Fungicides to control fungal competitors in Chinese straw mushroom[1]

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ABSTRACT

Laboratory and production bed observations reveal that the major fungal competitors of the Chinese straw mushroom growing on sugarcane bagasse are *Sclerotium rolfsii* Sacc., *Corticium* sp., *Coprinus* sp., *Aspergillus flavus* Link ex Fries and *Chaetomium globosum* Kunze ex Steud. Because of their perceived importance, these five fungi were selected for control studies.

The selectivity of fungicides for the control of these fungi in Chinese straw mushroom beds was tested in vitro. Benomyl, captan, carboxin, chloroneb, mancozeb and penthachloronitrobenzene (PCNB) were tested in poison agar tests at the rates of 1, 10 and 100 p/m a.i. with Chinese straw mushroom and the selective competitors.

Mushroom radial growth was reduced in all treatments of carboxin and at 10 and 100 p/m of chloroneb. Compared to these, PCNB was intermediately toxic. Chinese straw mushroom was tolerant to all concentrations of benomyl, captan and mancozeb. *A. flavus* radial growth was highly reduced with benomyl and slightly reduced by carboxin, chloroneb and PCNB. *S. rolfsii* showed growth reduction at 10 and 100 p/m of PCNB, chloroneb and carboxin. Growth of *Caprinus* sp., *Corticium* sp., *C. globosum* and *A. flavus* was reduced over 90% with treatments of benomyl at 100 p/m.

Fungicidal sprays were evaluated in vivo on sugarcane bagasse and coffee pulp beds. Poor pasteurization of the coffee pulp was associated with rampant development of *Aspergillus fumigatus* Fries. and *Mucor* sp. Partial superficial control of these fungi was obtained with mancozeb alone (50 to 75% reduction of visible growth). Benomyl alone and in combination with mancozeb gave excellent suppression of fungi on the mushroom beds (90% reduction of visible growth). None of the treatments controlled fungi within the beds.

INTRODUCTION

Fungal pathogens and competitors are known to cause considerable losses to the common cultivated mushroom *Agaricus bisporus* (Lange) Sing. (4). Competitors colonize compost and beds and suppress mushroom development much in the same way weeds suppress field crops. Composting techniques and modification of the mushroom bed environ-

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ment are used to manage fungal diseases and competitors. Nevertheless, complete control is rare and mushroom production relies on the intensive and costly use of steam, disinfectants and pesticides.

Few studies have focused on measuring the interactions of mushrooms with competitors, particularly for Chinese straw mushroom. Yee and Chang-Ho (9) studied the antagonism of weed fungi toward Chinese straw mushroom. Mycofloral composition and pH were important determinants of growth losses by Chinese straw mushroom.

Hayes (1) suggested that control of fungal diseases and competitors is difficult because of the physiological similarity of mushrooms and their fungal pests. Diseases caused by imperfect fungi are commonly controlled by benomyl (5, 8). Basidiomycetous competitors may be more difficult to control because of their greater physiological affinity to commercial mushrooms. Competitor control is also complicated by the bulky substrates. Chemical control usually depend on contact of the toxic principle with the pest. Therefore, these chemical must enter and diffuse within the substrate to effectively control fungal competitors within the beds. The fact that early mushroom growth stages are most sensitive to toxicity and to competition, complicates chemical control.

Experimental production of Chinese straw mushroom, *Volvariella volvacea* (Bull ex Fries) Sing., in Puerto Rico has been greatly inhibited by the low nutrient availability of sugarcane bagasse substrates and by fungal competitors (3, 6). Twenty genera and species of fungi were identified from bagasse mushroom beds. On the basis of laboratory and production bed observations, *Sclerotium rolfsii* Sacc., *Coprinus* sp., *Corticium* sp., *Aspergillus flavus* Link ex Fries and *Chaetomium globosum* Kunze ex Steud are the major competitors to *V. volvacea* on sugarcane bagasse (3).

Considering the perceived importance of weed fungi, we designed studies to determine fungicidal selectivity in Chinese straw mushroom and selected competitors in vitro and to test the use of fungicides on in vivo beds.

MATERIALS AND METHODS

Research was carried out in the College of Agricultural Sciences farm, Mayagüez Campus. Throughout the experiment a native strain of Chinese straw mushroom (*V. volvacea*) was used (ATCC 52932 and ATCC 52933 are strains of *V. volvacea* collected in Puerto Rico). The strain was isolated from the stipe of a mushroom in the egg stage growing on fermenting piles of sugarcane bagasse at the Coloso sugarcane mill in Aguada, Puerto Rico. This strain was cultured on potato dextrose agar (PDA) and preserved on slants under sterilized mineral oil.

Fungicide selectivity

Potato dextrose agar (PDA) culture plates were supplemented with 1, 10 or 100 p/m a.i. of several commercial fungicides; non-treated plates were used for comparisons. The commercial fungicides used were: Benlate 50 WP, active ingredient benomyl, methyl 1 (butylcarbamoyl)-2-benzimidazole carbamate 50% a.i., E. I. Du Pont de Nemours Co., Inc., Wilmington, Delaware; Demosan 65 W active ingredient chloroneb, 1,4-dichloro-2-5-demithoxybenze, at 65% a.i. by E. I. Du Pont de Nemours Co., Inc.; Manzate-D, active ingredient zinc salts of maneb (mancozeb), manganese ethylenibisdisthiocarbamate, 80% a.i. by E. I. Du Pont de Nemours Co., Inc.; Orthocide 4 F, active ingredient captan, (N-Trichloro-methyl-thio)-4-cyclohexene-1, 2-dicarboximide), at 34% a.i. by Chevron Chemical Co., Ortho Agricultural Chemicals Divisions, Richmond, California; Terraclor 75%, active ingredient pentachloronitrobenzene (PCNB) 75% a.i. by Olin Agricultural Division, Olin Corp., Little Rock, Arkansas and Vitavax 75 W, active ingredient carboxin, (5,6-Dihydro-2-methy-1-14-oxathin-3-carboxanilide) at 75% a.i. by Uniroyal Chemical Co., Div. of Uniroyal Inc., Naugatuck, Connecticut.

Pure culture agar disks (4 mm) of *V. volvacea* and selective competitors were placed at the center of 9-cm culture plates amended with fungicides as mentioned above. *V. volvacea* and "weed" fungi (*A. flavus*, *S. rolfsii*, *C. globosum*, *Coprinus* sp., and *Corticium* sp.) cultures were measured 1, 2, 4 and 8 days after incubation. Each treatment was replicated 10 times. Mean radial growth was measured at each date and the standard error of the means calculated.

Fungicide in vivo tests

Benlate at 0.5 a.i. per liter and Manzate-D at 1.4 g a.i. per liter of water were used either alone or in combination in mushroom trays filled with a bagasse-coffee pulp substrate (2 parts of coffee pulp, 1 part of sugarcane bagasse, 5% lime and 3% of montmorrillonite clay). Metal greenhouse seedling trays were used as test containers. Both substrate and trays were steamed for 1 hour on each of 2 consecutive days at 121° C and 1.1×10^5 N/m^2 for pasteurization. Spawn was grown on coffee pulp amended with clay (10%). Five hundred grams of active spawn mixture was used for each of 8 experimental trays. Five days after spawning fungicide was sprayed. Trays were incubated at 90% RH and 35° C. Each treatment was replicated twice.

RESULST AND DISCUSSION

Chinese straw mushroom was tolerant to all rates of benomyl, captan and maneb, whereas carboxin, PNCB and chloroneb were inhibitory at the same concentrations (fig. 1). Radial growth was reduced more by

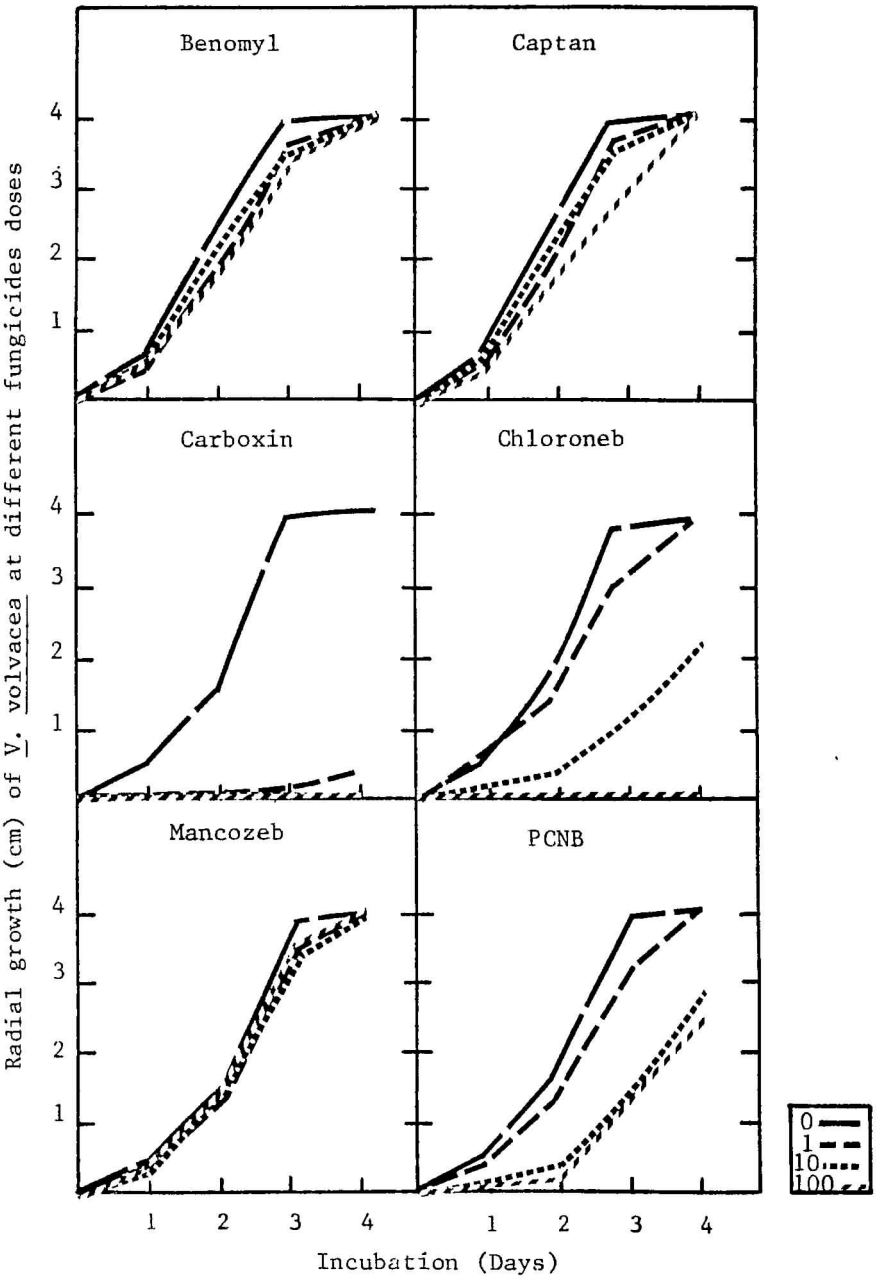

FIG. 1.—Mean radial growth of Chinese straw mushrooms (*V. volvacea*) in fungicide amended culture plates.

carboxin and chloroneb than by PCNB. Since the weed fungi *Corticium* sp., *Coprinus* sp. and *S. rolfsii* were basidiomycetes, selection of fungicides to control weed fungi poses a problem because of their physiological similarity to *V. volvacea.*

Selected fungicides showed activity in controlling compost fungi. Benomyl effectively controlled *Corticium* sp. (fig. 2), *Coprinus* sp. (fig. 3). *C. globosum* (fig. 4) and *A. flavus* (fig. 5). *S. rolfsii* (fig. 6) tolerated 10.0 p/m of benomyl but was sensitive to 100.0 p/m. Further studies of intermediate levels for 10 to 100 p/m and higher levels would be of interest in future investigation. Benomyl was the most effective fungicide tested on the basis of its broad spectrum action and high efficacy and selectivity. Gaps in benzimidazole activity include: oomycetes, some porosporic dematiaceous hyphomycetes such as *Alternaria* sp. and *Curvularia* sp. and some basidiomycetes (2, 5); Wuest et al. used benomyl (8) to control a number of fungal pathogens of *Agaricus bisporus* (Lange) Imbach. Rates of 1 and 2 lb/100 gal of benomyl (50% WP) in the different combinations tested did not reduce common mushroom yield in any of the strains tested. Carboxin was used in this trial of competitive fungi, on the basis of its activity against basidiomyctes. *V. volvacea* was sensitive to this product. Besides carboxin and benomyl, chloroneb has shown systemic activity. This fungicide may control *Sclerotium rolfsii* selectively in composts at low rates and should be studied further.

Besides benomyl, maneb is another broad spectrum fungicide used in common mushroom production (2, 5). Maneb and zinc (mancozeb) showed reduced phytotoxicity and improved fungicidal properties and pesticide compatibility. In our test the mycelial growth of neither *Corticium* sp., *Coprinus* sp., *Chaetomium globosum* nor *S. rolfsii* was affected significantly by mancozeb.

A benomyl and mancozeb, 1:1 mixture, used in the in vivo production experiments gave effecctive superficial suppressions of fungi on the mushroom beds but did not effectively control fungi within the beds. Mancozeb and benomyl showed the ability to control rampant development of *Aspergillus fumigatus* Fries. and *Mucor* sp. but only on the surface of beds of coffee pulp and bagasse. Benomyl alone and benomyl plus mancozeb are more effective than mancozeb alone. *A. fumigatus* and *Mucor* sp. appeared to originate from coffee pulp and indicated insufficient composting and pasteurization. Protection with fungicides like zineb and mancozeb was not adequate since weed fungal spores causing primary infection may be deep in the substrate beds. The bulky compost obstructs fungicide permeation and thus precludes effective and low cost chemical control.

Chloroneb showed good activity against *S. rolfsii, Corticium* sp., *Chaetomium globosum* and *Coprinus* sp. It could be selectively used at

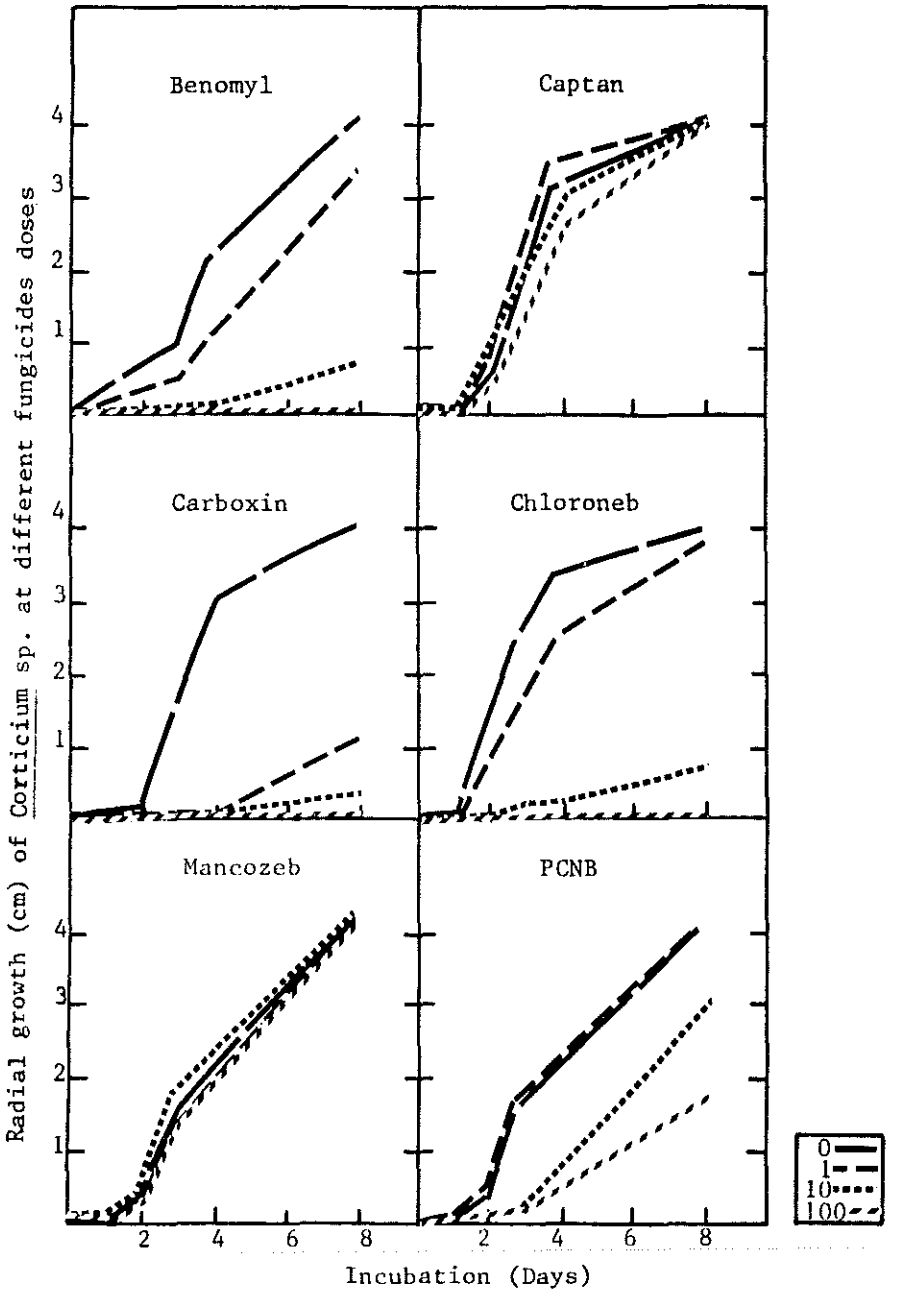

FIG. 2.—Mean radial growth of *Corticium* sp. in fungicide amended culture plates.

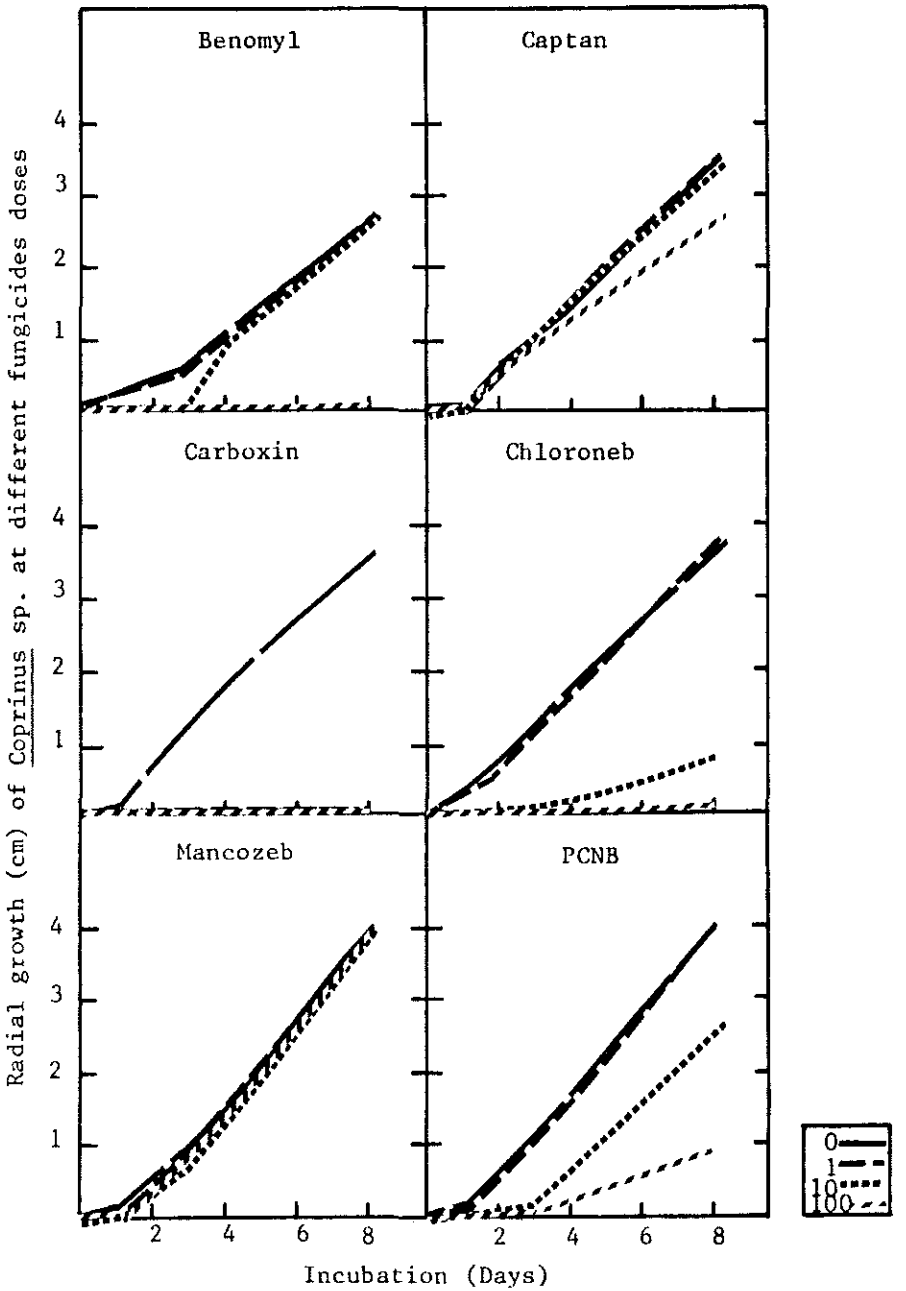

FIG. 3.—Mean radial growth of *Coprinus* sp. in fungicide amended culture plates.

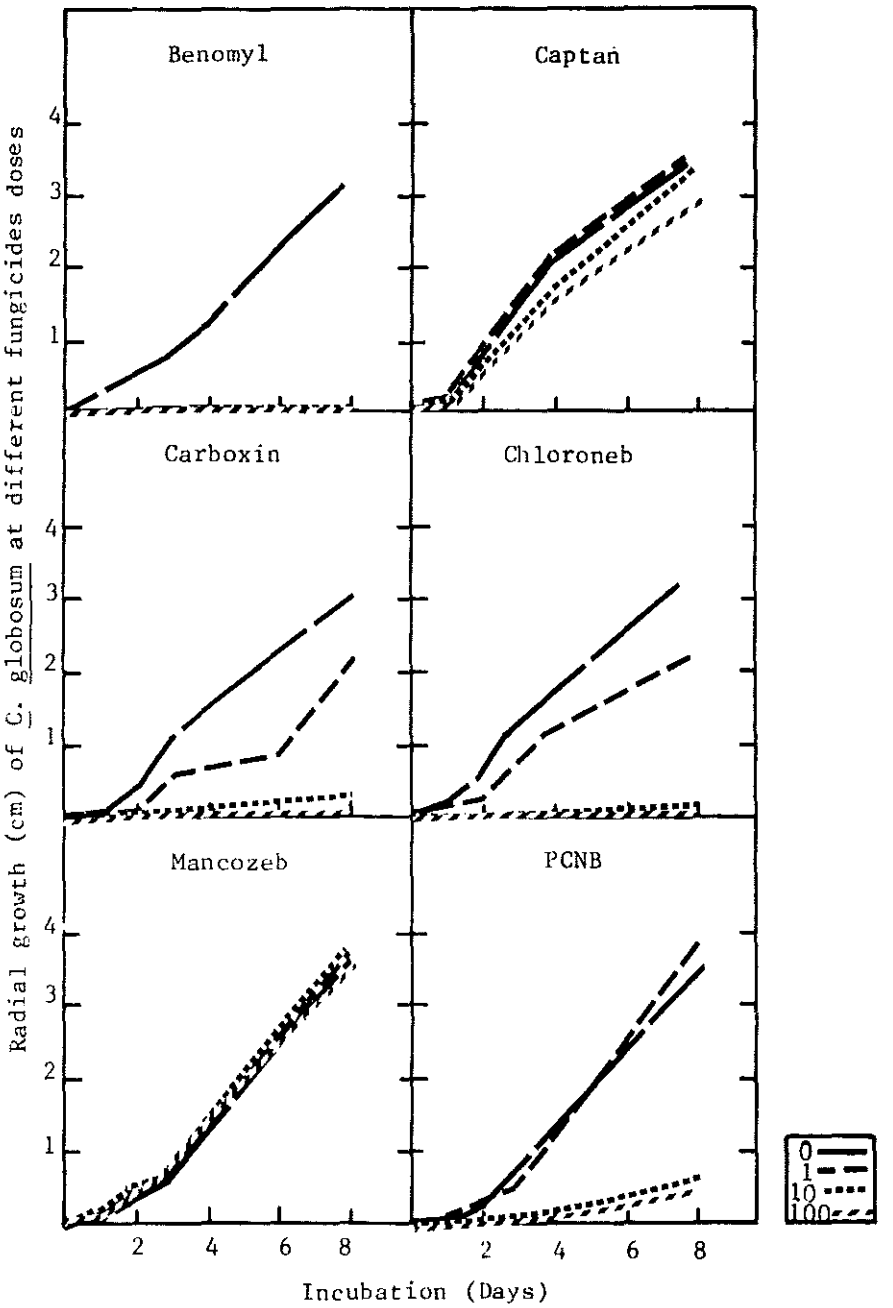

FIG. 4.—Mean radial growth of *C. globosum* in fungicide amended culture plates.

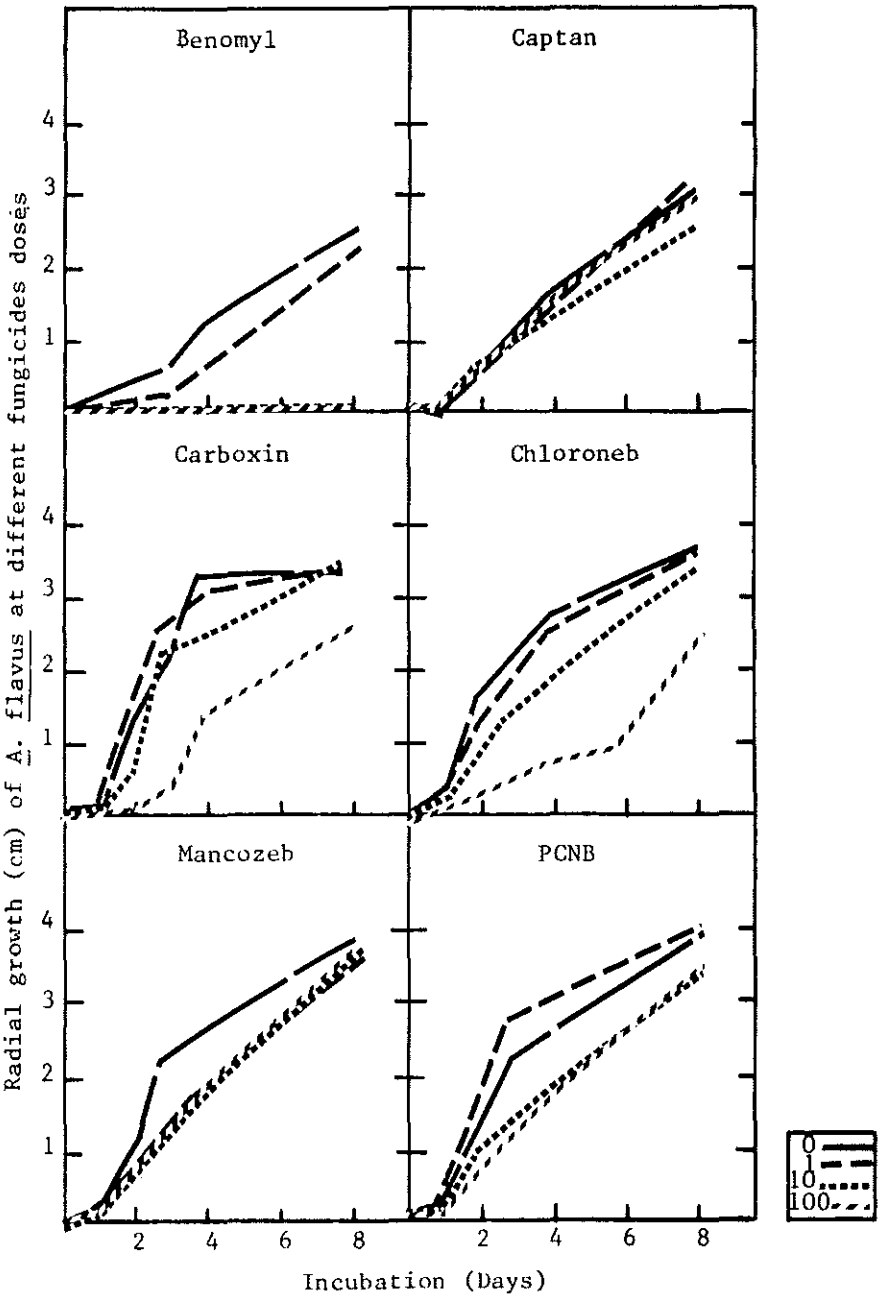

FIG. 5.—Mean radial growth of *A. flavus* in fungicide amended culture plates.

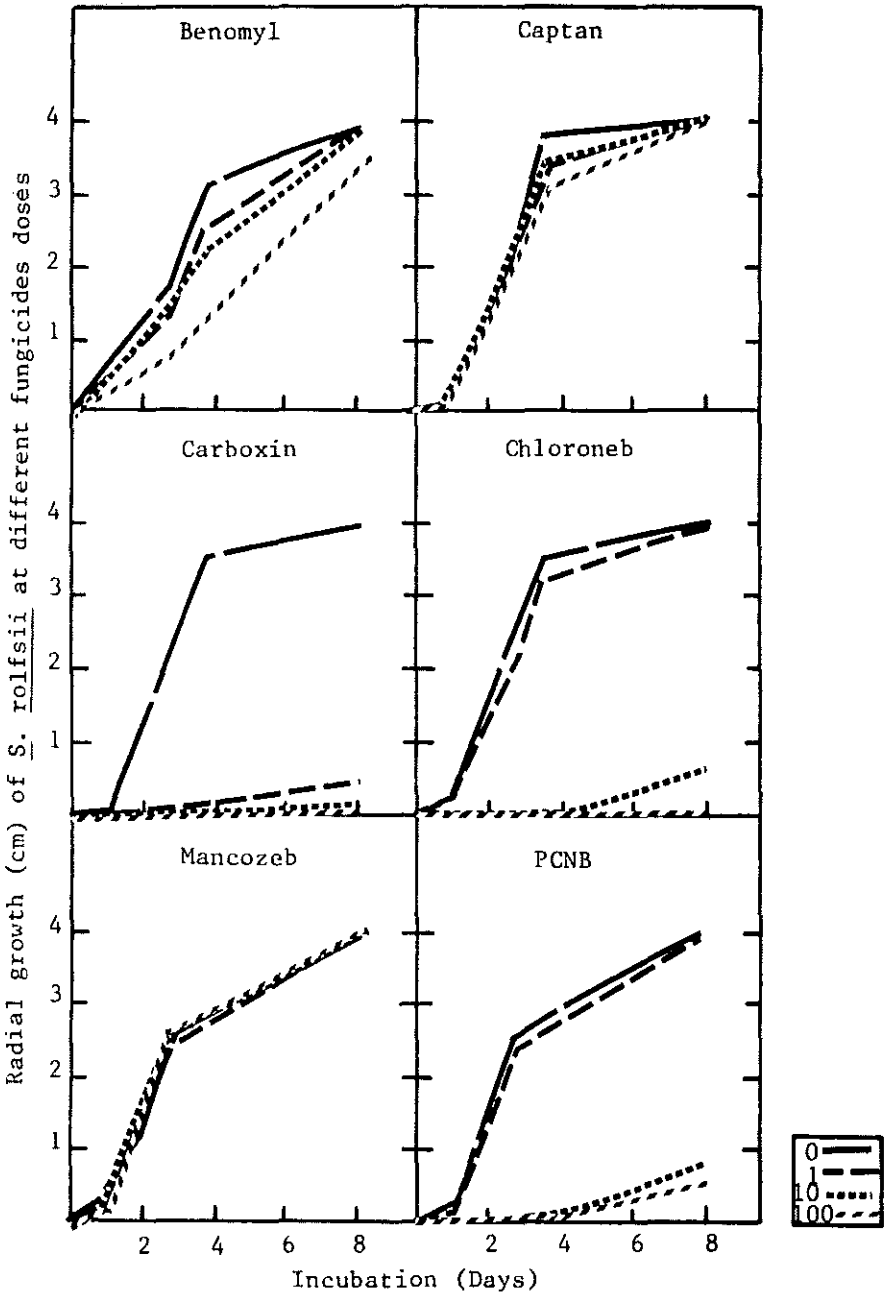

FIG. 6.—Mean radial growth of *S. rolfsii* in fungicides amended culture plates.

concentrations between 1 and 10 p/m. More testing of chloroneb concentrations and mixed applications with benomyl deserve further attention.

Several plant pathogens such as *Aspergillus* spp. and *Penicillium* spp. have developed strains that are resistant to certain fungicides, and therefore are unaffected by these. It appears that resistant strains of fungi can be expected to develop in the future because systemic fungicides are specific in their action. Selection of the *V. volvacea* strains resistant to fungicides or the adaptation of Chinese straw mushroom strains to fungicides on amended media might be helpful for enhancing commercial chemical control of weed fungi.

Further tests are needed to demonstrate the applicability of chemical control methods in Chinese straw mushroom production. Of particular importance is information on how to integrate management practices and control techniques.

The cultivation of mushrooms utilizes biological controls. Besides cultural and environmental management, effective chemical controls could improve commercial viability of Chinese straw mushrooms cultivation in Puerto Rico and other Caribbean countries.

RESUMEN

Fungicidas para reprimir los hongos competidores de la seta china

La producción de la seta china, *Volvariella volvacea* (Bull ex. Fries) Sing, aparentemente está limitada por la incidencia de otros hongos que crecen junto a ésta en las camas de bagazo de caña de azúcar. A base de 20 géneros y especies de hongos identificados en las camas de bagazo observados en el laboratorio y el invernadero, *Sclerotium rolfsii* Sacc., *Corticium* sp., *Coprinus* sp., *Aspergillus flavus* Link ex Fries y *Chaetomium globosum* Kunze ex Steud son los competidores más importantes de la seta china en el bagazo de caña. Por aparecer en el bagazo estas especies se seleccionaron para estudios de represión química.

Se hicieron estudios "in vitro" para seleccionar posibles fungicidas para reprimir los hongos competidores sobre las camas de bagazo. Se estudiaron los siguientes fungicidas: benomyl, captan, carboxin, chloroneb, mancozeb y pentacloronitrobenceno (PCNB) a diferentes concentraciones (1, 10 y 10 p/m). Las pruebas incluyeron las especies competidoras y la seta china (*V. volvacea*).

El crecimiento radial de la seta se redujo con concentraciones de 1 y 100 p.p.m. de carboxin y de 10 y 100 p.p.m. de chloroneb mientras que el PCNB tuvo un efecto tóxico intermedio. La seta china (*V. volvacea*) toleró dosis de 1, 10 y 100 p.p.m. de benomyl, captan y mancozeb. El crecimiento radial de *A. flavus* se redujo grandemente con benomyl y levemente con carboxin, chloroneb y PCNB. *S. rolfsii* mostró reducción de crecimiento con PCNB, chloroneb y carboxin a 10 y 100 ppm. El crecimiento de *Coprinus* sp., *Corticium* sp., *Chaetomium globosum* y *A. flavus* disminuyó en un 90% con los tratamientos de 100 p.p.m. de benomyl.

Los fungicidas se evaluaron en camas de bagazo de caña de azúcar y pulpa de café con una mezcla 1:1 de benomyl y mancozeb. La pobre pasteurización de la pulpa de café estuvo asociada con el desarrollo descontrolado de *Aspergillus fumigatus* Fries y *Mucor* sp. Los tratamientos de mancozeb sólo mostraron una represión superficial parcial (50 a 75%) de estos hongos. Benomyl solo y en combinación con mancozeb mostró una excelente represión superficial de estos hongos, no fue eficaz controlando hongos internos en las camas de pulpa de café y bagazo de caña.

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