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STUDIES ON *MORTIERELLA RAMANNIANA*

I. RELATIONSHIP BETWEEN MORPHOLOGY AND CULTURAL BEHAVIOUR OF CERTAIN ISOLATES

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(With Plate 27 and 4 Text-figures)

Isolates of *Mortierella ramanniana* from various British sources are separated into two varieties, *M. ramanniana* var. *ramanniana* and *M. r.* var. *autotrophica* var.nov., on the basis of gross and micro-morphology.

The two varieties retained their morphological identities under a variety of cultural conditions, including changes in the medium type, carbon source, temperature and pH, and differed inherently in their quantitative growth. They also differed in response to certain media, in amount and rate of spore production, and in the effect of light on sporulation, in pH optima for growth, and in vitamin requirement. *M. r.* var. *ramanniana* requires thiamine or thiazole for growth, but var. *autotrophica* has no such requirement. *M. r.* var. *angulispora* has a partial requirement.

he fungus was first isolated by Möller (1903) and named *Mucor ramannianus*; Linnemann (1941) transferred the name to *Mortierella* on comparison with *M. isabellina* Oud. The chief reason for the transfer was the similarity between sporangia of the two species. *M. ramanniana* has a distinctive mucoracean columella and, although the sporangia of this species are small in comparison with those of the genus *Mucor* in general, the ratio of the columella size to the size of the sporangium is of an order similar to that found in many species of *Mucor*. As Turner (1963) observed, the Isabellina group of the genus *Mortierella*, which includes *M. ramanniana*, has certain features characteristic of the genus *Mucor*; it should perhaps be considered a bridging group between *Mortierella* and *Mucor*.

MATERIALS AND METHODS

Isolation techniques

The species was isolated from soil crumbs using the technique of Warcup (1950) and from root segments and other plant material by plating out on complete media. Surface-sterilized roots were first held in 0·1 % mercuric chloride for 30s, washed for 5 min in each of three changes of sterile water and cut into segments using sterile instruments. All isolates were monospore cultured following their isolation.

Media

The following media were used in solid culture work: 2% malt extract (MA), Czapek-Dox (CD), Czapek-Dox + 0·5% yeast extract (CDY), oatmeal (OM), Melin + 2% malt extract (MM) and potato dextrose (PDA). For experiments involving liquid culture 2% malt extract (ML) and Czapek Dox (CDL) media were used. The malt extract and yeast extract were 'Oxoid' and 'Difco' dehydrated preparations respectively and the agar was 'Oxoid no. 3'. All media, other than MM, were made up as described by McLean & Cook (1941); the recipe for MM, received from Levisohn (personal communication), was as follows: potassium dihydrogen phosphate, 1·0 g; ammonium chloride, 0·5 g; magnesium sulphate, 0·1 g; glucose, 20·0 g; malt extract, 20·0 g; Oxoid no. 3 agar, 15 g; glass-distilled water, 1 l. For investigations of the effect of carbon source on growth, the medium was CDL plus a growth factor complex with the various carbon sources replacing the sucrose in equivalent percentage.

Inocula

Solid media were inoculated with mycelial point inocula. Liquid media were inoculated with spore suspensions made up in sterile distilled water immediately before use; spore counts were made with a Hawkesley haemacytometer cell with a Bürker graticule and the suspensions adjusted so that equivalent inocula were used with each experiment.

Techniques of measurement

Measurements of various morphological structures, other than spores, were made from slide cultures irrigated with water when necessary; the duration of measurement of any one preparation did not exceed 30 min. Spores were mounted on a thin film of water agar; tests revealed that there was no change in their size over a period of 3 h. A hundred structures of each type were measured using a Baker screw micrometer eyepiece.

Linear measurements of colony growth were made along two diameters and the mean was taken.

Dry-weight measurements of liquid-grown cultures were made by filtering off the colonies, which were then washed with distilled water and gently pressed between filter-paper pads to remove excess moisture; the colonies were placed on pieces of aluminium foil of known weight and oven dried for 12 h at 100 °C.

Pigment extraction

For pigment extraction large quantities of spores were amassed in sterile distilled water. Suspensions were centrifuged at 4000 ***g*** for 20 min and the supernatant was discarded; solvent was added and the spores were resuspended by shaking. The standard methods for pigment extraction were employed and the solution of the pigment was assessed by centrifugation or filtration. Ether, petroleum ether, ethyl alcohol, acetone, benzene and chloroform were used as solvents.

Estimation of sporulation

The method employed for estimating spore output gave the number of spores/cm^2 of colony surface which would come into suspension without the use of mechanical force other than rotation of the culture tube between the palms of the hands for a fixed period of time. Sterile water (10 ml aliquots) was added to each culture tube. The tubes were hand rotated and the resultant spore suspensions poured into 250 ml Erlenmeyer flasks containing 90 ml of sterile water which were mechanically shaken for 1 h. Spore counts were made using a Hawkesley haemacytometer cell with a Bürker graticule. The efficiency of the counting technique was tested by taking dilution plate samples of the estimated spore suspensions; the dilution plate counts revealed that the number of colonies which arose differed from the estimated number by a maximum error of 1·2% and a mean error of 0·6%. After the spore suspensions were prepared the cultures were removed from the tubes and their surface areas calculated.

Light treatment

Cultures were grown in a constant temperature room (25°) and fluorescent light banks (65 ft-candle) were used; dark-incubated cultures were placed in boxes lined with black paper. Cultures were incubated in continuous light, continuous darkness and alternating light/dark periods of 12 h duration.

Buffering, pH adjustment and measurement

Media were either buffered with McIlvaine's citrate–phosphate buffer or unbuffered, the pH being adjusted. The two components of the buffer and the medium were autoclaved separately and mixed after cooling. The pH of unbuffered media was adjusted by use of N hydrochloric acid (HCl) and N sodium hydroxide (NaOH). The pH of the media was measured colorimetrically, using B.D.H. indicators. Experiments were carried out in plate and static liquid culture on MA and ML media respectively; 100 ml Erlenmeyer flasks containing 25 ml aliquots of medium were used for liquid culture. All cultures were grown at 25°; the growth of the liquid cultures was measured after 10 days incubation.

Growth factors

Master solutions of thiamine and thiazole were made up in absolute alcohol and diluted, as required, with sterile glass-distilled water. The vitamins were added to sterile media and all glassware was chemically cleaned before use. Experiments were carried out in static liquid culture; 100 ml Erlenmeyer flasks containing 25 ml aliquots of CDL medium were used. Cultures were incubated at 25° for 12 days.

RESULTS

Morphology

The sources of isolates are shown in Table 1. Isolates can, on gross and micro-morphological criteria, be placed in two distinct groups, for purposes of clarity designated A and B. The descriptions of Möller (1903), Hagem (1910) and Müller (1941) of *Mucor ramannianus* (*Mortierella ramanniana*) tend to fit isolates of the B group and Hepple's (1958) description tends to fit the A group; the descriptions of Zycha (1935) and Gilman (1957) and the description of *M. ramanniana* var. *ramanniana* by Turner (1963) fit isolates of both groups to some extent.

Table 1. *Group type and source of isolates*

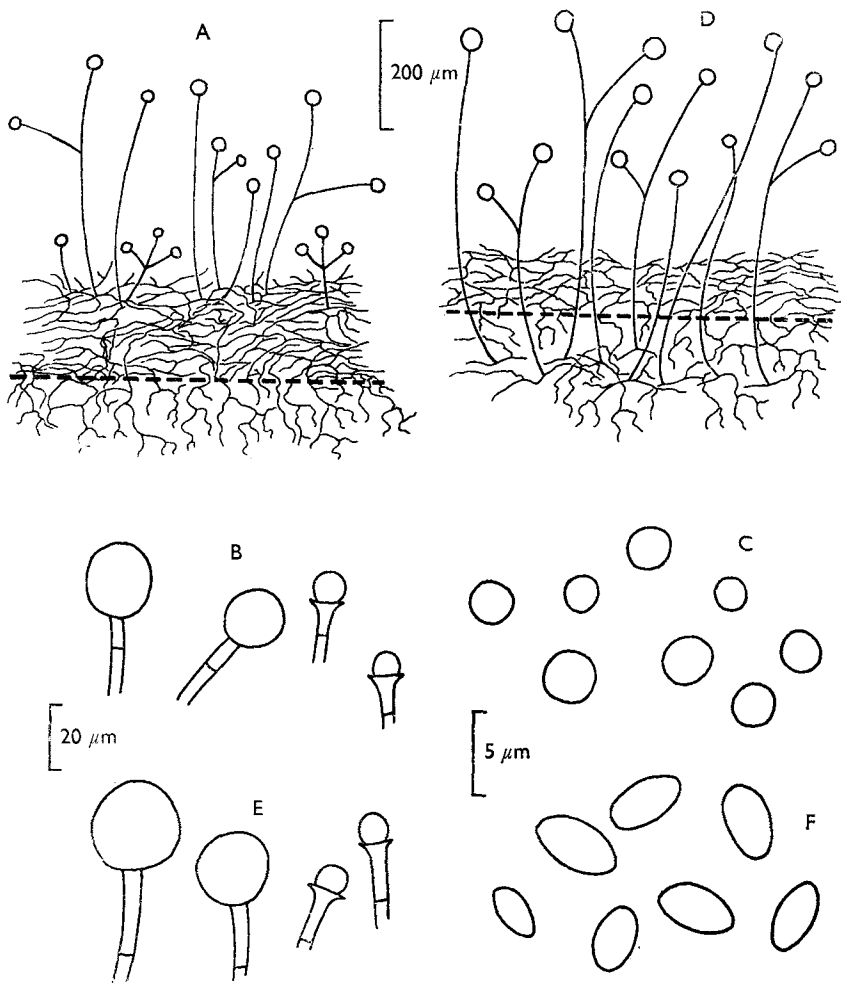
Group A (6 isolates)		Group B (6 isolates)	
A1	Soil plate—Leith Hill, Surrey	B1	*Pinus sylvestris* root surface washing—Oxshott Heath, Surrey
A2	Soil plate—Oxshott Heath, Surrey	B2	*Boletus scaber* sporophore—Bagshott Heath, Surrey
A3	Soil plate—Sugar Hill, Dorset	B3	*Boletus carpini* sporophore—Berkhampstead, Hertford
A4	Soil dilution plate—Wareham Heath, Dorset	B4	Decaying apple fruit—Belfast, N. Ireland
A5	Soil dilution plate—Forestry Nursery, Yorkshire	B5	*Calluna vulgaris* root segment—Leith Hill, Surrey
A6	Soil dilution plate—Forestry plantation, Glamorgan	B6	*Pinus sylvestris* root segment—Coedmor, Anglesey

On a natural medium (MA) and on a semi-synthetic medium (CDY) gross morphological differences between the isolates of the two groups are marked, whereas within the groups morphological differences between the isolates are comparatively slight. Some isolates do differ from other members of their group: A 1 (Table 1) exhibits delayed sporulation in which for the first 3–4 days of growth the colonies are white and then slowly become pink from the centre outwards, whereas other members of the group produce colonies which are pale congo pink from the start; B 6 (Table 1) tends to have longer sporangiophores and a less dense turf than other isolates of the group. The most striking difference between the two groups of isolates is in colour: the A group are pale congo pink whilst the B group are vinaceous brown. The descriptions of colour have been made by comparison with the colour standards of Ridgway (1912).

On MA and CDY the growth pattern of all isolates is uniform, the turf of the A group being dense, high and velvety to floccose whilst that of the B group is less dense, low and velvety (Pl. 27, figs. 1, 2). When cultured on a synthetic medium (CD) isolates of the A group produce an even higher, more fluffy turf than on MA or CDY (Pl. 27, fig. 3), together with a delay in sporulation readily detected since the pigment characteristic of the species is contained in the spores. For the first 4–5 days of incubation the colonies are more or less white, slowly becoming pale congo pink only when

sporangia form and mature. Isolates of the B group are incapable of growth on CD (Pl. 27, fig. 3).

The zonation described by Turner (1963) as resulting from the stimulation of sporulation due to small temperature fluctuations is evident in both groups of isolates; colonies of the A group show a similar zonation under alternating light/dark conditions, those of the B group do not.



Text-fig. 1. *M. ramanniana* var. *autotrophica* (A group). A, Mycelium and sporangiophores; B, sporangia and columellae; C, spores. *M. ramanniana* var. *ramanniana* (B group). D, mycelium and sporangiophores; E, sporangia and columellae; F, spores. (A and D drawn from slide culture.)

The pattern of hyphal growth and branching is the same in both groups. Although the turf of the B group is thinner than that produced by the A group the former has longer sporangiophores; there is a tendency for the sporangiophores of group B to arise from hyphae within the medium

whilst isolates of the A group produce sporangiophores on aerial hyphae (Text-fig. 1 A, D). A columella is present in all isolates as is a cross-wall a short distance below the sporangium; on average the sporangia of the B group are larger than those of the A group (Text-fig. 1 B, E). The spores of group A isolates are smaller than those of group B and are irregular to spherical in shape; those of group B are oval to ellipsoid (Text-fig. 1 C, F).

Isolates of group A occasionally produce distinct sectors in plate culture, those of group B do so only very infrequently. Generally the sectors differ only temporarily from the parent type and revert to the parent form after one passage (Pl. 27, fig. 5); on one occasion, however, two sectors developed in a group A plate culture which have remained constant in their difference from the parent type over a period of 12 years and innumerable passages (Pl. 27, fig. 4). Those mutant sectors and their progeny have all the tested characteristics of the group B isolates including the inability to grow on a synthetic medium.

Cultural characteristics

All isolates of the groups A and B were grown on six different media, MA, CDY, CD, OM, MM and PDA. Growth and sporulation of all isolates was best on PDA, MA and CDY; on OM sporulation was lower, particularly in the A group, and on MM isolates of both groups showed poor growth. The isolates of group, B failed to grow on CD whilst the isolates of group A grew well on this medium.

On a variety of carbon sources such as mono-, di-, tri- and polysaccharides, glucosides and alcohols there was no marked difference in response between the two groups of isolates in most instances. However, isolates of the B group grew, albeit poorly, on media containing aesculin and amygdalin, whereas isolates of the A group produced no growth on such media. It is of interest that all isolates grew well on sucrose, contrary to the observations of Margolin (1942) who recorded poor growth of the fungus on this disaccharide. In the present study the sucrose medium was sterilized by Seitz filtration to avoid possible hydrolysis during autoclaving.

The optimum temperature for the growth of all isolates was found to be 25°; group A isolates grew reasonably well at 35°, isolates of the B group failed to grow at this temperature.

Preliminary observations on the effect of the pH of the medium on the growth of all isolates were carried out using both buffered and unbuffered MA medium in plate culture. All isolates grew best under acid conditions; the optimum pH for group A was higher than that for group B, the values being in the region of 5·5 and 4·5 respectively.

A comparison of the growth rate and dry weight of mycelium produced by the respective groups revealed an inherent difference between them; under equivalent environmental and nutritional conditions and using equivalent inocula, isolates of the B group showed a significantly higher linear growth rate than did those of group A, whereas the dry weight of mycelium produced by group A isolates was significantly higher than that produced by those of group B (Table 2). Differences in the amounts of growth shown by isolates of the same group were not significant.

Preliminary investigations into the cultural behaviour of the two morphologically distinct groups of isolates indicated that they also differ physiologically; under the cultural conditions employed isolates of the same group showed uniformity of response to any particular treatment, whilst the inherent morphological and quantitative growth differences between the groups remained constant. The degree of similarity of response among isolates of the same group was such that randomly selected isolates were used for further investigation of certain of the cultural characteristics.

Table 2. *Growth of all isolates of groups A and B (results from four replicates)*

Colony diameter (cm)		Dry weight (mg)	
Group A	Group B	Group A	Group B
6·5 ± 1·2	8·6 ± 0·4	201·0 ± 15·3	160·0 ± 14·0
6·9 ± 1·1	9·1 ± 0·4	202·0 ± 3·4	128·0 ± 9·2
6·3 ± 0·2	8·6 ± 0·4	198·3 ± 5·8	153·6 ± 6·1
6·6 ± 0·8	8·2 ± 1·1	210·3 ± 7·3	162·0 ± 13·4
6·6 ± 0·2	8·9 ± 0·3	228·0 ± 6·6	160·0 ± 6·0
7·0 ± 0·3	9·0 ± 0·3	186·3 ± 10·1	176·3 ± 10·3

Pigmentation and sporulation

The pigment present in all isolates of *M. ramanniana* is a characteristic of the species recognized by all authorities who have described it. There has been general agreement that the pigment is present in the sporangia only, but some disagreement as to its exact location. Campbell (1938) maintains that the spores are 'tinged with pink' and Möller (1903) and Zycha (1935) are of the opinion that the pigment is contained in the sporangium wall. Gilman (1957) and Hepple (1958) state that the pigmentation of the sporangium is due to some interstitial material. In the present studies it has been observed that *M. ramanniana* is a 'wet-spored' fungus, the sporangial wall is evanescent and the spore drop is pigmented; microscopic examination reveals no pigmented fragments of sporangial wall and an absence of interstitial material. Centrifugation of spore suspensions gave a colourless supernatant and a densely pigmented spore pellet which on microscopic examination was found to consist of spores alone, the individual spores being a pale but distinct pink in colour.

Pigment extraction. The colour difference between the two groups of isolates remains constant under all the cultural conditions employed other than when sporulation is inhibited. Pigment extraction was attempted in the hope of determining whether the colour difference was due to pigment type but all efforts met with failure. Attempts to rupture the spores by homogenation with a silica abrasive in the hope of aiding the extraction also failed.

Relationship between pigmentation and sporulation. An attempt was made to assess whether or not the difference in colour was due to a difference in the level of sporulation exhibited by the two groups of isolates. Nine-day-old CDY slope cultures, incubated in intermittent light at 25°, of one randomly selected isolate from each group were used. Admittedly the method

employed for the estimation of spore production did not given an accurate absolute measure of sporulation but it did give a valuable measure for comparison. Results showed the spore output of the two isolates to differ markedly; the A isolate yielded considerably more spores per unit area of colony surface than did the B isolate (Table 3). There appears to be no relationship between spore output and pigmentation; the vinaceous brown of group B is a much stronger colour than the pale congo pink of group A, yet invariably isolates of the B type have been found to produce far fewer spores.

Table 3. *Estimated spore output per cm² colony surface (results from four replicates)*

Group A isolate	7 000 000 ± 168 000
Group B isolate	2 500 000 ± 304 139

Table 4. *Effect of light on sporulation, estimated spore output per cm² colony surface (results from four replicates)*

	Light	Dark	Light/dark
Group A isolate	598 140 ± 89 990	2 126 603	1 531 480 ± 111 397
Group B isolate	1 122 590 ± 262 571	1 195 520 ± 145 976	1 016 620 ± 72 002

Even if a correction factor is employed to allow for the larger size (approximately double) of the group B spores, the difference in pigmentation cannot be explained in terms of spore volume. It is therefore suggested that the difference in the pigmentation of the two groups is qualitative rather than quantitative.

Effect of light on pigmentation and sporulation. Plate cultures of all isolates of groups A and B were grown on CDY under the conditions previously described.

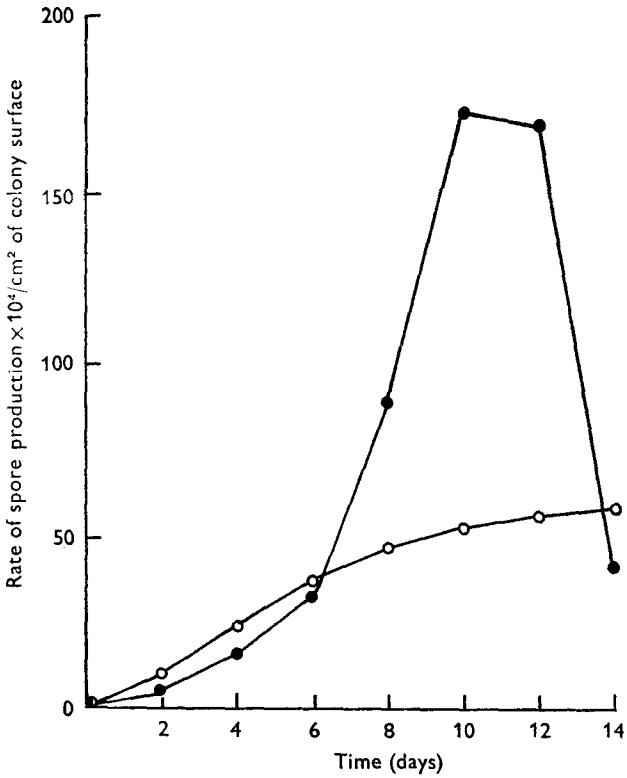
Different régimes had no effect on the degree of pigmentation in isolates of the B group and zonation was not obvious in the plates which had been grown under alternating light/dark conditions; the cultures of the A group, on the other hand, showed distinct zonation under alternating light/dark conditions and the dark-grown cultures of this group were more deeply pigmented than the light-grown ones.

CDY slope cultures of two randomly selected isolates, one from each group, were grown for 7 days under the lighting régimes described earlier; the spore output per unit area of colony surface was then calculated as previously described.

The results given in Table 4 indicate that the various lighting régimes had no significant effect on sporulation in the B isolate, whilst the effect was marked in the case of the A isolate. Whilst sporulation was not completely inhibited by either light or darkness, the spore output of the light-grown cultures of the A isolate was considerably lower than that of the dark-grown cultures; under an alternating light/dark régime the spore output of the A isolate was near the mean of the output of the light- and dark-treated cultures.

Rate of sporulation. Replicate CDY slope cultures of randomly selected isolates, incubated in intermittent light at 25°, were sampled for spore output at intervals of 48 h.

Müller (1941) found that sporulation in this species reached a maximum after 14 days incubation. The results of this experiment (Text-fig. 2) reveal a marked difference between the sporulation rates of the A and B isolates; the former reached a maximum rate in 10 days with a very sharp rise in rate during the period of 6–10 days following inoculation whilst the latter, which showed a more gradual increase in rate over this period, showed a levelling out of spore production at 14 days. Also, the difference in the rate of sporulation at various times is reflected in the results given in Tables 3 and 4; these totals are for 9-day and 7-day cultures respectively: at 9 days the rate of sporulation of both the isolates is higher (Fig. 2).



Text-fig. 2. The rate of sporulation of two isolates of *M. ramanniana*. ●, group A isolate; ○, group B isolate (results from four replicates).

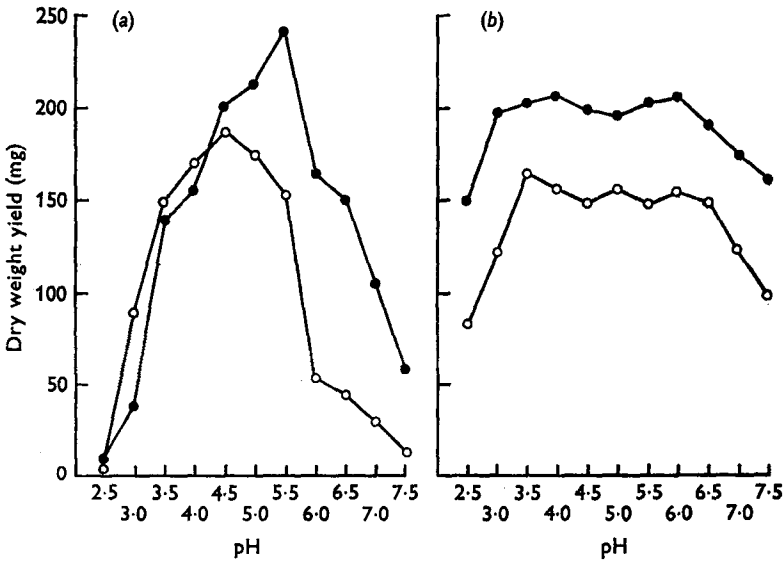
pH

M. ramanniana has been described as a fungus characteristic of acid sandy podzols (McLennan & Ducker, 1954). Pistor (1929) gave the pH optimum for growth in unbuffered media as 3·0 with relatively good growth at 6·0, the pH being adjusted by the addition of sodium carbonate or sodium citrate. Müller (1941) recorded best growth for the species between pH 3·3 and 4·1 using McIlvaine's citrate/phosphate buffer to obtain a pH range.

Effect of pH on growth. Preliminary investigation of the effect of pH on the growth of all A and B isolates of *M. ramanniana* indicated a marked difference in pH optimum between the two groups.

Two isolates, one from each group, were selected at random and their growth was measured over a pH range using both buffered and unbuffered adjusted liquid media.

The isolates of both groups produced maximum growth at pH values well to the acid side of neutrality; the optima for the two groups differed, the isolate of the A group produced maximum growth at pH 5·5, whereas the optimum for the growth of the group B isolate was pH 4·5 (Text-fig. 3*a*). On unbuffered adjusted media no clear optimum emerged (Text-fig. 3*b*).



Text-fig. 3. The effect of pH on the growth of two isolates of *M. ramanniana* in (*a*) buffered and (*b*) unbuffered media. ●, group A isolate; ○, group B isolate (results from four replicates).

Effect of fungus on pH of medium. In an experiment run parallel to the above, using the same conditions and the same quantities of inoculum, the pH of the medium was measured at 48 h intervals during the incubation period. Considerable fluctuations in the pH of the unbuffered adjusted media occurred (Table 5). The pH of the buffered media did not fluctuate in the same way but there was a drop in the pH of media with high starting values and also a slight lowering of pH in the media with the lowest starting value, namely pH 3·0, as the experiment progressed (Table 6). In some instances a fall in pH was coupled with a marked increase in sporulation as determined by increase in pigment intensity (Table 5). The dry-weight values were of the same order and pattern as those for the previous experiment; it is of interest that the greatest changes in pH were not of necessity correlated with greatest growth. The pH optima for the isolates were the same, namely pH 5·5 for isolate A and 4·5 for isolate B.

Table 5. *Fluctuations in the pH of unbuffered adjusted media (results from four replicates, pH tested colorimetrically)*

pH at start	Sampling time in days following inoculation: 2	4	6	8
Group A isolate				
3·0	3·4	2·8	3·0	2·8
3·5	3·3	3·3	2·8	3·1
4·0	4·0	4·5	4·0	4·6
4·5	4·5	4·0	3·6	3·7
5·0	5·1	4·6*	5·2	5·3
5·5	5·3	4·6*	5·2	5·3
6·0	5·6	4·5*	3·6	4·4
6·5	6·2	5·1*	3·6	4·6
7·0	6·9	6·9	5·0	5·0
Group B isolate				
3·0	3·3	3·1	3·3	3·1
3·5	3·4	3·0	3·1	3·1
4·0	4·3	4·4	3·6	3·6
4·5	4·3	4·0	3·6	3·5
5·0	4·5	4·0	3·4	4·6
5·5	5·3	4·3*	4·0	4·6
6·0	5·6	4·5*	4·6	4·7
6·5	6·6	4·6*	4·0	4·7
7·0	7·0	6·9	5·1	4·8

* Denotes sporulation burst.

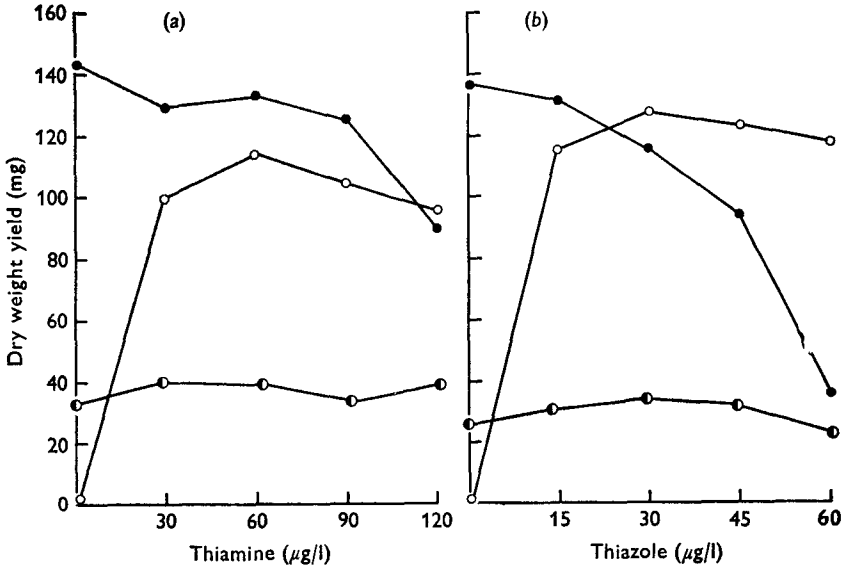
Table 6. *Fluctuations in the pH of buffered media (results from four replicates, pH tested colorimetrically)*

pH at start	Sampling time in days following inoculation: 2	4	6	8
Group A isolate				
3·0	—	—	2·8	2·8
3·5	—	—	—	—
4·0	—	—	—	—
4·5	—	—	—	—
5·0	—	—	—	—
5·5	—	—	—	—
6·0	—	5·9	5·8	5·6
6·5	6·3	6·1	5·9	5·8
7·0	—	6·9	6·7	5·9
Group B isolate				
3·0	—	—	2·8	2·8
3·5	—	—	—	—
4·0	—	—	—	—
4·5	—	—	—	—
5·0	—	—	—	—
5·5	—	—	5·4	5·4
6·0	—	5·9	5·7	5·6
6·5	—	5·9	5·7	5·5
7·0	6·8	6·8	6·8	6·8

— Denotes no change.

Growth factor requirement

Perhaps the most striking difference in the cultural behaviour of the two groups is the inability of isolates of group B to grow on a synthetic medium whilst isolates of group A grow well on such a medium. Müller & Schopfer (1937) found that the species required thiamine or more precisely its moiety thiazole for growth; Debrit & Schopfer (1946) observed that *M. ramanniana* var. *angulispora* was capable of growth on a synthetic medium. Debrit (1950) concluded that whereas *M. ramanniana* was heterotrophic in respect to vitamins its variety *angulispora* was incompletely autotrophic in this respect.



Text-fig. 4. The effect of (*a*) thiamine and (*b*) thiazole on the growth of: ●, group A isolate; ○, group B isolate of *M. ramanniana*; and ◐, *M. ramanniana* var. *angulispora* (results from four replicates).

Effect of thiamine and thiazole on growth. All isolates of group A are capable of good growth on a synthetic medium (CD), whereas all isolates of group B fail to grow on such a medium. When thiamine (60 μg/l) was added to CD or CDL the B type grew well.

A comparative study was made of the effect of thiamine and its thiazole moiety on the growth of two randomly selected isolates, one from each group, and an isolate of *M. ramanniana* var. *angulispora* obtained from a heathland soil near Turtagrø, Norway.

The group A isolate grew well on CDL in the absence of thiamine; addition of thiamine or thiazole to such a medium did not result in an increase in growth, in fact at concentrations of 120 μg/l thiamine and 60 μg/l thiazole the growth of this isolate markedly decreased (Fig. 4*a*, *b*). The group B isolate produced no growth on CDL, but grew when thiamine or thiazole was added to such a medium; the optimum vitamin level for

growth was found to be 60 μg/l for thiamine and 30 μg/l for thiazole (Text-fig. 4*a*, *b*). The isolate of *M. ramanniana* var. *angulispora* was found to be capable of growth on CDL; addition of thiamine or thiazole to such a medium resulted in an increase in growth; however the pattern of response bore no clear relationship to the concentration of vitamin present (Text-fig. 4*a*, *b*). Although the relationship between vitamin concentration and growth of *M. ramanniana* var. *angulispora* is unclear an indication of its partial vitamin requirement is obtained when its growth and that of isolates of groups A and B are compared on a natural medium (ML) and a synthetic medium (CDL). Comparison of the dry weights given in Table 7 shows that the growth of *M. ramanniana* var. *angulispora* is 70% higher on a complete medium than on a minimal medium, whereas the growth of the group A isolate is respectively only 3·5% higher.

From these observations there appear to be different levels of vitamin requirement exhibited by the species: isolates of the A group have no requirement, those of group B have a total requirement as observed by Schopfer (1934) and the variety *angulispora* has been found to have a partial requirement as observed by Debrit (1950).

Table 7. *Mean dry weight (mg) of* M. ramanniana *(group A isolate and group B isolate) and* M. ramanniana *var.* angulispora *on natural, synthetic and synthetic + thiamine liquid media (results from four replicates)*

	ML	CDL	CDL + 60 μg/l thiamine
Group A isolate	236·6 ± 11·9	226·0 ± 5·7	204·0 ± 12·1
Group B isolate	190·0 ± 14·5	—	171·0 ± 2·6
M. ramanniana var. *angulispora*	88·0 ± 1·7	34·0 ± 2·6	49·0 ± 2·0

— Denotes no growth.

DISCUSSION

Observations on the morphological and physiological characteristics of the isolates of *M. ramanniana* used in this study clearly indicate that there are at least two readily separable subspecific taxa apart from the variety *angulispora*.

There are numerous descriptions of the species (Evans, 1961), some of which quite adequately fit isolates of both types; others tend to fit more readily either the A or the B type. The major drawbacks of the existing descriptions are the lack of precision in describing colony colour and the failure to link spore shape and other morphological features with colony colour. Thus significant variants within the species, apart from the variety *angulispora*, have not been obvious. The non-requirement and requirement for thiazole by the A and B isolates respectively gives yet another level of difference between the two groups as do the physiological differences described earlier. It is of interest that Peberdy & Turner (1968) found that the esterase pattern of typical strains differed from that of their strain 163 which they noted also differed from the typical strains morphologically; strain 163 which was sent to Turner by the author is an isolate of the A group.

Although the two groups within the species are quite distinctive their close association has been confirmed by the spontaneous mutation of an A type isolate resulting in the production of B type sectors.

Isolates of the B group, in both morphological and physiological respects, are like the cultures **IMI** 29661 and 35044 (a) which are generally considered to be the typical form of the species. However it is isolates of the A type that have invariably been obtained from the B horizons of podzolic soils, of which the species is considered to be characteristic (McLennan & Ducker, 1954). Furthermore, from Hepple's (1958) description of the species, including the ability of her isolates to grow, albeit slowly, on a minimal medium and from her comments in discussion it is clear that her isolates were of the A type.

There is ample evidence that various workers have isolated and identified both A and B forms as *M. ramanniana*; however, apart from the references by Turner (1963) and Peberdy & Turner (1968) to the author's work, the two forms have not been distinguished from each other in the literature and the vitamin autotrophism of the A group has not been noted.

Peberdy & Turner (1968) point out that the species included in the Isabellina group (Turner, 1963) to which *M. ramanniana* belongs, are frequently somewhat difficult to distinguish from each other morphologically; they also suggest a similarity between strain 163 and *M. vinacea*. In view of their comments concerning this strain three points should be made clear: the author has never found isolates of the A type producing the angular spores characteristic of *M. vinacea* and has always found that such isolates have sporangiophores of two types; all isolates have been monospore cultured at frequent intervals to confirm their purity. The fact that the spores of *M. ramanniana* are uninucleate (Evans, 1961) precludes the possibility of inherent heterokaryotic states in monospore cultures.

On morphological and associated physiological criteria there appear to be sufficient invariable differences between the two groups within the species, referred to as A and B, to merit their being recognized as separate taxa. It is proposed that they be given varietal status and that the A and B forms be called *M. ramanniana* var. *autotrophica* var.nov. and *M. ramanniana* var. *ramanniana* respectively.

Mortierella ramanniana (Möller) Linnem. var. *ramanniana*

Colonies on MA and CDY vinaceous-brown with a whitish margin. Mycelium velvety 2–3 (2·5) mm high. Sporangia globose 18–29 (24) μm diam, columella present. Sporangiophores 300–700 (500) μm long, simple or branched with a single or occasionally a few branches and with a cross-wall 12–26 (20) μm below the sporangium. Spores pink, oval or ellipsoid, 1·5–3 × 2·5–5·5 (2 × 4) μm in size. Hyphae and sporangiophores hyaline. Chlamydospores and giant cells present.

Mortierella ramanniana (Möller) Linnem. var. **autotrophica** var.nov.

Coloniae in agaro malti extracti et agaro Czapekii-Doxii cum fermento extracto subroseae albido cum margine. Mycelium velveti simile vel floccosum 3–5 mm altum.

Sporangia globosa 13·5–20 μm diametro, columella praesenti. Sporangiophora bipartito, 200–400 μm longa, simplica aut ramosa cum septo 12–24 μm infra sporangium, aut rarius 50–110 μm longa semper ramosa et cum septo 7–10 μm infra sporangium. Sporae roseae, sphericae vel irregulares 2–3 μm diametro. Hyphae et sporangiophora hyalina. Adsunt chlamydospora.

Habitant solum Oxshott Heath in Surrejano Comitatu. Typus generis a solo ab E. Howell Evans seiunctus et in Herbario CMI (**IMI** 150941) et U.C.N.W. (MR, 01) positus.

Colonies on MA and CDY pale congo pink with a whitish margin. Mycelium velvety to floccose 3–5 (3·5) mm high. Sporangia globose, 13·5–20 (18) μm diam, columella present. Sporangiophores of two kinds: 200–400 (350) μm long, simple or branched with a cross-wall 12–24 (20) μm below the sporangium, and less frequently 50–110 (75) μm long, always branched and with a cross-wall 7–10 (8) μm below the sporangium. Spores pink, spherical to irregular 2–3 (2·5) μm diam. Hyphae and sporangiophores hyaline. Chlamydospores present.

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EXPLANATION OF PLATE 27

Fig. 1. *M. ramanniana* (group A and group B) on 2% malt agar.

Fig. 2. *M. ramanniana* (group A and group B) on Czapek-Dox+0·5% yeast-extract agar.

Fig. 3. *M. ramanniana* (group A and group B) on Czapek-Dox agar.

Fig. 4. Mutation resulting in permanent change in *M. ramanniana* (A group parent, B group sectors).

Fig. 5. Sectoring of temporary nature in A group colony of *M. ramanniana.*

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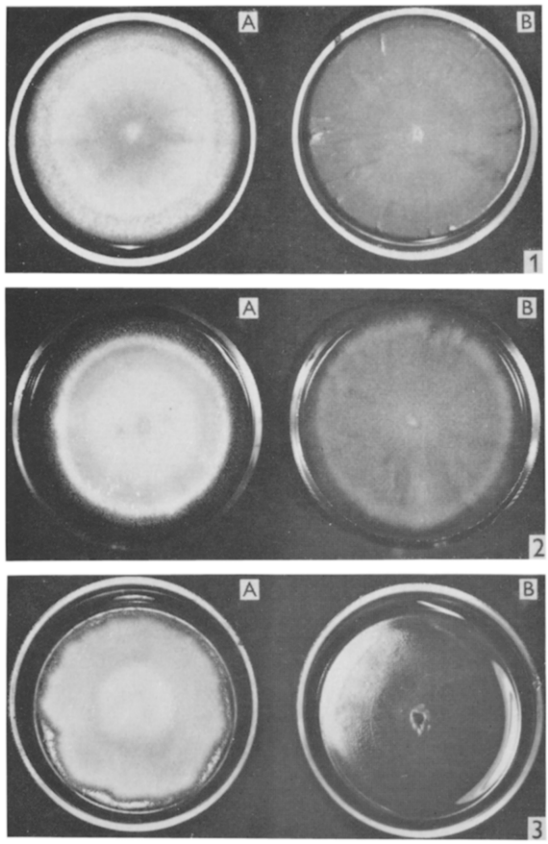

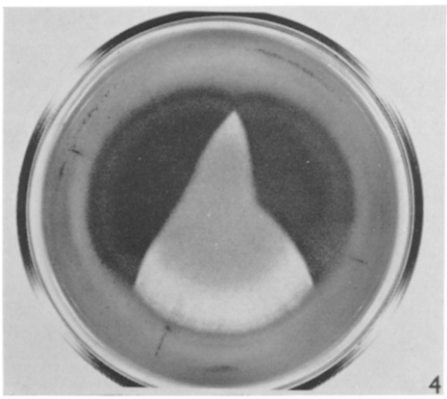

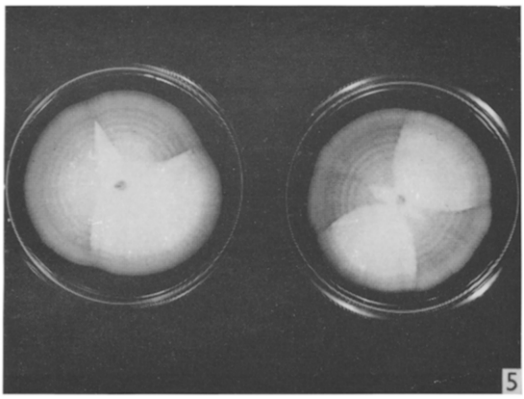

(*Facing p.* 216)