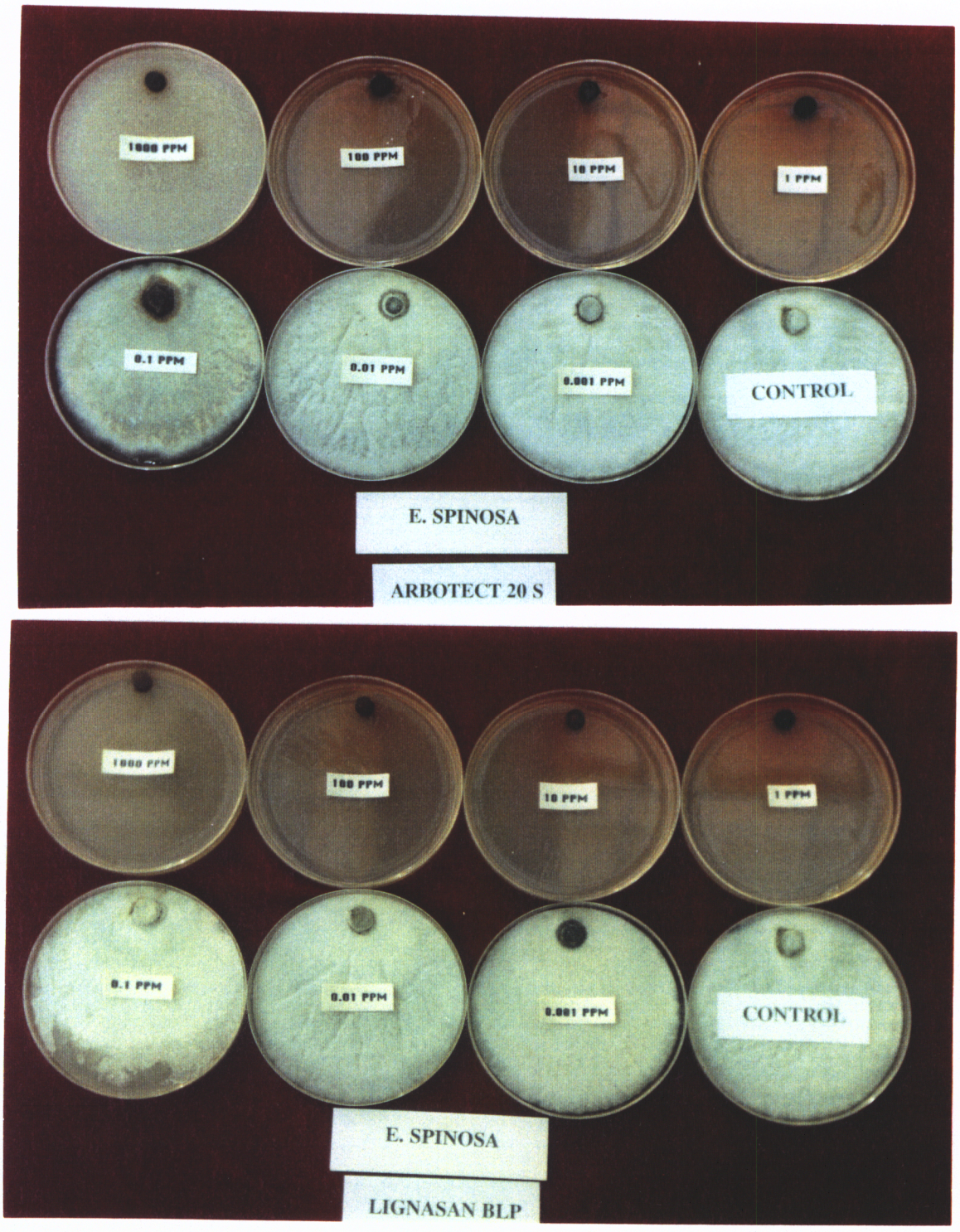

Figure 3. Growth responses of *Eutypa spinosa* to the fungicides Arbotect 20S (upper picture) and Lignasan BLP (lower picture).

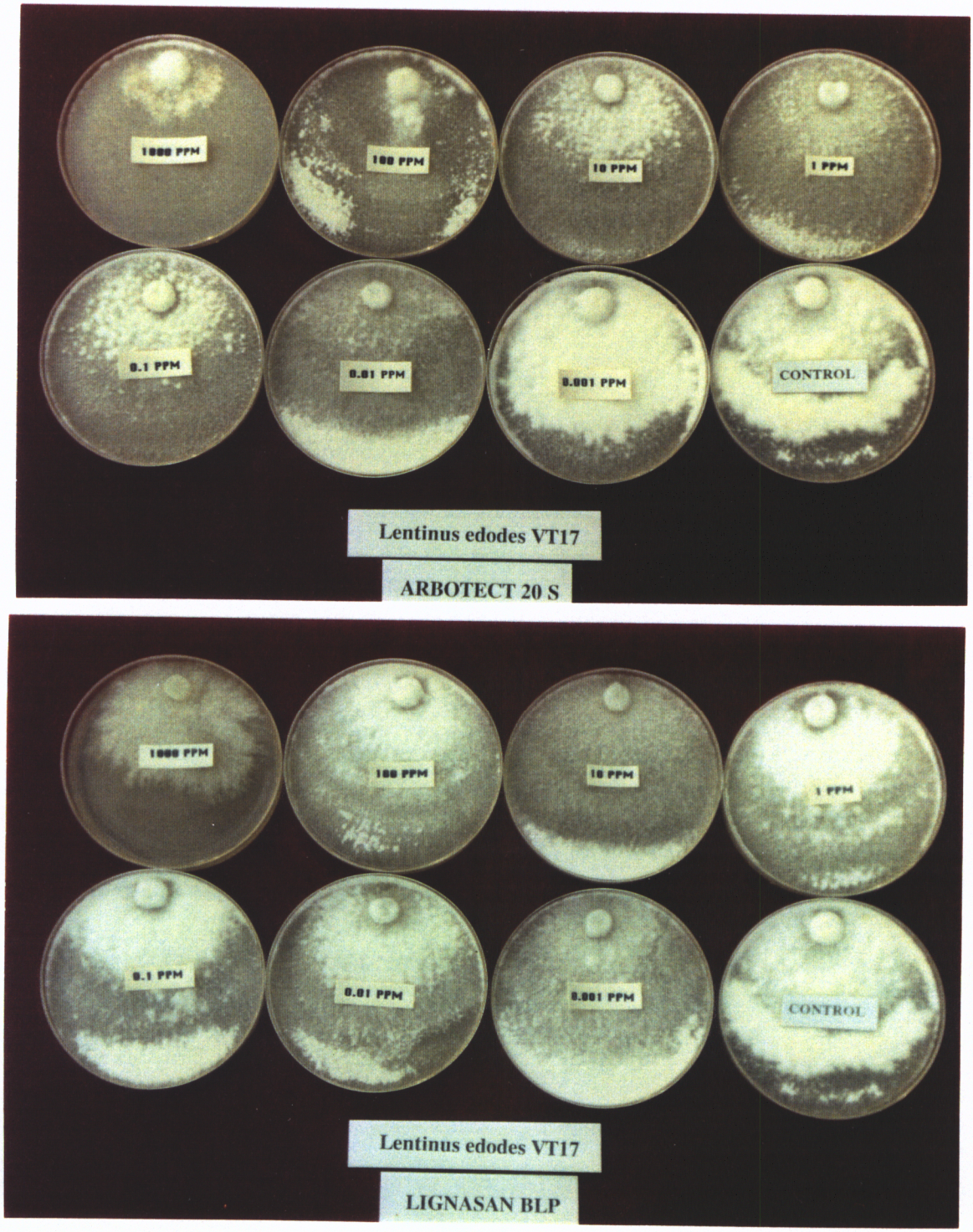

Figure 4. Growth responses of *Lentinus edodes* strain VT17 to the fungicides Arbotect 20S (upper picture) and Lignasan BLP (lower picture).

TABLE 1. Sensitivity of *Graphostroma platystoma* to selected fungicides incorporated in potato dextrose agar.*

	fungicide concentration ppm (µg/ml)							
Fungicides	Ctl.	0.001	0.01	0.1	1.0	10	100	1000
Arbotect 20 S	75	75	75	60	6	0	0	0
Lignasan BLP	75	75	75	57	6	0	0	0
Borax	75	75	75	75	75	75	75	0
$CuSO_4$	75	75	75	75	75	75	75	3
NaCl	75	75	75	75	75	75	75	75

* Mycelial growth in mm after 16 days of incubation at 28° C.

TABLE 2. Sensitivity of *Eutypa spinosa* to selected fungicides incorporated in potato dextrose agar.*

	fungicide concentration ppm (µg/ml)							
Fungicides	Ctl.	0.001	0.01	0.1	1.0	10	100	1000
Arbotect 20 S	75	67	61	54	0	0	0	0
Lignasan BLP	75	64	69	67	0	0	0	0
Borax	75	75	75	75	75	75	75	75
$CuSO_4$	75	75	75	75	75	75	75	74

* Mycelial growth in mm after 16 days of incubation at 28° C.

TABLE 3. Sensitivity of *Lentinus edodes* strain VT17 to selected fungicides incorporated in potato dextrose agar.*

	fungicide concentration ppm (μg/ml)							
Fungicides	Ctl.	0.001	0.01	0.1	1.0	10	100	1000
Arbotect 20 S	68	68	70	70	65	70	41	2
Lignasan BLP	68	72	70	71	67	69	51	29

* Mycelial growth in mm after 16 days of incubation at 28° C.

TABLE 4. Sensitivity of *Stereum* aff. *complicatum* to selected fungicides incorporated in potato dextrose agar.*

	fungicide concentration ppm (µg/ml)							
Fungicides	Ctl.	0.001	0.01	0.1	1.0	10	100	1000
Arbotect 20 S	75	75	75	75	75	75	75	75
Lignasan BLP	75	75	75	75	75	75	75	75
$CuSO_4$	75	75	75	75	75	75	75	75
Borax	75	75	75	75	75	75	75	75

* Mycelial growth in mm after 16 days of incubation at 28° C.

TABLE 5. Suppressive potential of the fungicide Arbotect 20 S at 1000 ppm (μg/ml) to fungal competitors in logs inoculated or non-inoculated with shiitake after 6 mo of incubation at room temperature.

	Control	Logs dipped	Cut ends treated	Shiitake inoculated logs
Fungus ** *Graphostroma platystoma & Hypoxylon* sp.	50%	0.0%	20%	60%
Eutypa spinosa	0.0%	0.0%	0.0%	0.0%
Gliocladium	40%	0.0%	10%*	40%
Basidiomycetes	0.0%	100%	50%	0.0%
Trichoderma	20%*	0.0%	0.0%	80%*
Graphium	10%*	0.0%	0.0%	0.0%
Zygomycetes	0.0%	0.0%	0.0%	0.0%
Deuteromycetes	10%	0.0%	20%	0.0%
Sporothrix	0.0%	0.0%	0.0%	10%*
Penicillium	0.0%	40%*	10%	0.0%
Helminthosporium	0.0%	0.0%	20%*	0.0%
Paecilomyces	0.0%	0.0%	0.0%	0.0%

* Fungi growing along with other fungi.
** Fungi obtained from sapwood samples cultured on glucose-yeast extract agar.

TABLE 6. Competitive potential of weed fungi versus shiitake in inoculated logs after 6 mo of incubation at room temperature.

	Control logs	Shiitake versus *E. spinosa*	*Eutypa spinosa*	Shiitake versus *G. platystoma*	*Graphostroma platystoma*
Fungus ** *Graphostroma platystoma* & *Hypoxylon* sp.	50%	20%	70%	30%	70%
Eutypa spinosa	0.0%	0.0%	0.0%	0.0%	10%
Gliocladium	40%	10%*	30%*	10%*	0.0%
Basidiomycetes	0.0%	0.0%	0.0%	10%	0.0%
Trichoderma	20%*	70%	60%*	60%	70%*
Graphium	10%*	0.0%	0.0%	0.0%	10%*
Zygomycetes	0.0%	0.0%	0.0%	30%*	0.0%
Deuteromycetes	10%	10%	10%	10%*	20%
Sporothrix	0.0%	0.0%	0.0%	0.0%	0.0%
Penicillium	0.0%	0.0%	0.0%	0.0%	0.0%
Helminthosporium	0.0%	0.0%	0.0%	0.0%	0.0%
Paecilomyces	0.0%	0.0%	20%	0.0%	0.0%

* Fungi growing along with other fungi (numbers indicate percent of logs).
** Fungi obtained from sapwood samples cultured on glucose-yeast extract agar.

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CHAPTER 3. COMPARATIVE SAPWOOD ROTTING POTENTIAL OF THE MAJOR "WEED FUNGI" *G. platystoma, E. spinosa,* AND THE EDIBLE MUSHROOM *L. edodes.*

ABSTRACT

The comparative white rotting potentials of pin-oak (*Quercus palustris*) sapwood by *Lentinus edodes* (shiitake) (LE) and the two major shiitake log "weed fungi" (*Graphostroma platystoma* (GP) and *Eutypa spinosa* (ES) were evaluated. We used the 1971 ASTM (Amer. Soc. for Testing & Materials) soil-block decay test method which consisted of the exposure of wood samples (wood blocks) in decay chambers at 28° C for 12 wk to pure cultures of fungi. The fungi were grown on filter paper placed on the substrate in the chamber. Pre-dried test blocks were weighed before and after exposure, and loss in weight was a measure of decay. Moisture and temperature were carefully regulated during the experimental period. GP and ES caused 20% and 16% losses, respectively, while LE strains VT17, U8, W4 and WG induced weight losses of 16%, 14%, 19%, and 16% respectively. A control standard, *Trametes versicolor,* produced 19% loss while the non-inoculated control was unchanged. The weight loss induced by these "weed fungi" reduces long-term fruiting of shiitake on the logs of many of Virginia farmers.

INTRODUCTION

A wide variety of organisms occur in decaying wood, and although fungi may be regarded as the prime movers in wood decomposition process, their roles differ in wood decomposition (Rayner & Boddy, 1988). Exploitation of decay fungi has so far been to some extent limited, the most general practical application perhaps being in cultivation of edible fungi such as those of *L. edodes, Pholiota nameko,* and *Pleurotus ostreatus.* Some

wood decaying fungi have other commercial uses. *Phlebia gigantea* controls *Heterobasidion annosum*, *Coriolus versicolor* soften wood in the production of softwood pencils. *Fistulina hepatica* causes the disease, "brown oak", in oak heartwood which makes timber ten times better in the production of veneers. *Chlorosplenium aeruginascens* is used in production of a variety of artifacts and decorative ware. Wood-decaying fungi uses will increase considerably particularly in those that have the ability to convert lignocellulosic resources into proteins, fermentable sugars, organic compounds, medicines, and degrading certain organic pollutants as well.

There are many wood-inhibiting fungi that produce edible fruiting bodies of which most notable is *Lentinus edodes* (Berk.)Sing. (Rayner & Boddy, 1988). Shiitake is a white rot-fungus, i.e. decays lignin mainly but also cellulose and hemicellulose so that the wood acquires a bleached appearance (Oki et al. 1981: Rayner & Boddy, 1988). The biology of this wood-decaying fungus is well known, and a chemically defined medium for fruiting of *L. edodes* has been published by Leatham (1983) and Song et al. (1987).

There is much information about Basidiomycetes and some about Ascomycetes and other fungi that decay wood ranging from taxonomic monographs, biological, ecological and physiological studies, to lists of wood-inhabiting fungi. However, no information was found about comparative quantification and qualification of wood decay by the "weed fungi" *Graphostroma*, *Eutypa*, and *Coriolus* versus shiitake (*L. edodes*), in shiitake production logs.

Among previous studies (in chronological order), the nature of decay in wood was studied by Longyear (1926), who developed a method of measuring the rate and stage of decay in wood. In 1937, Sheffer & Livingston (1930) related oxygen pressure and temperature to growth and carbon dioxide production in the fungus *Polyporus versicolor*, reporting that a minimal oxygen pressure for growth was about the same for all the tested

temperatures, being between 1.5 to 10 mm, and the optimal temperature for growth was about 29° to 33.5°C; at 33.5°C *P. versicolor* evolved carbon dioxide most rapidly (Sheffer & Livingston, 1937). Blaisdell (1939) (in Nilsson & Geoffrey, 1989) reported significant losses in three different hardwoods caused by *Daldinia concentrica*, *Hypoxylon* sp., and *Xylaria* sp.

Relative durability of ash wood has been studied by exposing wood blocks to wood decay fungi *Daldinia concentrica* and *P. versicolor* (Kispatic 1956 in Merrill et. al. 1964). The percent weight losses in the test blocks provide a measure of the relative decay susceptibility or inversely, of decay resistance of the sampled wood or material (Amer. Soc. for Test. & Mat. 1986). Kispatic found that *D. concentrica* caused weight losses of 5.8 and 2.8%, compared with *P. versicolor* which caused weight losses of 27.8 and 23.8% respectively after 3 mo of incubation. Cartwright & Findlay, 1958 (in Merrill et al. 1964) reported that *Xylaria polymorpha* caused weight loss of 14.0% after 4 mo of incubation in beech blocks.

Roff & Whittaker (1963) studied decay resistance of red-stained in lodgepole pine caused by *Stereum pini*, *Fomes pini*, and *S. sanguinolentum*. They concluded that this stain does not reduce the decay by *Poria incrassata*, *Lentinus lepideus*, and *Lenzites saepiaria*.

Rajagopalan (1966) (in Nilsson & Geoffrey 1989) registered weight losses of 16.0% in aspen wood caused by *Xylaria digitata* and *Hypoxylon rubiginosum* after 3 mo of exposure. Butcher (1968) studied the ecology of fungi by infecting untreated sapwood of *Pinus radiata* reporting the frequency of fungi in above-ground, ground-line, and below-ground zones and soft-rot capability of the main soft-rot fungi found. Smith (1969) among others, measured the decay potential of ponderosa pine sapwood blocks impregnated with chemicals (preservatives) by *Poria monticola* and other fungi. When the blocks, were treated with 0.30% pentachlorophenol, 0.0-0.4% of decay was observed while untreated blocks decayed 43-53% after 12 wk incubation. Clark (1969) tested the natural decay resistance of several

exotic woods, finding that *Lophira alata*, *Eperua falcata*, and *Eucalyptus marginata* were decayed between 0 and 10% by *Polyporus versicolor and Lenzites trabea* and therefore were very resistant. In a similar study, Highley & Scheffer (1970) reported that *Dipteryx alata*, *Aspidosperma cylindrocarpon*, and *Caryocar coccineum* were decayed 1, 8, and 9% (average weight loss) respectively, by *L. trabea*, *P. monticola*, and *P. versicolor* in this order.

The effect of a brown rot and a white rot fungus on the microstructure of red oak heartwood was studied by Toole (1972), finding that anatomic changes in wood have a role in the advance of decay. Decay associated with fire wounds was the major cause of defect of oaks in the southeast after 20 yr (Hepting & Shigo, 1972; Shigo, 1971, 1972).

Merrill et al. (1964), reported weight losses from 10.0 to 86.0% in aspen and red oak in 8 mo by *Daldinia concentrica*, *Hypoxylon atropunctatum*, *H. mediterraneum*, *H. pruinatum*, *H. punctulatum*, *Lenzites trabea*, and *Polyporus versicolor*. In another study, Merrill and French (1965) studied the histology of wood fibers after being exposed to decay by soft rotters which are Phycomycetes and Ascomycetes. These fungi did not occur within the secondary walls but were limited to the cell lumina. Furthermore, Merrill and Cowling (1966a, 1966b, and 1966c) investigated the role of nitrogen in wood deterioration by fungi.

The effect of alkaline treatment on decay resistance of wood was studied by Highley (1973), who found that alkaline-treated wood was readily attacked by brown-rot fungi when the soil block test was used. The pH and ammoniacal nitrogen content of the wood affected decay resistance. When the pH of hydroxide-treated southern pine and sweetgum was lowered, decay by *Poria monticola* was observed. On the other hand, when ammonia-treated southern pine was leached in acid to lower ammoniacal nitrogen content, decay by *P. monticola* was very easy.

The utilization of oxygen and weight loss associated with decay was studied by Toole

(1973), who found that weight loss increased at a relatively constant rate with increase in incubation time, while the amount of oxygen reached a peak after 2 wk incubation and then decreased with time at constant rate in most fungi. He also noted that these relationships vary with fungus and host species. Manganese concentrations were found to be higher in those tissues at the interface between discolored wood and wood that decayed frequently yielded Hymenomycetes in culture than contiguous clear tissue (Shortle & Shigo, 1973).

One hundred ninety species of wood-rotting Basidiomycetes from New Mexico were reported by Gilbertson et al. (1975). Succession of microorganisms during wood decay in contact with soil was studied by Kaarik (1975). He summarized his findings as follows: first sap stain fungi, sap stain fungi with soft rot characteristics, and finally Basidiomycetes. The interaction of light, moisture, and decay by fungi was studied by De Groot (1975) finding that these parameters affect the rate of decay in *Peniophora gigantea*. Twenty two hardwood species were tested for decay resistance against *Lenzites trabea* by Carter et al. (1976) who reported that decay ranged from 8% for blackjack and white oak to 67% for red maple.

The influence of oxygen and carbon dioxide on wood decay by heartrot and saprot fungi was studied by Highley et al. (1983) reporting that decay by both groups of fungi was reduced by low oxygen and high carbon dioxide. The structure of hyphae sheath in white-rot and brown-rot was studied by Palmer et al. (1983a, 1983b). Structurally speaking, the hyphal sheath in white-rot fungi did not differ from that in brown-rot fungi but functions may differ (Palmer et al. 1983a, 1983b).

In other wood decay tests, *L. edodes* caused the greatest weight loss in wetwood while *Hypoxylon truncatum* caused the greatest loss in dry wood. This suggests that in shiitake cultivation, *H. truncatum* had adapted to dry conditions which were unsuitable for *L. edodes* (Abe, 1989). Weight losses between 13.0 and 20.0% in pin oak sapwood blocks

were produced by *Graphostroma platystoma*, *Eutypa spinosa* and *Trametes versicolor* versus *L. edodes* strains (Guevara et al. 1991). Advantages in producing shiitake on sawdust, as opposed to natural logs includes less time required to complete the growing cycle as well as greater biological efficiency (Royse & Bahler, 1986, Royse et. al. 1990).

In the following experiment the comparative decay potential of pin oak sapwood by *Lentinus edodes* strains versus the "weed fungi", *Graphostroma platystoma* and *Eutypa spinosa*, was evaluated.

MATERIALS AND METHODS

Decay by *L. edodes* (strains VT17, WG, U8, and W4), *G. platystoma*, E. *spinosa*, and *Trametes versicolor* (FP 72074-R ATCC-12679) were inoculated on pin oak sapwood in the ASTM (1971) soil-block test. Strains of shiitake (WG, U8, and W4) were obtained from Fungi Perfecti, Inc., strain VT17 originated in Korea and was obtained from Elix Corp., Arvonia, Va. *Graphostroma platystoma* and *Eutypa spinosa* were obtained and isolated from shiitake logs in Virginia by Guevara and Stipes. The standard white rot test fungus *Trametes versicolor* was obtained from the American Type Culture Collection. Sapwood blocks were obtained from Dr. Stipes' research plantation pin oak plot (*Quercus palustris*) at VPI & SU; the trees were about 18 yrs old. The fungi were grown on filter paper placed on the substrate in the chamber. The test blocks were weighed before and after exposure, and any loss in weight was the measure of decay. The white-rotting potential of shiitake (*L. edodes*), and two of the major shiitake log "weed fungi", *G. platystoma* and *E. spinosa*, as well as *T. versicolor* was compared.

Sapwood samples measuring about 30 x 30 x 10 mm were used in this test. The blocks were of normal growth and with same orientation and free of knots, abnormal

amounts of resin, gums, or other exudates, and without visible evidence of fungus infection of other structures by other organisms. Eighty blocks were used with 10 replications for each fungus including control. The samples were oven-dried for 12 hr to get the initial weights (R1). Final weights (R2) were obtained after the block exposition which were the reference to calculate the decay percentage. These blocks were put into culture bottles which were prepared before the decay exposure. The test bottles were prepared by first putting water into the culture bottles, then soil, and finally the filter paper feeders in this order.

Determination of amount of water to be added required several steps. First the amount of water in the soil was calculated. A volume of about 120 ml of air-dry-soil (about half filling the bottle) was weighed and then oven dried (at 105°C for 12 hr) to enable calculation of percentage of water in the soil. Water holding capacity (WHC) of the soil was another parameter needed to calculate the amount of water to put in the bottles. Using the ASTM (1971) procedure to calculate the WHC, a filter paper (Whatman, No. 2., 4.25-cm diam) was placed into a Buchner funnel measuring 50 mm in diameter by 25 mm in depth. Funnels were filled with air-dry soil previously passed through a 4.78 mm opening sieve (U.S. standard sieve series, Dual Manufacturing Co.). The soil was compacted by smoothly dropping the funnel several times on a soft surface. Then the filled funnel was placed into a 400-ml beaker and retained in a upright position by using masking tape as supporters. Water was added to the beaker to a depth slightly above the level of the filter paper. The soil was wetted by capillarity with water added until wetting was evident at the top of the funnel and then soaked over night. After this, the funnel was placed in a suction flask which was connected to a vacuum pump at 0.5 atmospheres (G.E. Mod. 5KH33DN66) for 15 min. The soil was then weighed before oven drying to determine the water-holding

capacity. The amount of water to be added to a bottle was calculated by using the following formula: grams of water to add = (1.30 A-B) [D/(100+B)] where A = water-holding capacity in %, B = water content of air-dry soil in %, and D = grams of air-dry soil to used in culture bottles. Approximately 120 ml of air-dry soil gave 100 gm of soil to the bottle. 1.30 = constant. After adding water, loam soil (from the Duck Pond at Va. Tech) was added. This soil with a pH of 7.0 - 7.6 and a water-holding capacity of about 26.65% was sieved through a sieve with openings of 4.77 (A.S.T.M.). The sequence of first water and then soil leaves the water surface above the soil level in the bottles. The water diffuses upward through the soil. Therefore the soil surface was leveled before it became wet by smoothly shaking the bottle. Then the (feeder) filter paper (4.25 cm diam) was placed on the soil surface. Then the tops of the bottles were capped with cotton cheese-cloth to permit air-exchange and covered loosely with Reynolds aluminum foil. The prepared bottles were sterilized in the autoclave for 30 min at 15 psi and 121°C.

After cooling the bottles were inoculated with four different strains of *L. edodes*, and one strain each of the "weed fungi", *G. platystoma*, E. *spinosa* and *T. versicolor* (inoculated control). The inoculum was prepared by growing the test fungi from either vegetative hyphae or multispores (ascospores, basidiospores) on PDA (potato dextrose agar) amended with a micronutrient solution and incubated at 28°C in darkness. Fresh fungus inoculum of approximately 1 cm^2 from the growing edge of the colonies was placed on the soil and in contact with the edge of the filter paper (feeders). Then the inoculated bottles were incubated at 28°C in darkness for approximately 4 wk. Once the filter papers, called feeders strips, were covered by the fungi, the previously weighed and recorded wood blocks were exposed to decay. Before placing the blocks into the bottles, they were sterilized by placing into a closed container after which the container was steamed at about 100°C for 30 min.

After cooling, blocks were put into the bottles, one block per bottle, with the cross section facing down on feeder strip under a laminar hood using sterile forceps.

Cotton cheese-cloth caps were used to cover the bottles and foiled to permit a better air-exchange. The bottles then were put in darkness for incubation. To keep a relative humidity close to 100%, several containers with distilled water were put into the incubator in darkness.

To prevent losing data on the blocks due to severe decay, the identity of the block, the number of bottle, and the fungus identity, were recorded on both the bottle as well as on data sheets. After 8 wk of incubation, two reference blocks from each fungus series were removed. Mycelium was brushed off with a microdissecting scalpel before being oven-dried to obtain the weight loss. Data were recorded as R2 for calculation of decay using the following: weight loss, % = [(R1-R2)/R1] x 100.

Such determinations were made weekly for every fungus series. The ten replicates per fungus (series) permitted weekly removals for an additional 4 wk. The water content of the soil in the bottles was monitored at weekly intervals after determining the weight loss of the decayed blocks. Water loss in the soil of the bottles was adjusted to between 20 and 35% under the laminar hood by adding sterile distilled water with a sterile syringe having a cannula needle.

RESULTS

Results of the comparative maximum decay potential of pin oak (*Quercus palustris*) sapwood by *Lentinus edodes* strains (VT17, U8, WG, and W4), *Trametes versicolor* (ATCC-12679), and the two major shiitake log "weed fungi" *Graphostroma platystoma* and *Eutypa spinosa* are given in Table 1 and shown in Fig. 1.

In March, 1991, (after 8 wk of block exposure to these decaying fungi), two blocks from every fungus series were withdrawn to calculate the percentage of decay. At weekly withdrawals (5 in all), *E. spinosa* had decayed pin oak sapwood in the following percentages. In the first withdrawal (1) 13.31, in the second one (2) 14.03, in the third (3) 14.66, fourth (4) 15.89, finally (5) 15.65% respectively. *G. platystoma* decayed (1) 15.21, (2) 15.59, (3) 15.06, (4) 19.94, (5) 19.73%. *L. edodes* VT17 decayed (1) 12.55, (2) 12.40, (3) 14.30, (4) 15.88, (5) 15.27%. *L. edodes* U8 decayed (1) 8.74, (2) 8.86, (3) 7.14, (5) 12.69%. *L. edodes* WG rot (1) 10.97, (2) 12.43, (3) 15.51, (4) 15.91, (5) 18.01%. *L. edodes* W4 did (1) 12.17, (2) 13.40, (3) 15.20, (4) 19.33, (5) 18.68%. *T. versicolor* used (1) 6.90, (2) 9.17, (3) 15.67, (4) 11.22 (5) 10.86%. Finally, control blocks were not decayed in any of the withdrawals by any of these wood decomposing fungi.

Visual block appearance caused by fungal colonization was recorded in this assay. Generally, blocks exposed to *Trametes versicolor* and *Eutypa spinosa* were poorly colonized when compared with those blocks exposed to *L. edodes* strain WG. *L. edodes* strain WG was the best colonizing fungus when compared with the other wood decaying fungi. Blocks exposed to *L. edodes* strain W4 were very well to well colonized. Block colonization by *G. platystoma* was good to very good when compared with blocks colonized by *L. edodes* WG. The appearance of blocks colonized by *L. edodes* VT17 and U8 was good to very good.

DISCUSSION

The standard soil-block decay test and statistical analysis indicated that these "weed fungi" *G. platystoma* and *E. spinosa* do in fact decay oak sapwood when compared with the decay caused by strains of *L. edodes*. Therefore, shiitake production in logs may be decreased by these "weed fungi". *G. platystoma* decayed the sapwood as much as *E. spinosa*

and *L. edodes* strain WG and strain W4 (T test, $\alpha = 0.05$) and more than *L. edodes* strains U8, VT17 and *T. versicolor.*

Since many of these "weed fungi" are present in or on logs before inoculation with shiitake spawn, "weed fungi" management measures must be taken by shiitake grower to manage this problem. The following recommendations are suggested: (1) healthy and non-stressed trees that show less colonization by wood decaying fungi should be selected; in previous studies we found that some wood-decaying fungi were present in living, stressed trees, and so are poor choices for shiitake logs; (2) selection of oak species with low pH in the sapwood will improve the colonization of the shiitake fungus which is acidophilic but many "weed fungi" would not be able to compete; (3) shiitake log inoculation would be better done during the late winter/early spring when temperatures are lower and therefore more suitable for the growth of shiitake than for growth of competing fungi; and (4) a dip treatment of logs with the fungicide Arbotect 20S before inoculation with shiitake spawn should decrease colonization by undesirable wood decaying fungi.

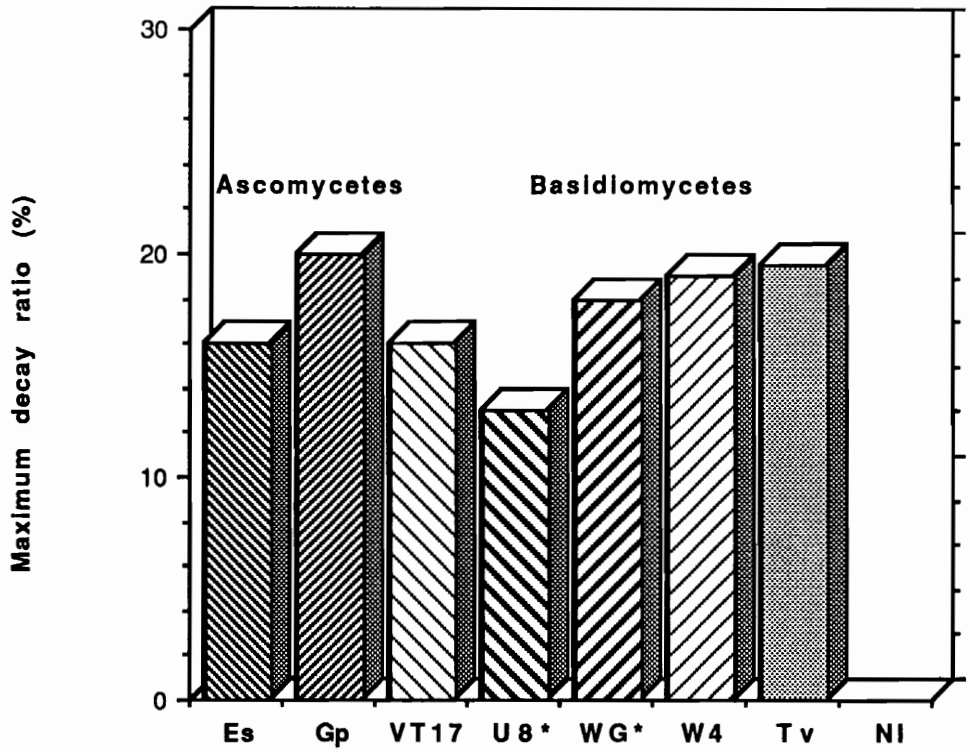

Fig. 1. Maximum decay of pin oak sapwood by isolates of shiitake, oak weed fungi, and a common white rotter during 15 weeks. Es = *Eutypa spinosa*, Gp = *Graphostroma platystoma*, VT17 = *Lentinus edodes* strain VT17, U8 = *L. edodes* strain U8, WG = *L. edodes* strain WG, W4 = *L. edodes* strain W4, Tv= *Trametes versicolor*, NI= non-inoculated. * = decay continues.

TABLE 1. Comparative maximum decay potential of pin oak (*Quercus palustris*) sapwood by strains of *Lentinus edodes* and the major "weed fungi" in shiitake logs after 8 wk of incubation in a soil-block decay test.*

	1 wk.	2 wk.	3 wk.	4 wk.	5 wk.
Fungus	Decay (%)				
G. platystoma	14.6, 15.8	14.6, 16.5	16.2, 13.9	18.9, 20.9	24.3, 15.1
E. spinosa	15.8, 10.8	14.7, 13.3	14.2, 15.1	15.0, 16.8	16.7, 14.6
L. edodes VT17	13.6, 11.4	13.1, 11.7	10.7, 17.8	15.9, 15.5	15.8, 14.7
L. edodes U8	9.8, 7.7	8.3, 9.4	7.2, 7.1	11.0, 11.2	14.1, 11,3
L. edodes WG	9.8, 12.1	12.4, 12.4	14.9, 16.1	15.9, 15.5	16.5, 19.5
L. edodes W4	12.6, 11.7	14.9, 11.9	15.3, 15.1	19.3, 19.1	19.2, 18.1
T. versicolor	6.9, 6.9	16.1, 2.2	19.5, 11.8	11.2, 11.5	7.5, 14.2
Control	0.0, 0.0	0.0, 0.0	0.0, 0.0	0.0, 0.0	0.0, 0.0

*Statistical analysis (T test, α=0.05), showed that *G. platystoma* decayed as much as did *E. spinosa* and *L. edodes* strain WG and strain W4, but decayed more when compared with *L. edodes* strain U8, strain VT17 and *T. versicolor* as well as control blocks.

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Vita.

Gonzalo Guevara Guerrero, son of the late Félix Guevara and Filadelfa Vda. de Guevara, was born January 10, 1962 in Monterrey Nuevo León, México. He was married to Amy Wiley, and to them a son was born, Félix Alberto on July 22, 1989. He received his elementary education at Esc. Alfonso Montemayor Lozano. His secondary education was obtained at Esc. Concepción Treviño de Montemayor, and his preparatory education at Preparatoria # 3, Universidad Autónoma de Nuevo León. At an early age, he showed interest in biological science. In 1979, he was admitted at Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León. He did research on mushrooms from northern México to obtained his bachelor degree in biology in 1984. He is senior author of several national and international papers on economically important fungi. He has participated as a research assistant in several projects on Mexican fungi. He has obtained several national and international scholarships, e.g., Agency for International Development (AID), Secretaria de Educación Pública (SEP) and Consejo Nacional de Ciencia y Tecnología (CONACyT). In 1989, he was accepted as a graduate student at Virginia Tech. to work on a master of science degree in plant pathology. His thesis research was on the edible fungus shiitake (*Lentinus edodes)*, learning invaluable experience under the advice of Dr. R. Jay Stipes. Two years latter, he was awarded the M. S. degree in plant pathology. He was repatriated by his government and nationally recognized as a member of the Sistema Nacional de Investigadores (SNI). Currently, he is a researcher and professor of plant pathology and mycology at Instituto Tecnológico de Victoria in México.