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Chemical induction of trap closure in *Dactylella brochopaga*

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Summary. Constricting ring traps can be sprung by exposure to dilute alcohol solutions and even by the vapours of higher alcohols. The greater the lipid solubility of the alcohol, the lower the concentration required to spring 100% of treated traps. This implies first that their primary action is at the plasma membrane, and second that some traps in a population are more reactive than others. Over 80% of traps could be activated between pH 5.5 and 8.2 suggesting an adaptation to permit prey capture even in unfavourable micro-environments.

Keywords: Fungus; Constricting ring trap; Chemical stimulus.

Introduction

Among the most interesting of the microbes that inhabit the soil are the predatory fungi, particularly those employing cellular snares. And of these implements of capture the type which has aroused the widest scientific interest is the constricting ring trap of the predatory hyphomycetes. This is a torus of three excitable cells, attached by a stalk to the parent hypha. When a nematode pushes itself partially through the ring the cells expand so as to triple their volume in about 0.1 sec thus firmly gripping the worm. Then the trophic hyphae grow from the expanded cells, penetrate the integument of the immobilized prey and rapidly digest and absorb its tissues.

It is important to distinguish between two aspects of trap action: first the stimulus and second, the inflation process. Muller (1958) made the valuable contribution of separating these operationally. He stimulated the cells with heat while preventing inflation with a hypertonic medium, which he then gradually diluted so as to prolong the process from 0.1 to 10 sec. His study demonstrated that it is water which enters to effect inflation; even water vapour alone can do this and the water or vapour enters all three cells from their immediate surroundings (Insell and Zachariah 1978, Barron 1979). We do not yet understand the stimulus-receptor mechanism. Couch (1937) made the important discovery that heat could trigger inflation and Comandon and de Fonbrune (1938) duplicated the mechanical stimulus which the prey provides by using a microneedle. Lawton (1967) found that acetylcholine facilitated trap closure implicating a site of action at the cell membrane. Insell (1975) in testing this and other neurotransmitters reported that a clinical preparation of adrenalin at 10^{-5} g/ml would spring ring traps. However, I could not repeat this with pure samples of the catecholamine, and in a later study showed that chlorobutanol (1,1,1-trichloro-2-methyl-2-propanol) which is used as a preservative in the clinical preparations was very effective in springing traps, even as vapour. It was significant that treated traps were quite viable and could proliferate readily (Zachariah 1981, 1982). Thus chlorobutanol probably mimics the mechanical stimulus which the nematode supplies in nature. Accordingly, other alcohols were tested with the results reported in this paper.

Materials and methods

The strain of *Dactylella brochopaga* (American Type Culture Collection, ATCC 13897) was grown in replicate on corn meal agar plates, as centrally inoculated cultures. Cultures about 10 days old were provided with the free-living nematode *Panagrellus silusiae* as prey, so as to induce ring trap formation. Approximately 10 days later blocks of agar (1 cm^2) were singly excised, care being taken that all blocks from different plates were taken from regions the same distance from the central inoculum site so as to be physiologically comparable. In turn, each block was placed in a new, sterile plastic

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Petri dish under the microscope and either photographed at once, or in some experimental series, first rinsed with a drop of deionized water (not sterilized) and then photographed. Then a drop of the chemical solution to be tested was placed on the block and after about 10–15 seconds another photograph was taken. Then another block of agar with traps was excised, placed in a distant part of the same dish and the response of the traps to a higher concentration of the same chemical was recorded photographically. At the end of a series of such "before" and "after" photographic records, a written description of the concentrations used was photographed with the same camera so that this frame terminated the roll of film. This obviated confusion about the sequence of concentrations used in any experiment. Each "before" print was numbered and placed under an overhead transparency sheet. Each spontaneously closed trap (Fig. 2, arrow) was marked with one colour. Next the transparency was placed over the corresponding "after" print and each ring that had closed as a result of treatment was marked with a second colour. The accumulated transparencies from each series of treatments were scored to determine the fraction of rings which had closed due to treatment with the test chemical.

In treatments such as with methanol, which induced gradual swelling of ring cells or swelling of fewer than three cells per ring, photographs were taken at varying times after application of the chemical. When very volatile substances (chlorobutanol, 1-butanol, ether, chloroform) were to be tested, traps were exposed to their vapours by holding a drop of the fluid at the end of a Pasteur pipete a short distance above the field or in the case of chlorobutanol, affixing a small crystal with silicone grease to the end of a fine glass needle (Zachariah 1981, 1982).

The effect of variations in pH on the trap trigger mechanism was examined by first rinsing the agar block with a buffer, blotting up the excess and then adding a drop of the test chemical. Buffers used were citric acid/phosphate (0.1 M citric acid, 0.2 M Na_2HPO_4) and glycine (0.1 M glycine, 0.1 M NaCl, 0.1 M NaOH) for the lower and upper halves of the scale respectively. All chemicals used were reagent grade.

Results and discussion

Four alcohols were used to induce ring trap closure; in order of increasing lipid solubility they were methanol, ethanol, 1-propanol and 1-butanol. Correspondingly, they were capable of inducing closure of 100% of treated rings at the following minimal concentrations: methanol, 40% solution; ethanol, 16% solution; propanol, 4% solution; butanol, vapour sufficed to induce closure (Fig. 1 a, b). This recalls classical permeability studies which established that generally, molecules with the largest partion coefficients most rapidly penetrated living cells (Collander and Barlund 1933); it suggests that these agents act to trigger ring closure by perturbing the lipid bilayer of the plasma membrane or at the boundary of some intracellular compartment. In most previous studies of the trap mechanism (Muller 1958, Lawton 1967) heat was used to induce ring closure; it is probable that heat acts to produce similar perturbations by increasing the fluidity of the membrane lipid component. Like heat and mechanical stimulation, all the alcohols tested except methanol induced instantaneous closure.

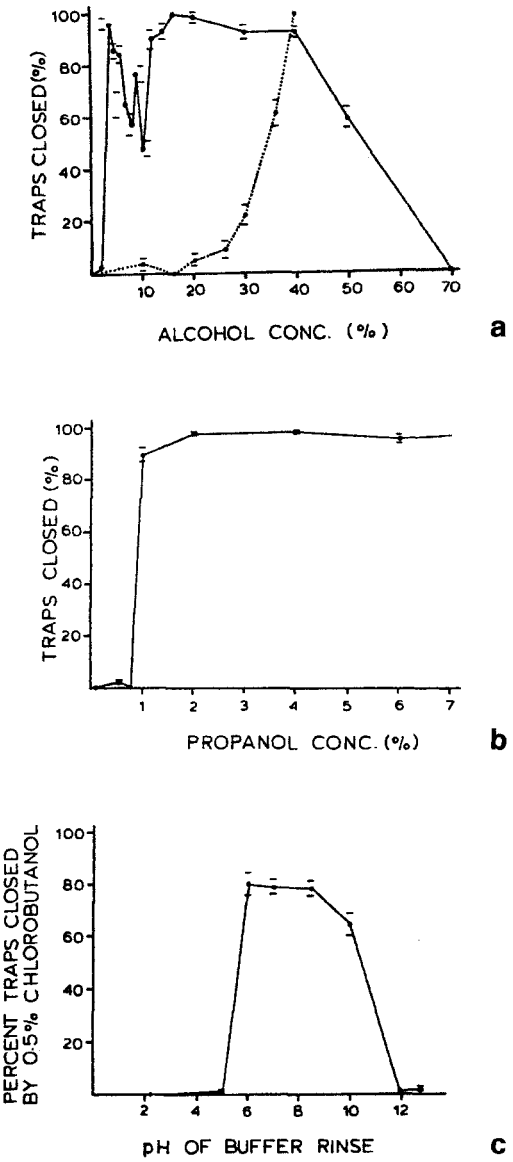

Fig. 1. **a** Effect of increasing methanol concentrations (·····) and ethanol concentrations (————) on inducing 3-cell closure of ring traps about 15 seconds after application of alcohol. **b** Effect of increasing propanol concentrations on inducing 3-cell closure of ring traps about 15 seconds after application of propanol. **c** Effect of 0.5% chlorobutanol on inducing 3-cell closure of ring traps held at various pH values. The traps had been rinsed with the appropriate buffer about 1 minute before application of the chlorobutanol solution. Each point represents mean ± SD in a randomly selected field of about 50–150 ring traps, respectively

Muller (1958) attempted to induce ring closure with several chemicals, including chloroform, ether and benzene, with no success. His finding was confirmed in the present investigation which raises the question of why these strong lipid solvents should be ineffective. All four of the alcohols used are nonionic polar compounds

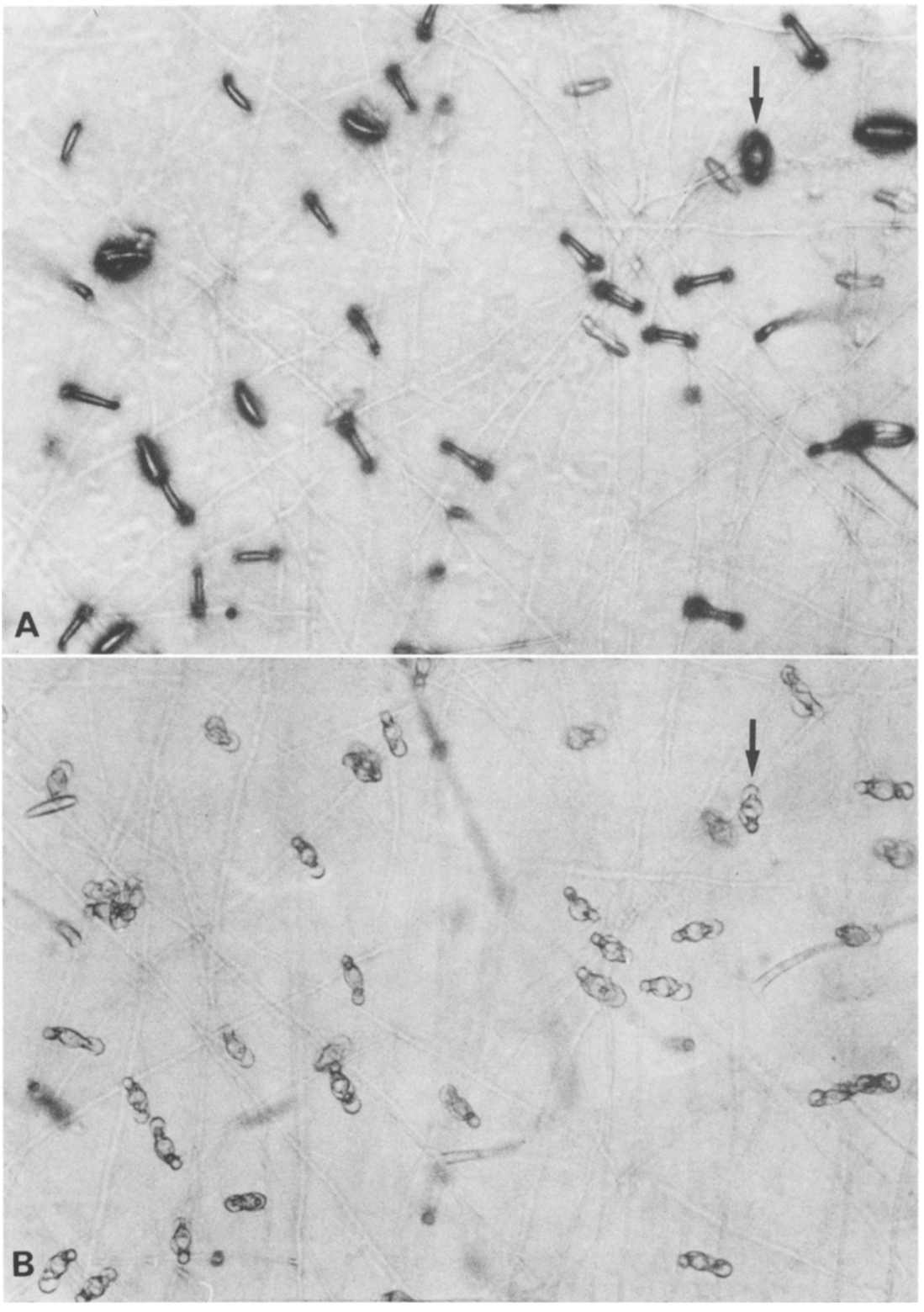

Fig. 2. Ring traps before (**A**) and about 15 seconds after (**B**) application of 6% propanol. Arrow indicates spontaneously closed ring trap which was not scored in making estimate. × 280

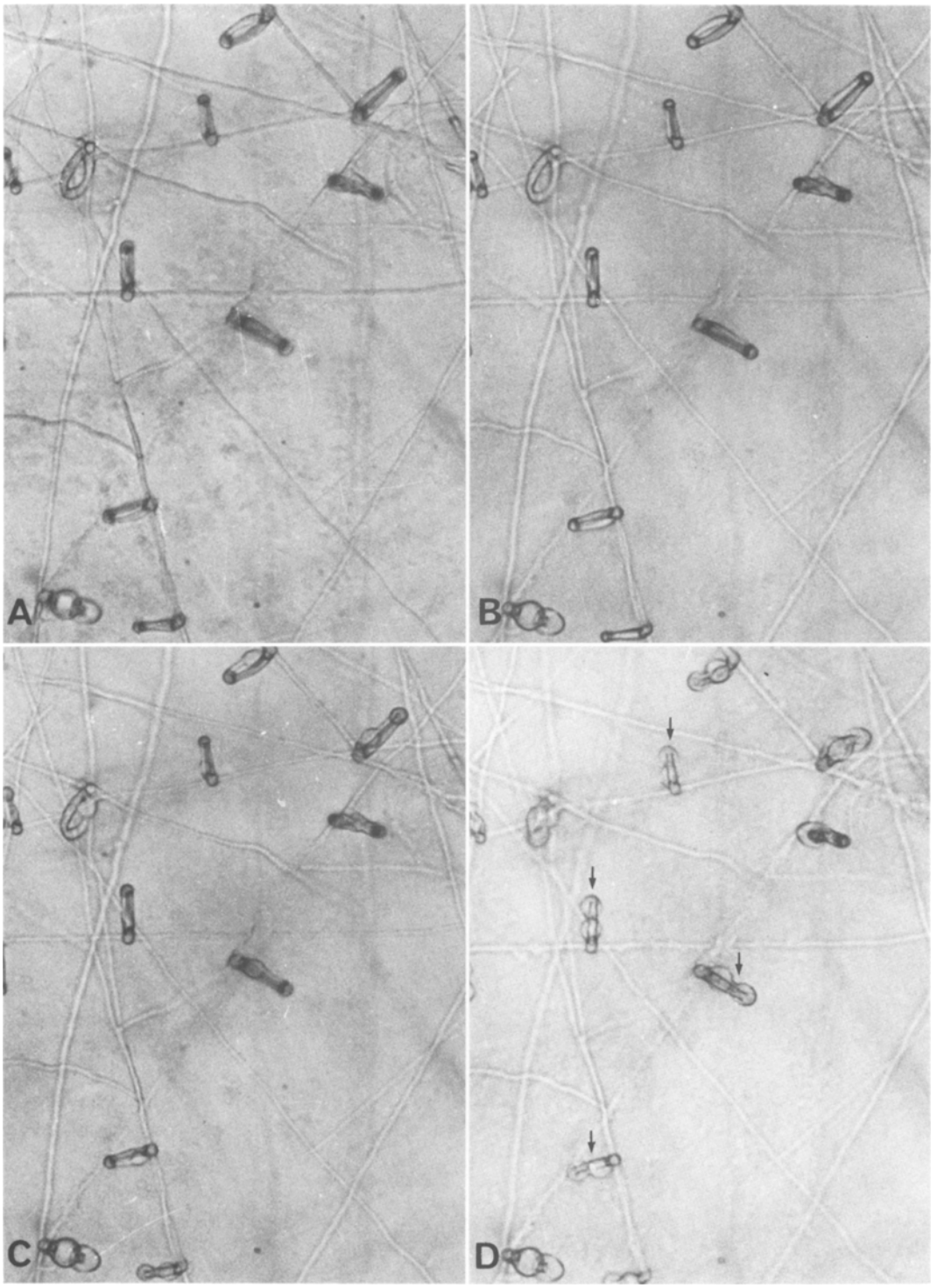

Fig. 3. Time-lapse photos of ring traps after addition of (**A**) water, (**B**) 30% methanol, (**C**) 36% methanol, and (**D**) one minute after addition of 36% methanol. Arrows in **D** show traps exhibiting gradual expansion of the two youngest cells; the unexpanded first-formed cell is seen as two parallel lines. × 280

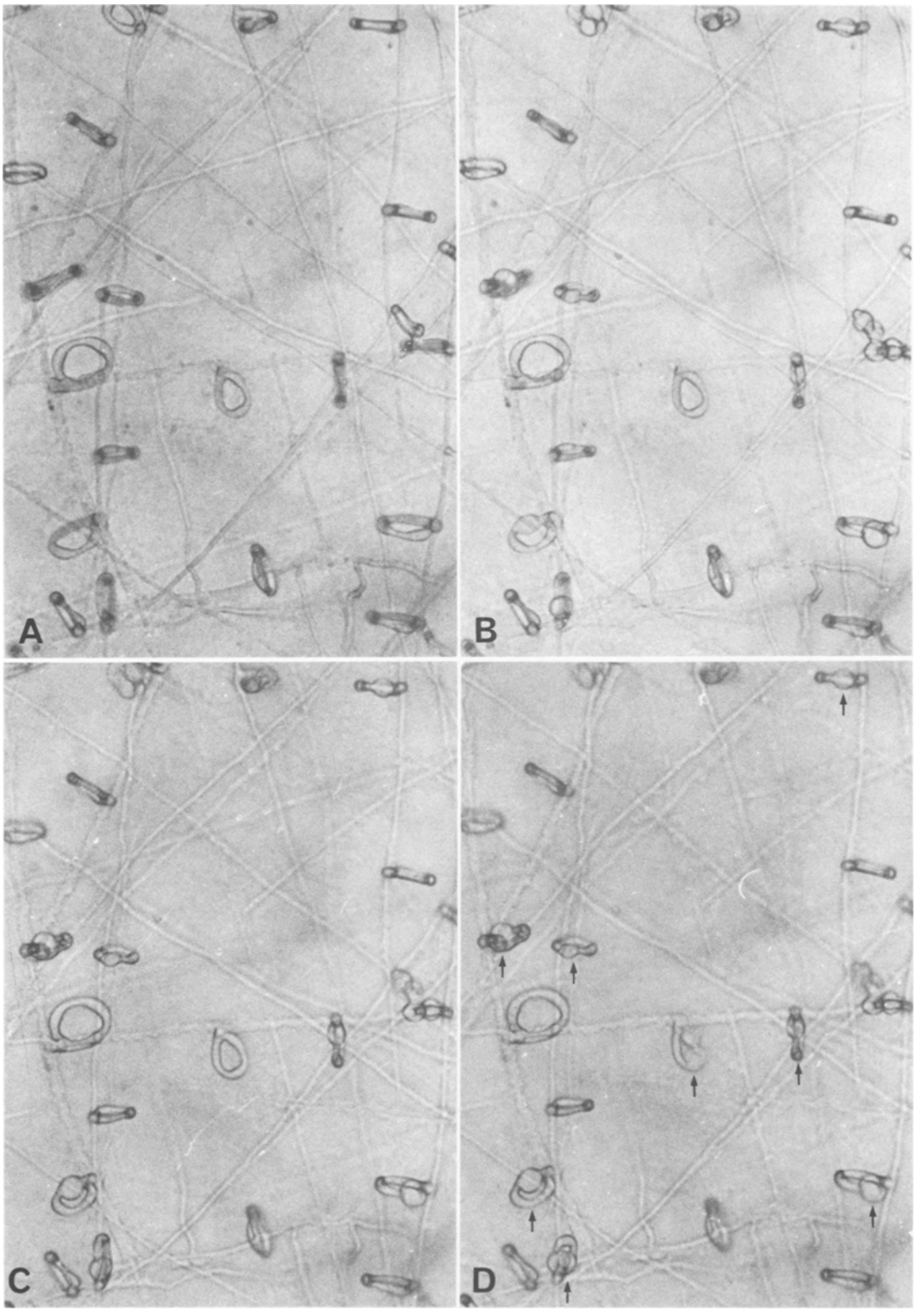

Fig. 4. Time-lapse photos of ring traps after addition of (**A**) water, (**B**) 26% methanol, (**C**) and a few seconds later, and (**D**) 30% methanol. Arrows in **D** show traps in which only one of the three cells expanded. Usually it is the youngest cell but in some cases it is the oldest. × 280

by virtue of their strongly electronegative oxygen atoms; this probably allows them to interact with the polar head groups of cell membrane phospholipids in such a way as to bring about a change in the permeability of the cell membrane to water. They probably act directly on the cell membrane; however, an alternative target might be the accumulation of dense membrane bounded vesicles just within the membrane of the ring cell (Heintz and Pramer 1972, Dowsett et al. 1977). It is possible these contain a substance which once releaed through the agency of mechanical irritation, heat or polar solvents, increases cell membrane permeability to water (Insell and Zachariah 1978). It is relevant that the electron micrographs referred to above confirm the disappearance of these vesicles in the expanded ring trap cell. All other polar solvents used in the present study effected trap closure at appropriate concentrations; these included acetone and ethylene glycol monoethyl ether (cellosolve). It is probable that the nonpolar compounds, ether, benzene, chloroform, employed by Muller (1958) rapidly penetrate the cell and destroy some cytoplasmic component of the system controlling the triggering and inflation processes.

A remarkable feature of this study is that with all three of the lower alcohols, increasing concentrations induced an increasing fraction of the traps to close; this identifies subsets of differing sensitivity in a trap population, responsiveness perhaps being related to cell age and trap age. The effect was most noticeable with methanol (Fig. 1 a) where response was clearly a power function of alcohol concentration. With logarithmic scales, the slope is 2.80 (or about 3); a possible interpretation is that the three ring cells successively respond to an increase in stimulus strength. This was first noted by Comandon and de Fonbrune (1938) and later by Muller (1958) and Rudek (1975). Most often the last formed (youngest) of the three cells expands first (Fig. 4), followed by the next youngest and finally the oldest cell (Figs. 2 and 3). Moreover, with methanol the swelling was not instantaneous but gradual. This indicates that the sensitivity of the triggering and inflation mechanisms varies with cell maturity.

The response to propanol was dramatic; few rings were closed by concentrations below 0.75% but as the concentration was increased there was closure of about 90% of the rings over a very narrow range of 0.8–1% (Fig. 1 b). Ethanol was tested over a wider range than the other alcohols; the threshold concentration was higher than with propanol, about 2–4%, but thereafter the response was difficult to interpret. About 85–95% of the rings were closed by concentrations of 5 or 15% but concentrations of 8–11% were much less effective (Fig. 1 a). Since the sample sizes here were usually about 100 rings each it is likely that these changes were not due to disproportionate numbers of young or aged rings; pending further experiments this effect must remain unexplained. Concentrations of about 40% increasingly inhibited ring closure, probably through protein denaturation. It is interesting that whereas hot water at 50 °C induced ring closure, gradual heating to 70 °C prevented it and also killed the cells (Muller 1958). The pH of the trap environment affected ring closure markedly below pH 5.5, and less drastically above pH 8.2. It was remarkable that even at pH 10, about 70% of the rings could close normally (Fig. 1 c). This could clearly be adaptive in the natural state, allowing capture of prey even in microenvironments which did not encourage hyphal growth. It is likely that the cell membrane is a mosaic of positively and negatively charged regions (Giese 1973); external hydrogen ion concentration, by altering this pattern, would affect the number of hydrogen bonds that have to be broken in the movement of a polar permeant like chlorobutanol into the membrane (Stein 1967). Such compounds have been useful in elucidating ring trap physiology (Zachariah 1981, 1982) and future studies should take the maintenance of external pH into consideration.

Muller (1958) on the basis of his careful experimental study proposed that the primary result of the stimulus was a decrease in wall pressure. Another plausible but even simpler idea is that the outer of the two wall layers ruptures along an existing line of weakness (Barron 1981). The results I report here point to the cell membrane also as a site of primary action. A cell's membrane allows rapid water exchange; but it can always acquire an increased permeability if its lipid barrier is perturbed. It is probable that the alcohols effect this through conformational changes in both the lipid bilayer and membrane proteins.

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