Tiscia (Szeged) Vol. X, pp. 39—44 (1975)

MASS-PRODUCTION OCCURRENCE OF THE BOTRYDIUM SPECIES IN THE INUNDATION AREAS OF THE TISZA AND MAROS IN THE ENVIRONS OF SZEGED

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(Received 12 January 1975)

In the inundation areas of the Tisza and Maros, in a permanently sunny autumn period, the „soil-efflorescendes" of green, bluish-green or brownish-green, engendered by the mass-productions of various algae, are of frequent occurence. Among them, the appearance of *Botrydium* is not an everyday sight but if it appears every now and then, it usually calls the attention to itself with an enormous mass-production. The polyenergidic cells of Botrydium, visible even to the naked eye, take densely place beside one another, and the picture is as if the surface of soil were strewed densely with green poppy-seeds. Walking on a surface like that, one may hear crackling-rattling sounds, as well. These sounds are similar to crackling to be heard on the occassion of treading on salt or granulated sugar strewed on a smooth floor.

The occurrence of *Botrydium* earlier, in the environs of Szeged, was reported on by A. SZABADOS (1933). The occurrence of *Botrydium pachydermum* and *Botrydium granulatum* in mass-production in the confines of Deszk, Újszeged, and Szentmihálytelek was observed by him in the Autumn of 1930. These species induced their mass-production independently of others or associated. He investigated the material of these, originating from four biotops, both from morphological and cytological points of view.

I observed these two *Botrydium* species in the inundation areas of the Tisza and Maros in the environs of Szeged, in the course of the latter nearly four decades, on eight occasions altogether. A short characterization of these mass-productions is as follows:

1. Szeged, Boszorkánysziget, October 19th 1932. It formed smaller or larger mass-production spots in a length of 25—30 m, on crumbling banks. These were formed by *Botrydium granulatum* alone.

2. Újszeged, September 19th 1935. The "soil-efflorescence" of *Botrydium granulatum* appeared in the form of a 30 m long and 1 to 2 m broad greyish-green surface, on a flat bank, opposite to the Bertalan-memorial.

3. On the left bank of Maros, October 10th 1973. About 2 to 3 km above the mouth of the river, on a flat bank, the grass-green "soil-efflorescence" of *Botrydium granulatum* appeared in a length of about 200 m, on the flat bank, with several discontinuities. This phenomenon could be observed for a fortnight or so.

4. On the left bank of Maros, November 7th 1957. About 4 km above the mouth, in a 800—900 m flat riverside sector, the stands of *Botrydium granulatum* and *B. pachydermum* formed 1—2 m broad spots. The changes in that enormous mass production could be followed with attention till the end of the month.

5. "Sárga"-sands at the Tisza, October 27th 1963. At the northern end of the sands, *Botrydium granulatum* appeared in dense masses, on five rather small spots. Its state could be followed with attention for a fortnight.

6. Újszeged, November 2nd 1970. About one km north of the Bertalan-memorial, on the partly ragged, partly flat bank, in a 25 to 30 m stripe, *Botrydium granulatum* and *B. pachydermum* appeared associated.

7. "Sárga"-sands at the Tisza, November 11th 1970. In its southern part, in an about 20 m long and 2 to 3 m broad sloping riverside sector, mass-productions were formed in dark bluish-green spots mainly by *Botrydium pachydermum*. Among them, the lighter spots of *B. granulatum* spread on a minor surface.

8. On the right bank of Tisza, south of the ferry of Tápé, October 31st 1971. On the somewhat flat bank between the ferry of Tápé and the "Sárga"-sands, in an about 100 m length, in spots connected together, *Botrydium granulatum* and *B. pachydermum*, associated with each other, were forming some mass-production spots. Among these there appeared some different shapes, as well, such as not observed earlier. This mass-production series has perished till the middle of November. In the meantime we have investigated it two times. It could be established that the individual number of different forms was increasing continuously.

In the following, both Botrydium species are shortly characterized and we are reporting on the different forms and the ecological investigations.

Botrydium granulatum (L.) GREVILLE

The overground parts of plants seem from above to be globular but observed from the side they seem to be rather elongated, oval or pear-shaped. Their lower part, narrowed more and more, turns into a rhizoid-system with a manifoldly dichotomous ramification. The macroscopical polyenergidic cell is 1 to 1.2, rarely 1.5 mm long and 0.8 to 1 mm broad. Its basal part above the ramification is 35 to 50 μ thick. The young cells have a light green, the older, more developed ones a darker, grass-green hue. On their surface mostly appears a calciferous ($CaCO_3$) incrustation, softening, making rather grey the fresh, green colour.

The rhizoid-system that can be prepared but with difficulty by means of an ejector jet pump, equally surpasses the size of the overground cauloid part both in length and in projective breadth. Its final ramifications are of fourth to fifth grade, their thickness is 5 to 10 μ.

In the overground part the cell-wall is particularly thin, rather rendible, the globules sometimes burst to pieces even if touched slightly. After that the membrane opened, a protoplastic, green, thick mass is pouring out. In the fixed and smeared protoplast the nuclei can be demonstrated by staining well. Its chloroplasts are elliptic or oblongly elliptic, they seem to be of almost homogeneous substance, there couldn't be any pyrenoid demonstrated in them. In the protoplast mass, that in the beginning was pressed out of the carefully perforated green capitulum, there were comparatively more nuclei to be seen than in the plasma-mass getting out later. It is possible that the nuclei are not divided in equal proportion in the plasma-mass. (Close to the vacuolum the energid-system may be rarer?). As to the size of the vacuolum we could not obtain any sure data. We may anyway get some information concerning its presence by the comparative shortness of nuclei in the plasma-mass coming out later.

The multiplication of *Botrydium granulatum* by zoospores could be observed

but on a single occasion only, in the material of November 1957. The older mother-cells that are ripening the zoospores burst in their apical or lateral part and the zoospore-mass gets outside. We could not observe the motion of the oblong ovoid zoospores, only their weak metabolizing change in shape could be noticed. Their length is 15 to 20 μ. At sampling point 4, in the second half of November 1957 there were already to be found overwhelmingly only burst plants. It was shown by the stem of their majority that the cell-wall can burst not simply in the apical part alone but in the lower part, as well, respectively the upper larger part of the globule can be torn into pieces all at once, dispersing in pieces in the vicinity. In a case like that, the cell-wall-remains of the part of stem are turning out. At the end of November, in the rhizoid parts of second and third grades also the formation of persistent spores or cysts (Dauersporen, Hypnosporen) could be observed. These formulae are a little condensed, containing more nuclei. In the laboratory material their further development could not be observed either on the soil surface or in agar culture medium.

Botrydium pachydermum MILLER

In the eight occurrences discussed, this species had a part only on four occasions, associated with *B. granulatum*. In occurrence 7 (November the 12th 1970), *Botrydium pachydermum* played a dominant part opposite to *Botrydium granulatum*. In the other occurrences, *B. granulatum* appeared in larger masses.

This species significantly differs from *B. granulatum* in morphological respect. Its overground, cephalic part is completely globular or sometimes a little appressed, in the direction of its longitudinal axis and it is only 0.4 to 0.7 mm in diameter, as well. Its cephalic part is quite protruding from the soil, below it becomes quickly comparatively narrow, continued in a relatively long and thin cervical part. And sometimes this cervical partis, apart from being thin, strikingly short, as well. The cephalic part of *Botrydium pachydermum* mostly of bluish-green or dark bluish-green colour, considerably paled by the excretion of $CaCO_3$ granules, also selected on its surface.

The rhizoid-system is dichotomic but it is not at all as rich in ramifications as *B. granulatum* is. Even after preparing it the most carefully, there was to be observed only its third grade ramification. And it does not penetrate as deep into the soil as seen in case of the former species. The thickness of its branches of third grade varies between 5 to 12 μ. These final branches are not so easily torn, either, as in case of the former species.

The cephalic part of *Botrydium pachydermum* does generally not tear by being touched and even it is able to bear a slighter pressure, as well. The whole overground part of the small plant is comparatively tough, hardy since its cell-wall is thick enough and multilayered. The whole membrane is thick, not only in its cervical part but also in the cervical continuation. The membrane of the cervical part often suffers deep notchings, ruptures; owing to that, the cervical part has the character of constriction. That, however, can never be observed in case of Botrydium granulatum. In the final ramifications the membrane is comparatively thick. But it is questionable if it does not continue in thinner and more rendible ramifications.

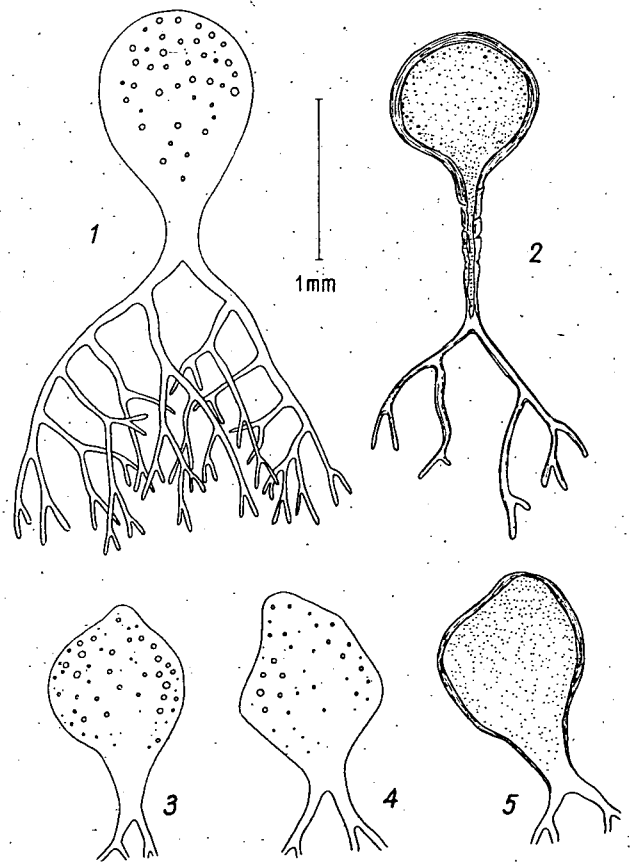
We have not observed the zoospore formation of Botrydium pachydermum on any occasion. The cyst formation could, however, be observed on two occasions: in the second half of November 1957, as well as in November 1970 (occurrences 4

and 7). Its cysts are dark bluish-green. On their surface we did not notice any calcium carbonate selection. They always contain several cell nuclei but the number of these is varying.

Botrydium-forms unnoted earlier

They could only be observed on one occasion, at the very end of October 1971 and at the beginning of November (occurrence 8), after extraordinary circumstances, in the year following the Great Inundation Defence in the Lower Tisza Region. It is known that in Spring 1970 an emergency situation was lived through in the Lower Tisza Region. The water level of the flooded Tisza and Maros was reaching such a height as never before since carrying out recordings and even from time immemorial.

It is a common characteristic of the different forms that their overground part is not globular or ovoid, like in case of Botrydium pachydermum and *Botrydium granulatum*, but it is irregularly deformed, their apical part being mostly slightly protruding. On the basis of the thickness of the cell-wall, however, in case of these, too, there were two types to be distinguished: the forms of thin cell-wall (Figs. 3, 4) and those of thicker cell-wall (Fig. 5).



The overground part of the type with *thin* cell-wall did generally not reach the length of 1 mm. The shape of its cephalic part reminds us a little of the shape of *Botrydius tuberosum* IYENGAR. Its size agrees with that of the latter one by and large. Its rhizoid-system was, however, in decay, in crumbling. In its final ramifications it did not show, in traces either, the features of the species, described by IYENGAR, the ending in globules. The slight protrusion of the apical part is a little similar to the pointed form of *Botrydium cordiforme* VODENICHAROV, being anyway not at all horn-shaped but rather definitely stocky. In this form-group, the individual shown in Figure 4 proved to be the most slender and acute. The rather acute forms like this appeared in the second week of November when even the number of these forms was considerably increasing. It could be observed that the overground part of it is not too rendible, being not completely turgescent. This cephalic part seemed to be a little wilted.

The membrane of the type with *thicker* cell-wall has mostly only two to three layers and that cannot be compared with the membrane of *Botrydium pachydermum* having as many as five to six layers. The number of these was higher, as well, in the cultivation in the secnod week of November than on the first occasion, on October the 31st 1971. Its form is similar to the former type of thin wall. The rhizoid-system of this type, as well, was in the state of disintegration.

The problem of the types of thicker and thinner cell-walls of different shapes cannot be decided, therefore, quite definitely. The fact that the rhizoid-system is easily torn in both cases and that the cephalic part is not quite turgescent, either, makes us think that from among the cells of different shapes those with thin walls are the forms of *Botrydium granulatum* and those with thick walls the forms of *Botrydium pachydermum* only that they are modified for certain reasons and have partly lost their turgescence. All this could only have been decided with physiological and genetic investigations founded on cultivation. The physiological and genetic investigations of the distortedly differing forms like these are justified by the fact, too, that their presence proved rare in the mass production found on October 31st 1971; later on, however, in the second week of November they occurred more and more frequently. That is to say: this *character of distortion was decidedly growing*, raising the reasonable thought of a kind of virus-effect, as well.

Ecological investigation of Botrydium mass productions

The mass multiplication of both *Botrydium* species fell, at all the eight occurrences, on the autumn period. That corresponds to Szabados's earlier results concerning similarly the environs of Szeged. It is not impossible that this seasonal uniformity is cloaking deeper demandable physiological connections. Concerning the two species, the following comparative ecological (partly phisiological) observations, as well, are to be mentioned:

a) The granular calcium-carbonate coating of the membrane is an excretory product. In case of *Botrydium pachydermum* the excretion is of major degree than at the cells of *B. granulatum*. In addition, the excretory coating is often strengthened by the condensation of clay-colloids, too, from the soil surface. These can be wiped away, together with the possible fine grains of sand, without the danger of ripping up the cephalic part.

b) The rhizoid-system of *Botrydium granulatum* is more developed than that of the individuals of *B. pachydermum*, and it penetrates, too, deeper into the soil. Their

preparation can mostly be carried out only with the method of water-jet maceration. As a result of water, however, the cephalic parts of *B. granulatum* often bursted, while *B. pachydermum* could endure, without any visible lesion, the macerating or mechanical effect of water.

c) At all the eight occurrences, we carried living material, as well, from the mass productions for being observed in the laboratory. The transportation with the 1.5 cm thick soil layer of the habitat, and the covering in Petri dishes, provided the transport without damage. We have observed that the capitula of *B. granulatum* in the covered Petri dishes mostly burst till next day, *B. pachydermatum*, on the other hand, could endure well the laboratory conditions, as well.

d) The formation of cysts or persistent spores fell on the late autumn period in the culture of both species. Their development may have been in connection with the effects of cold, resp. of a slight frost. That was observed both by SZABADOS (1933). and by us. On the other hand, in case of the individuals brought into the laboratory and accomodated to the conditions there we have learned that, after being dried or illuminated with a rather strong light, the protoplast or at least a part of that migrates into the rhizoids, beginning to be transformed there into cyst-like formations. A further development of these formations could, however, not be observed under laboratory conditions, even in some weeks or months. The further development of these multiplying formations must probably claim a certain period of rest and more suitable conditions of environment.

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