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The need for methods of controlling mite infestations of fungus cultures is felt in every mycological laboratory. Whenever plant or animal material is brought in from the field, there is danger of bringing in also mites or their eggs. If their presence is not recognized in the infested culture and they are not exterminated there, they may pass from culture to culture, mixing and contaminating them all.

Smith (17) recommended that cultures be kept in cupboards on shelves supported on cork legs set in dishes of 1:1000 bichloride of mercury. He found that when the solvent evaporated, the crystals remaining were a sufficent barrier to mites, so that more water need not be added.

Barnes (2) devised a water-barrier system for dish cultures set on tripods, the water level to be maintained by occasional refilling of the tank in which the tripods are set.

Hansen and Snyder (11) and Snyder and Hansen (19) used barriers of cigarette papers sealed to the mouths of tubes by means of gelatin, to prevent entrance of mites to stock cultures.

Carpenter (6) in 1914 reported that a solution, having bichloride of mercury as the active ingredient, devised by Kellerman for the treatment of cotton stoppers to prevent invasion of cultures by "molds", also served to prevent invasion by mites. Thom (20) recommended treatment of stoppers with the same or a similar solution to keep mites out of stock cultures.

Putoni (15) exterminated mites in tube cultures by dipping the stoppers in petroleum ether and reinserting them. He recommended use of stoppers previously dipped in "petrole" and air-dried to prevent subsequent entrance of mites. Emmons (10) modified Putoni's method by moistening the tip of the stopper with petroleum ether from a pipette, thus avoiding the withdrawing of the stopper. Emmons also tested the effects of petroleum ether vapors on several fungi, and in no case was the fungus killed.

Jewson and Tattersfield (13) tested the action of eleven volatile organic compounds on fungi and on mites. A "green *Penicillium*" was exposed to each compound for three days, and then the growth of a subculture was measured after seven days. Mites were exposed to each chemical in two series, of four hours and sixteen hours respectively, and then examined for recovery over an extended period. They found ammonia most effective against mites, but also extremely toxic to fungi. They recommend its use to wash laboratory equipment and to fumigate rooms. Of those compounds only slightly toxic to fungi, they found pyridine to be most effective against mites.

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They found paradichlorobenzene only slightly toxic to fungi, but because of the short exposures they used, concluded that it is merely anaesthetic to mites.

Shafik and Page (16) tested several fumigants in an attempt to find a means of controlling mites in fungus cultures and insect cultures. Of those tested, only trichlorethylene and carbon tetrachloride were satisfactory for their purposes. Representatives of several groups of *Aspergillus* and a species of *Syncephalastrum* were unharmed by concentrations of these fumigants which killed mites.

Smith (18) reviewed many recommended fumigants, the work on pyridine, carbon tetrachloride, and trichlorethylene in particular. Of these, he states a preference for carbon tetrachloride in industrial applications.

Buell and Weston (3) found that mites could be eliminated by covering the infested cultures with mineral oil for two months. Subcultures from infested cultures so treated were always free of mites. No attempt was made to determine the minimum time necessary to eliminate mites by this method.

Caesar (4) recommended use of paradichlorobenzene to rid commercial mushroom beds of mites. Paradichlorobenzene may be placed directly on the bed or on muslin, and is covered with newspapers for the 48-hour fumigation period. The treatment must be repeated ten days later to kill any newly hatched mites. An attempt to fumigate an entire mushroom house by placing pans of paradichlorobenzene in front of fans resulted in killing of very few mites and delaying the next crop of mushrooms for a week.

Pease (14) used paradichlorobenzene to control mites in bacterial cultures. Depending upon minimum exposures to kill adults, she stated that eggs were not killed, and so repeated exposures must be made to kill new adults when they hatch.

Crowell (7) made extensive tests on the use of paradichlorobenzene. He placed contaminated tubes under a bell jar in an atmosphere saturated with paradichlorobenzene vapors. Also he placed crystals of paradichlorobenzene between the stopper and the wall of contaminated tubes. In no case were living mites observed after treatment. "About two hundred fungi of all classes were treated without apparent injury to any." Also, cultures were made with a crystal of paradichlorobenzene placed on the agar at the time of inoculation. In these cultures the fungi grew normally.

Horsfall (12) mentioned a report that damping off due to *Pythium* was reduced by fumigation of the seed-bed with paradichlorobenzene. Since this compound had been reported only slightly toxic to other forms, Horsfall suggested that its toxicity may be specific for Phycomycetes.

Paradichlorobenzene has long been used in the mycological laboratories of the State University of Iowa to control mites. A few crystals are placed on a square of sterilized paper directly in an infested dish, or a few crystals are dropped in an infested tube. Approximately two-tenths of a gram evaporate in two days; and after

such treatment neither recurrence of mites nor permanent injury to fungi has ever been detected.

Any satisfactory method of controlling mites in fungus cultures must kill both the mites and their eggs, must do no permanent damage to the fungi, and should be reasonably simple to apply. Previous reports have indicated that paradichlorobenzene does kill adult mites and does not kill any of the fungi tested against it. The following study was made to determine the extent of the toxicity to fungi of a dosage of paradichlorobenzene sufficient to kill the eggs of mites.

METHODS AND MATERIALS

Paradichlorobenzene (PDB) melts to form a colorless liquid at 53° C. It was found that a piece of ordinary white desk blotter one inch square would absorb about one-half gram of PDB when soaked in the liquid at temperatures a few degrees above the melting point. One-half gram proved to be large enough dosage for the purposes of these tests. PDB was heated in a beaker in a water bath. The temperature was controlled at between 55° C. and 60° C. Blotter squares were soaked about fifty at a time and stored in sterile petri dishes. It was found unnecessary to sterilize the blotters before soaking, and they retained sufficient dosage for as long as two months in storage. The blotters were handled with flamed forceps, and no contamination in any dish was ever traceable to them.

Mites of the genus *Tyroglyphus,* found in cultures of material brought in from the field, were tested against PDB. The mites were kept at all times in petri dish cultures of *Alternaria* sp. on which they thrived. Six pairs of mites in the act of copulation were transferred from the stock dish to each of several fresh plates of *Alternaria* sp. These plates were examined daily with the binocular until numerous eggs were found in every plate. Then PDB blotters were placed in the plates. The PDB was removed from the various plates at the end of eighteen, twenty, twenty-two, twenty-four, forty-eight, and seventy-two hours, respectively. Since contamination of these dishes with other fungi was not objectionable, the dishes were left uncovered for some time when the PDB was removed, to dissipate the remaining vajors in the dishes as quickly as possible. After the PDB was removed, the plates were examined every day until living mites were found, or for two weeks. In no case was the revival of a mite detected. Eggs hatched and living mites were eventually found in those plates subjected to eighteen and twenty hours treatment. No living mites were ever found in plates which received more than twenty hours treatment.

In order to allow a margin of safety in the killing of mites in infested cultures, the fungi tested were subjected to three times the minimum time required to kill eggs of mites as determined above. One myxomycete plasmodium and fifty-seven fungi were tested as to the effects of such dosage of PDB upon their rates of growth.

The myxomycete plasmodium was placed on Knop's agar and fed sterile oats until it was of such size that several transfers could be made. The tests were made with the plasmodium on Knop's agar.

All the fungi excepting the Basidiomycetes were cultured on a medium in regular use in this laboratory, known as IIa agar. None of the Basidiomycetes except *Pseudocoprinus* sp. grew rapidly enough on IIa agar to give useful measurements; so all members of this class were grown on a malt extrac agar. Ingredients of the three media used are:

Knop's Agar

$Ca(NO_3)_2$	1.0 gram
KNO_3	0.25
KH_2PO_4	0.25
$MgSO_4$	0.25
$FePO_4$	Trace
Agar	75.
Water	5. liters

IIa Agar

Glucose	10. grams
Peptone	2.
KH_2PO_4	0.5
$MgSO_4$	0.5
Agar	15.
Water	1. liter

Malt Extract Agar

Malt Extract	10. grams
Peptone	5.
Glucose	2.
KH_2PO_4	0.5
Agar	15.
Water	1. liter

In making the tests, each fungus was inoculated into three petri dishes at the same time, four inoculations on each plate, evenly spaced on two perpendicular diameters. Inoculi were made as small as possible in order that growth might be measured as from a point source. Growth was measured as the diameter of the colonies in millimeters. Whenever a colony was not approximately circular, the shortest line across the colony, passing through the inoculum, was taken. The figure recorded was the average of the four colonies on the plate.

The three plates thus made were labelled A, B, and C. Plate A was the untreated control. Plate B was treated with PDB at the

time of inoculation, one blotter being placed in the center of the dish. The blotter was removed at the end of three days, or in a few cases, at the end of four days. Plate C was untreated until substantial growth was attained, and then was treated with PDB for three days in the same manner as plate B.

The myxomycete plasmodium was handled in exactly the same way as the other fungi, excepting that the measurements taken were the distance the plasmodium crawled each day. However, the plasmodia in plates B and C were apparently dead soon after the PDB was introduced, and no further measurements could be taken. They did not revive after the PDB was removed.

RESULTS AND DISCUSSION

PDB is at least slightly toxic to all the fungi tested. On the basis of degree of response to PDB, these fungi are divided into three groups: I, those forms which grew at least a trace while the PDB blotter was in the dish; II, those which remained static while the dose was present but resumed growth almost immediately after its removal; III, those which were static while the dose was present and continued so for at least 24 hours after the dose was removed. In most forms, the reactions of the B and C cultures were nearly identical, but a few forms showed a considerable difference of response between the two. For example, *Neurospora tetrasperma,* in the B culture treated at the time of inoculation remained static for a day after the dose was removed, but grew considerably during treatment in the C culture which was established before subjection to PDB. On the contrary, *Penicillium notatum* remained static in both cultures during treatment, but while the B culture grew considerably during the first day after the dose was removed, the C culture showed no growth until the second day after treatment. In view of the fact that in actual practice cultures would not be treated for mite infestations at the time of inoculation, only the C cultures, those established before treatment, have been considered in making the following classification into groups I, II, III, as defined above. The fungi are listed in taxonomic order within each group. Numerical data and growth curves based on these data are given in an unpublished paper on file in the library of the State University of Iowa (9).

Group I

PHYCOMYCETES
Absidia scabra
Mucor racemosus
Mucor varians
Rhizopus arrhizus
Choanephora sp.

Haplosporangium parvum
Delacroixia coronata

ASCOMYCETES
- Aspergillus niger
- Penicillium italicum
- Physalospora cydonii
- Sordaria humana
- Neurospora tetrasperma
- Endothia parasitica

BASIDIOMYCETES
- Auricularia auricularis
- Coniophora cerebella
- Polyporus compactus

FUNGI IMPERFECTI
- Diplodia sp.
- Alternaria sp. 1
- Curvularia sp.
- Fusarium sp. 1
- Fusarium sp. 2

Group II

PHYCOMYCETES
- Mucor globosum
- Mucor ramannianus
- Zygorrhynchus vuilleminii
- Mycotypha microspora

ASCOMYCETES
- Aspergillus ustus
- Penicillium glaucum
- Penicillium roquefortii
- Chaetomium trilaterale

BASIDIOMYCETES
- Exidia glandulosa
- Exidia recisa
- Stereum gausapatum
- Fomes everhartii
- Coprinus sp.
- Pseudocoprinus sp.

FUNGI IMPERFECTI
- Phoma sp. 1
- Phoma sp. 2
- Pestalotia sp.
- Sporobolomyces salmonicola
- Botrytis terrestris
- Gonatobotrys sp.
- Trichoderma sp.
- Streptomyces griseus
- Alternaria sp. 2
- Alternaria sp. 3
- Beltrania rhombica

Macrosporium sp.
Memnoniella echinata
Pullularia pullulans
Tetracoccosporium sp.
Myrothecium glutinosum
Tetracrium incarnatum
Sclerotium rolfsii

Group III

PHYCOMYCETES
Pythium sp.
Rhizopus nigricans
ASCOMYCETES
Penicillium notatum
Chaetomium indicum

None of the fungi tested except the myxomycete plasmodium was permanently affected by the PDB. The response to it is variable from one fungus to the next, and two species of the same genus may react very differently. As grouped in the preceding lists on the basis of response of the C cultures, twenty-one of the fifty-seven fungi tested grew more or less rapidly while under treatment. None of the rest grew at all while the dose was present. Of these, only four did not resume growth as soon as the dose was removed. In every case, however, the treated cultures at least eventually resumed growth at a rate equivalent to the control culture.

PDB is only very slightly soluble in water. Little of it would be dissolved in the medium even though the dosage were placed directly on the agar. However, this small amount proved to be enough to prevent the growth of some fungi. *Trichoderma* sp. in culture B grew at an extremely rapid rate after the PDB was removed, but no hyphae could be found in the square inch where the blotter with PDB had been in contact with the agar. The remainder of the plate was green with spores at the end of forty-eight hours, but no growth in that one square inch was detected for five days. This same situation prevailed with the B and C cultures of *Haplosporangium parvum,* and to some extent, though not so marked, with others. The lag of a day or more in the resumption of growth by the four forms in Group III was probably due to their hypersensitivity to the fungistatic effect of the residual vapors of PDB in the atmosphere of the dish and to the very small amount dissolved in the medium.

It is notable that *Pythium* sp. is in Group III. The reduction of damping off by use of PDB fumigation of seed-beds as reported by Horsfall may have been due to a slowing down of *Pythium* rather than the destruction of *Pythium.* As for the toxicity of PDB being specific for Phycomycetes, seven of the thirteen Phycomycetes tested are in Group I, some of them being barely slowed at all by the treatment.

Examination of mycelium, fruiting structures, and spores, both macroscopic and microscopic, revealed no differences between untreated cultures and those subjected to PDB. Microscopic examinations were made on temporary mounts; phloxine was the only stain used. Carey and McDonough (5) produced polyploidy in the roots of *Allium* by treatment with PDB, which was evidenced macroscopically by stunting. It is possible that cytological studies would have revealed nuclear changes in the fungi treated in these tests, but such examinations as were made indicated that no such changes were to be suspected.

CONCLUSIONS

Paradichlorobenzene is a material readily available and simple to handle. Though extended exposure to its vapors is toxic to man, the vapors are easily avoided even when heated to the melting point, because the vapors are much heavier than air and so do not readily fill the room. The tests herein reported show that exposures of as little as one day to these vapors will kill mites and their eggs. Conversely, these tests indicate that though paradichlorobenzene is fungistatic to most forms, it is fungicidal to none except the one Myxomycete exposed.

The technique devised for these tests makes it very easy to put the paradichlorobenzene into the infested culture and to remove it at will. Ordinary white blotting paper, cut into pieces large enough to absorb half a gram of paradichlorobenzene, may be used. A large supply may be soaked at one time, as there is no loss of paradichlorobenzene from the prepared blotters even after extended storage if the container is air-tight.

Paradichlorobenzene does, then, provide an effective and convenient method of controlling mites in fungus cultures.

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