(0·8 mm wide) of the etched slide were counted under the microscope, spore-groups of 2–8 being ignored. When the asci of *A. viridulus* burst, most of the projectiles are single spores, but 2–8-spored projectiles also occur as in *Sordaria*, though they are not nearly so numerous.

The results are illustrated in Fig. 1. In this, the scale above 100 is reduced to a tenth. This greatly depresses the height of the peak at a distance of 1·0–1·5 cm from the fungus, but gives curves which are easier to represent; further it is the lateral extent of the spore-deposit which is of immediate concern.

In the experiments illustrated in Fig. 1 A and B, discharge was entirely in the dark whilst for those shown in Fig. 1 C and D the 2-day dark treatment was followed by a beam of blue light to induce puffing. The considerably increased lateral extent of the spore deposit, involving greater distance of discharge, is evident. This extension may reasonably be attributed to a puff. The peak position of discharge for spores liberated during the dark period, when there is no puffing, is just over 1 cm whilst the peak distance for the puffed spores is around 5 cm. Experiments have been most consistent. Four of the five carried out are illustrated in this note. The fifth also involved a puff, but in this, although the lateral extension of the spore deposit was even greater than that shown in Fig. 1 C and D, there was no clear peak for the puffed spores.

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SELF-INHIBITION OF SPORE GERMINATION IN ASPERGILLUS NIDULANS

Decreased germination at high spore densities has been called self-inhibition or auto-inhibition. Self-inhibition has been attributed to inhibitory substances produced by the spores (Allen, 1957), and to lack of oxygen (Doran, 1922). In the present investigation self-inhibition of conidial germination was demonstrated in *Aspergillus nidulans* (strain 13, Aberystwyth).

Conidia were washed off slopes in 200 ml medical flats with 10 ml of 0·05 % Manoxol solution followed by 10 ml of sterile, distilled water. The spores were centrifuged down, washed by resuspending them three times in Ringer's solution (pH 6·5), and finally resuspended in a synthetic medium which contained 0·5 % glucose as the carbon source. A conidial suspension prepared in this manner was serially diluted with equal volumes of the nutrient solution and 5 ml of each dilution was poured into a 9 cm Petri dish. The percentage of spores which had germinated in each treatment (200–400 conidia counted per Petri dish) is shown in Table 1.

Conidial germination was enhanced when the spore suspensions were aerated with air containing carbon dioxide (Table 2) or when sodium bicarbonate was incorporated in the medium (Table 3).

Table 1. *Influence of spore density on germination*

Spore concentration, conidia/ml of medium	Percentage of conidia which had germinated after 6 h incubation at 37 °C
$2{\cdot}0 \times 10^7$	34
$1{\cdot}0 \times 10^7$	44
$5{\cdot}0 \times 10^6$	52
$2{\cdot}5 \times 10^6$	65
$1{\cdot}25 \times 10^6$	79
$1{\cdot}57 \times 10^5$	84

Table 2. *Influence on germination of aerating spore suspensions (spore density* $1{\cdot}0 \times 10^7$/ml) *with air and carbon dioxide-free air*

Treatment	Percentage of conidia which had germinated after 6 h incubation at 37 °C (mean of two experiments)
Spore suspension aerated with air containing carbon dioxide	75
Spore suspension aerated with carbon dioxide-free air (carbon dioxide removed by bubbling air through N-NaOH).	14

Five ml volumes of the conidial suspension in test tubes were aerated by bubbling the gas at a constant rate through pieces of 2 mm bore capillary tubing.

Table 3. *Influence of bicarbonate on spore germination*

Molarity of $NaHCO_3$ in the nutrient solution	Percentage conidia which had germinated after 6 h incubation at 37 °C
0	19
1/1000	36
1/750	62
1/500	63
1/250	68

Five ml volumes of the conidial suspension ($1{\cdot}4 \times 10^7$ conidia/ml) were introduced by a hypodermic syringe into sealed, 50 ml Katz flasks. The Katz flasks had previously been flushed out with carbon dioxide-free air. The pH in each treatment was 7·5.

This study indicates that the self-inhibition of conidial germination observed at spore densities above 1×10^6 conidia/ml is probably due to an insufficient supply of carbon dioxide during the early stages of germination.

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AN EFFECT OF TEMPERATURE CHANGE ON HYPHAL BRANCHING

While the influence of temperature on zonation in fungal colonies has been studied by several workers (Ellis, 1931; Hafiz, 1951; Sagromsky, 1952), the behaviour of the hyphal tips under these conditions has not been described. During a study of the effect of bacterial toxins on *Aspergillus terreus* (to be published), temperature changes in themselves were noted to have a marked morphogenetic effect on the fungus.

Experiments were carried out in liquid culture in a shaking water bath, incubated at 37 °C. It was found that when the culture flasks were removed from the water bath, allowed to stand at room temperature (*c.* 20°) or 4° for a time and then returned to 37°, a check in the growth of the hyphal tips took place, with subsequent subapical branching (Fig. 1 A). This response took place in both nutrient and glucose–peptone broths and it was subsequently found to occur also in agar containing either of these media. In the broth cultures, however, the developing branches arose a little distance from the apex, while with those in the agar media many of the branches were so close to the tip as to appear to be apical in origin (Fig. 1 B, C). Although a small number of hyphal apices showed subapical branching following 30 min at room temperature, after 4 h at this temperature all tips were affected. Transfer to 4° for 1 hr was sufficient to cause all tips to be affected. *A. niger* was found to respond similarly to alternate lowering and raising of the temperature.

This morphogenetic response of the fungi to a check in growth is similar to that described by Robertson (1958, 1959) when he exposed hyphae to osmotic changes. The replacement of a single hypha and its lateral branches which normally develop some distance behind the tip, by a tuft of hyphae, is probably the mode of growth which causes the macroscopic effect of zonation in many fungi, whether induced by temperature or other factors. Hafiz (1951) observed that a change of temperature of 1 hr duration was sufficient to cause zonation in *Ascochyta rabiei*. Ellis (1931), working with *Pleospora herbarum*, found that a daily exposure of the fungus for 1 hr to temperatures above or below the optimum