stitutes approximately half the aliphatic aldehyde present in auto exhaust and atmospheric aldehydes (*6*). Consequently, the propionaldehyde and higher-molecular-weight aldehydes make up only a small portion of the atmospheric aldehydes and ketons analyzed by the bisulfite method. The limit of 0.20 ppm given by Brennan *et al.* (*1*) may have little relation to the actual atmospheric concentration of aldehydes that form phytotoxicants. It does not follow a priori that these particular aldehydes are linearly related to the analytical measurement obtained by the bisulfite method. The more specific analytical techniques available for aldehydes (*6*) are preferable.

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19 August 1964

Spore Discharge Mechanism in Basidiomycetes

Abstract. *Spore discharge in basidiomycetes is effected primarily by the explosion of a small gas bubble in the area of the apiculus of the spore and by pressure of residual gas (probably carbon dioxide) that has accumulated between the inner wall and outer membrane of the spore apparatus. A somewhat similar mechanism of discharge has been discovered in an undescribed mycetozoan.*

Ever since the discovery (*1*) that the basidiospores of club fungi are discharged forcibly from the sterigmata bearing them, there have been various theories attempting to explain the phenomenon. Both Ingold (*2*) and Prince (*3*) have summarized these theories and have presented their own. Almost all the hypotheses have been based on the idea of discharge resulting from increased turgor within the spore apparatus to a point at which the wall in the narrow region connecting spore with sterigma is ruptured and the pressure causes the spore to be shot away. Fayod (*4*) was probably the first to note that a droplet appears at the apiculus near the base of the basidiospore just before discharge, and Buller (*5*) elaborated upon this in his extended observations.

Because Buller studied the process more extensively than any other investigator, the so-called "droplet mechanism" of spore discharge came to be associated primarily with his name. Although Buller thought that the droplet had something to do with spore discharge, neither he nor any other investigator could satisfactorily explain how a liquid droplet could be exuded from the spore apparatus at the very moment when the greatest amount of pressure is needed within for spore discharge. Buller also concluded that the droplet was discharged along with the spore, but this idea was supported by tenuous circumstantial evidence.

Recently, during studies of a new and as yet undescribed mycetozoan, I observed that the single spore is shot away forcibly from the short stalk bearing it. Upon closer observation I noted that, just before discharge, a gas bubble, apparently arising from an accumulation of a gaseous layer between inner spore wall and an outer membrane, develops on one side of the spore. After a short interval, the bubble bursts, and this minute explosion appears to be primarily responsible for dislodging the spore from its stalk and generally propelling it for some distance across the surface of the agar medium. The gaseous nature of the bubble (probably CO_2 of metabolism) was verified by the observation that it sometimes bursts without effecting discharge of the spore. Occasionally, bubbles appear and burst repeatedly without causing discharge (*6*).

Since spore discharge in basidiomycetes bears considerable resemblance to that of the new mycetozoan, observations were accordingly begun to determine whether a similar mechanism might be operable in this large group of higher fungi. Such is the case. Spore discharge was examined in the basidiomycetous yeast, *Sporobolomyces*, which was also studied extensively by Buller, and in several agarics. *Sporobolomyces* sp. was observed both in agar plates and on thin agar drops inverted on cover slips in van Tieghem cells. The medium used was Difco cornmeal-dextrose agar supplemented with 0.1 percent yeast extract.

Sporobolomyces reproduces both by budding and by the production of ballistospores. In the latter, a cell produces a sporogenous hypha which extends into the air from the agar surface and produces from one to several ballistospores (successively) on tapered extensions or sterigmata from the upper part of the hypha. Both the hypha (including sterigma) and spore assume an opacity that is due at least partly to the accumulation of a gas, again probably CO_2 of metabolism, between an inner wall and an outer, rather pliable, membrane (Fig. 1). The wall and membrane of sporogenous hypha and spore are continuous. Where the hypha is in contact with the agar or is submerged in the film of water that often collects on the agar surface in van Tieghem cells, there is no accumulation of gas and the outer membrane is not readily discernible. However, where the agar surface is dry, that part of a sporogenous hypha which sometimes lies on the surface of the agar may show an accumulation of a gaseous layer between inner hyphal wall and outer membrane on the side not in contact with the agar.

Just prior to spore discharge there no longer appears to be any connection between the inner walls of sterigma and spore. The mature spore is asymmetrically inclined away from the apicular side. There is a small but distinct pocket of gas in the region of the apiculus. At the time of discharge the outer membrane in the apicular region, which is probably its weakest spot, suddenly expands under pressure from the gas and enlarges over a period of several seconds, or sometimes longer, then bursts. This small explosion, in addition to some residual pressure in the remaining gas layer, is apparently the main force that ruptures the outer membrane just below the spore and propels the spore from its sterigma. The asymmetrical position of the spore would, of course, assist in unbalancing it in the direction of the force exerted by the bursting bubble.

When a micromanipulator needle is touched to attached spores that appear mature, some of these seem to become dislodged partly by force from within

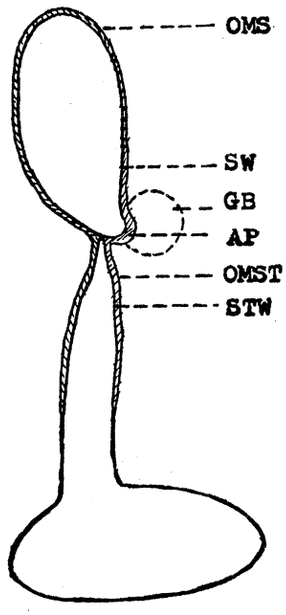

Fig. 1. Diagram of a cell of *Sporobolomyces* producing a sporogenous hypha with ballistospore. (*OMS*, outer spore membrane; *SW*, inner wall of spore; *GB*, gas bubble; *AP*, apicular region; *OMST*, outer membrane of sterigma; *STW*, inner wall of sterigma; the gaseous layer is cross-hatched).

the sporogenous system, even though no bubble is formed.

Several abnormalities that have been observed offer the best evidence that the swelling at the apiculus is a gas bubble and not a liquid droplet. In one instance a bubble burst and knocked the whole spore apparatus over onto the agar surface without causing discharge. On another occasion, as a spore was discharged from one sporogenous hypha, the explosion of the bubble was sufficient to dislodge a nearby spore from its sporogenous hypha and cause it to lodge on the inverted agar surface of a van Tieghem cell culture. No bubble formed on the latter spore before discharge. Quite frequently, under the very humid conditions prevalent in our cultures, the apicular bubble fails to appear altogether, but the entire outer membrane of the spore, probably weakened by excessive humidity, expands at once and envelops the whole spore. This large bubble may eventually burst and dislodge the spore, or it may continue to expand and even envelop one or more nearby spores. On one occasion, an apicular bubble appeared but suddenly deflated like a balloon as the entire outer membrane of the spore expanded. Buller observed these large bubbles but again mistook them for liquid droplets, an assumption which made it especially difficult to explain spore discharge in such cases.

The size of the bubble that is formed around the spore when the whole outer membrane expands indicates that, when the relatively small bubble bursts in normal discharge, there must be sufficient gas left inside the spore apparatus to exert some pressure below the spore and thereby contribute to its discharge.

Thin sections of the gills of various agarics, including species of *Russula, Marasmius*, and *Clitocybe*, as well as *Agaricus campestris* f. *bisporus*, were studied in van Tieghem cells. Although the detailed structure of the spore apparatus in these forms was less distinct than in cultures of *Sporobolomyces*, the discharge mechanism was the same, even to the common occurrence of abnormally large bubbles. Such bubbles may enlarge until they envelop all the spores on a basidium. Large bubbles frequently collapse without effecting spore discharge.

In these agarics, too, there appears to be a gaseous layer between inner wall and outer membrane of spore and sterigma. The gas bubble is normally small and develops opposite the apiculus, which is always pointed toward the central axis of the basidium. Typically, the bubble persists for only a few seconds, and when it bursts the spore is discharged. As noted by other investigators, the spores of a basidium (usually four in number) are discharged successively and never simultaneously. The best evidence of the gaseous nature of the bubbles has come from the observation that they sometimes burst without causing spore discharge.

The outer membrane of the spore apparatus has some very interesting properties. When surrounded by air it becomes impervious to the gas that accumulates between it and the inner wall. This property would likewise protect the spore apparatus and the discharged spore from desiccation. When wet, however, the outer membrane apparently permits free passage of the gas, since there is no accumulation of it in submerged cells or portions of sporogenous hyphae in contact with a wet surface (*Sporobolomyces*). Also, the membrane is quite pliable and stretchable, as demonstrated in the development of both normal and abnormal bubbles.

Since the liquid droplets described by Buller and others were not observed in my study, it seems likely that the earlier investigators mistook gas bubbles for droplets. The bubble discharge mechanism is probably characteristic of all higher basidiomycetes with ballistospores. Since it has also been observed in a mycetozoan, the mechanism should be looked for elsewhere. Also, the possibility of discharge without bubble formation but entirely by gas pressure from within the discharge apparatus should not be overlooked.

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Infestation of the Copepod Acartia tonsa with the Stalked Ciliate Zoothamnium

Abstract. *An entire population of the copepod* Acartia tonsa *in the Patuxent River, Maryland, was infested with a stalked protozoan of the genus* Zoothamnium. *Each copepod had 25 to 200 ciliates attached around the appendages. The infestation occurred at the time when* Acartia tonsa *was being replaced as the dominant copepod by* Acartia clausi.

As part of a large-scale ecological investigation of the Patuxent River estuary of the Chesapeake Bay system, periodic zooplankton collections are made with a towed ½-meter No.

Table 1. Average number of copepods per cubic meter and temperature and salinity ranges during period of infestation.

A. tonsa	*A. clausi*	Temp. range (°C)	Salinity range (0/00)
	5 March 1964		
13,000	900	2.34–6.12	4.65–14.22
	24 March 1964		
8,000	7,800	8.06–8.89	2.0 –12.10
	14 April 1964		
980	11,000	10.27–14.10	0.9 –10.3