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Benomyl sensitivity of Neurospora on solid medium and in liquid cultures.

Benomyl, methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate (E. I. duPont de Nemours and Co., Inc.), inhibits hyphal elongation of Neurospora but has no effect on germination of conidia or ascospores. N. crassa SF 26 (Gratzner and Sheehan 1969 J. Bacteriol. 97:544) was used to study the

Solid medium. Vogel's minimal N medium containing 1.5% sucrose and Benomyl was inoculated with conidia and linear

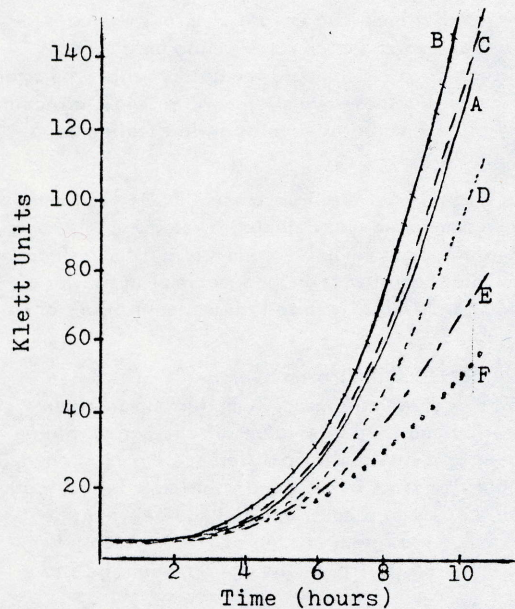


Figure 1. Effect of various concentrations of Benomyl on growth of *N. crassa* in liquid cultures. A, control; B,  $0.1 \mu\text{g/ml}$ ; C,  $0.15 \mu\text{g/ml}$ ; D,  $0.2 \mu\text{g/ml}$ ; E,  $0.25 \mu\text{g/ml}$ ; F,  $1.5 \mu\text{g/ml}$  Benomyl.

growth was measured in Ryan tubes over a period of 4 days at  $34^\circ\text{C}$ . Using aseptic precautions, Benomyl was dissolved in absolute ethanol and added to the autoclaved medium. The aliquot of Benomyl solution added to each flask was adjusted to equal the volume of ethanol added to the control. The ethanol concentration never exceeded  $0.7\% \text{ v/v}$  in the medium. No contamination was observed in cultures on solid medium or in liquid cultures when aliquots were plated on nutrient agar or BHI. Growth rates of mycelia at  $0.1 \mu\text{g/ml}$  Benomyl were slightly greater than those of the control, perhaps indicating a slight stimulatory effect at low concentration of the fungicide. Fifty percent inhibition of growth was obtained at about  $0.2 \mu\text{g/ml}$  and complete suppression of growth occurred at  $0.3 \mu\text{g/ml}$ .

Liquid medium: Nephelometer flasks containing 200 ml of Vogel's minimal salt solution with 1.5% sucrose and various concentrations of Benomyl (added as described above) were inoculated with equal volumes of a filtered conidial suspension to give equivalent Klett readings with filter #54. Cultures were incubated at  $34^\circ\text{C}$  with vigorous aeration and growth was followed for 10 hours by measuring Klett units (Bowman and Jones 1966 *Neurospora* Newsl. 9: 17). Figure 1 illustrates the results obtained. Some stimulation was again observed at  $0.1 \mu\text{g/ml}$  Benomyl. In general, however, inhibition was not as great as that observed on solid medium over a longer period of time. There was some increase in Klett readings even at high concentrations of Benomyl, implying that germination was not prevented. Microscopic examination of cultures indicated that ca. 95% of the conidia did germinate, but further incubation in the presence of inhibitory concentrations of Benomyl prohibited additional growth and produced gross alteration of hyphal morphology. Hyphae became distorted and bulbous with a densely granular appearance of the cytoplasm.

We have observed a similar effect on morphology and growth of 74-OR23-1A (FGSC#987), ad-1 (FGSC#672), pyr-3 (FGSC#835) and slime (FGSC#1118). Slime is inhibited by slightly lower concentrations of Benomyl, as expected from its lack of cell wall. The mechanism of action of Benomyl is currently under investigation in this laboratory.

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