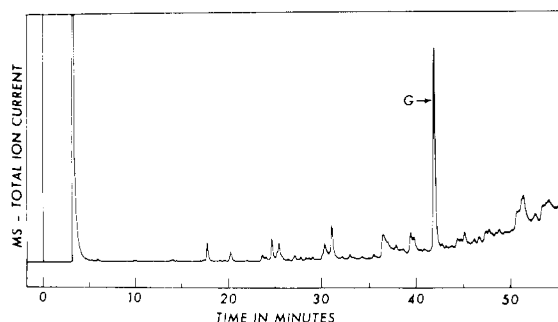

Figure 1.

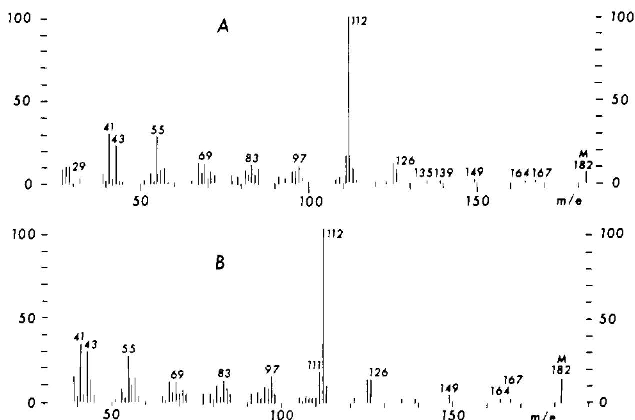

Figure 2.

essence and the basic fraction had a potato-like odor. The acid fraction was almost odorless. Figure 1 shows the gas chromatogram obtained from the concentrated neutral fraction of the beet essence. The strongest odor detected in this chromatogram was an earthy odor associated with peak G. The mass spectrum of peak G shown in Figure 2A is virtually identical with the spectrum of authentic geosmin, Figure 2B (Buttery, 1974; Kikuchi et al., 1972).

The retention time of synthetic geosmin prepared according to Marshall and Hochstetler (1968) and chromatographed in a packed column was 28.7 min and its mass spectrum and odor were virtually identical with that of peak G which had a retention time of 28.6 min on the same column. The *cis*-9-decalol isomer of geosmin had a very similar spectrum to that of geosmin but a retention time of 29.5 min and an odor similar to camphor or cedar. Thus, the character of beet odor can be described as a combination of a potato-like odor, from the basic compounds, and the earthy odor of geosmin.

The fact that geosmin is known to be produced by soil organisms raises the question of its origin in beets. In the experiments reported here the washing and blanching procedures eliminate the possibility of contamination by soil. However, beets may absorb geosmin from soil during growth or storage. If so then why do not other root crops such as carrots and turnips also have a strong earthy odor due to the absorption of geosmin? The possibility that geosmin is absorbed by beets from the soil rather than produced metabolically by the beet is presently being investigated.

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Uptake of Benomyl by the Cultivated Mushroom, *Agaricus bisporus*

Fruiting bodies of *Agaricus bisporus*, grown under commercial conditions in substrates treated with 40 μg/g of benomyl at different stages of cultivation, were found to contain from 0.67 to 3.7 μg/g of benomyl residues. Mushrooms from substrates treated by drenching contained the highest quantities of residues, whereas those treated by mixing benomyl with the spawning material contained the least. Although translocation from vegetative mycelium into fruiting bodies occurred, accumulation of residues in the mushrooms was not indicated.

Although the systemic fungicide benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate) has been shown to be effective for control of the major fungal diseases of the cultivated mushroom, *Agaricus bisporus* (Gandy, 1971; Holmes et al., 1971; Peake, 1972; Snel and Fletcher, 1971), and is widely used by growers in Europe and England, little published information is available concerning the presence or absence of residues in fruiting bodies harvested from treated cultures. Snel and Fletcher (1971), in a study which dealt mainly with disease control by benomyl, mentioned that residues of the fungicide were not present in mushrooms harvested from pots treated with 5 or 10 μg/g of a wettable powder formulation. In contrast to this, preliminary studies in our laboratory

Table I. Residues of Benomyl Recovered from Fruiting Bodies (Cap + Stipe) of *Agaricus bisporus* after a Single Treatment, at 40 μg/g (=20 μg/g Active Ingredient) during the Spawning, Casing, or Fructification Stages of Cultivation[a]

Benomyl treatment	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Mushroom yield,[b] kg/m^2
None	0	0	0	0	12.20
At spawning	1.00 ± 0.17	1.95 ± 0.24	0.67 ± 0.17	1.98 ± 0.13	13.59
At casing	0.95 ± 0.25	2.27 ± 0.23	2.41 ± 0.17	2.45 ± 0.88	11.92
At fructific.	3.61 ± 1.02	2.81 ± 0.22	1.99 ± 0.08	1.80 ± 0.31	10.13

[a] Results, which are in micrograms of residue/gram fresh weight of mushroom, are the averages ± standard deviations of triplicate cultures. Fresh mushrooms contained to 90 to 93% by weight of H_2O. [b] Mass of compost per m^2 = 60 kg.

indicated that benomyl residues could be recovered from mushrooms harvested from cultures grown and treated with benomyl under commercial conditions. To confirm these results, and to determine if treatment with benomyl during different stages of *Agaricus* cultivation had an influence on the quantities of residue present in the fruiting bodies, the following investigations were conducted.

MATERIALS AND METHODS

Cultures of *Agaricus bisporus* were grown on composted horse manure (Sinden and Hauser, 1950), in wooden trays, as described by Grabbe and Haider (1971). A single application of benomyl, at a rate of 40 μg of wettable powder (50% active ingredient) per gram of wet compost (70% H_2O), was added at the spawning (powder mixed with the spawn into the compost), the casing (powder mixed only into casing soil), or the initial fructification (H_2O suspension of powder applied as a drench) stages of cultivation. Triplicate cultures were prepared for each method of treatment. Mushrooms from the first four flushes were harvested, washed in running water, chopped to a paste in a blender, and then extracted and analyzed for the benomyl conversion product, methyl 2-benzimidazolecarbamate (= MBC: benomyl converted to MBC during extraction), by uv spectroscopy and thin-layer chromatography (TLC) as described by White and Kilgore (1972). Standard curves for each quantitative analysis were prepared by supplementing aliquots of mushrooms from fungicide-free control cultures with known quantities of benomyl just prior to their extraction. Recoveries from mushrooms supplemented with 2, 4, 8, or 12 μg/g of benomyl (two experiments), and immediately extracted, as determined by comparison to standard curves prepared from MBC, averaged 79.6 ± 7.2% of the applied dosages. In addition to the above analyses, spots tentatively identified as MBC by cochromatography with authentic compounds were bioassayed for their toxicity using an MBC-sensitive fungus, *Cladosporium cladosporioides*. For this assay, the aluminum foil TLC plates were cut into sections with a scissors and the areas containing the spots were placed into sterile petri dishes, with 2% malt agar containing a heavy inoculum of *C. cladosporioides* conidia, and incubated for 2 to 4 days at room temperature. After incubation, inhibition zones, indicating the presence of MBC, were evident as areas which were free of the dark-colored mycelia.

RESULTS AND DISCUSSION

Residues of benomyl (benomyl + MBC) were detected in mushrooms harvested from all cultures which had been treated at 40 μg/g with the fungicide (Table I). The quantities of residue recovered, which varied from 0.67 to 3.6 μg/g fresh weight of mushroom, were not high, and definite trends toward increasing or decreasing contamination of the fruiting bodies from harvest to harvest were not evident. On the average, mushrooms harvested from cultures treated at spawning contained the least residue, whereas those from cultures treated by drenching at the onset of fructification contained the most. The results which showed the presence of benomyl in the mushrooms of cultures treated at spawning also indicated that the fungicide was translocated from the vegetative mycelium into the fruiting bodies. In those cultures, the fruiting bodies, which develop on top of the casing layer, were separated from the fungicide-contaminated compost by a 5-cm thick layer of fungicide-free casing soil. Although translocation occurred, no tendency toward accumulation of residues in the mushrooms was evident. At maximum, only 2.3% of the benomyl applied could be recovered in the fructifications.

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