# NUCLEAR DISTRIBUTION AND BEHAVIOUR THROUGHOUT THE LIFE CYCLES OF *THANATEPHORUS, WAITEA,* AND *CERATOBASIDIUM* SPECIES

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*Summary*

Nuclear distribution and behaviour throughout the life cycles of *Thanatephorus*, *Waitea*, and *Ceratobasidium* species was studied in both living and stained preparations.

In the vegetative phase young cells of *Thanatephorus* and *Waitea* commonly contained 4–12 nuclei, whereas those of *Ceratobasidium* were binucleate. The multinucleate condition of the vegetative cells was independent of the origin of the isolates, whether naturally occurring in the field or derived from single basidiospores. In all three genera nuclear division in the vegetative cells was found to be conjugate, followed by an even segregation of the daughter nuclei. Frequent malfunction of the conjugate division resulting in uneven segregation of the daughter nuclei was almost certainly the reason for different numbers of nuclei in successive cells of young hyphae. No nuclear migration through septa was observed. In older hyphae, secondary septa formed without nuclear division, resulting in reduced numbers of nuclei per cell.

The change from vegetative to reproductive phase was associated with septation of hyphae cutting off cells with only two nuclei. In the basidia karyogamy and meiosis occurred, resulting in four haploid nuclei which migrated through the four sterigmata to form four uninucleate spores. Aberrations also occurred in the reproductive phase; three nuclei instead of two were sometimes included initially in the basidium or two nuclei sometimes migrated from the basidium into one spore. These aberrations complicate any genetical analysis based on single-spore cultures.

## I. Introduction

### (*a*) *Taxonomy*

The ecology, variation, and pathogenic specialization of *Rhizoctonia solani* and *Rh. praticola* have been studied in this Laboratory for several years. During these studies the basidial stage of many isolates formed regularly, either on agar culture or on the surface of inoculated soil. In our early work we followed the nomenclature of Rogers (1943) for the basidial stages. Many isolates agreed with Rogers' description of *Pellicularia filamentosa.* The remainder were identical with the organism described by Kotila (1929) as *Corticium praticola* and, to conform with Rogers, the combination *Pellicularia praticola* (Kotila) Flentje, 1956 was proposed. Donk's (1954, 1956, 1958) more recent work indicates, however, that the name *Pellicularia* is untenable for this group and, as we are not in agreement with Olive's (1957) transfer of these

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species to *Ceratobasidium*, we propose to follow Donk's nomenclature and use the name *Thanatephorus cucumeris* for those isolates previously described as *P. filamentosa*. For those previously described as *P. praticola* we propose the new combination apparently envisaged by Donk, *Thanatephorus praticolus* (Kotila) Flentje (= *Corticium praticola* Kotila, 1929).

Our studies on these two species have been handicapped by lack of information on nuclear distribution and behaviour throughout their life cycles. Several papers over the last 10 years have contributed information, especially in regard to *T. praticolus*, but it is still inadequate. The studies reported in this paper were undertaken to obtain the information. Studies on isolates of *Waitea circinata* and a species of *Ceratobasidium* were included for comparison with those on *Thanatephorus*.

### (*b*) *Literature Review*

Olive (1953), in his comprehensive review of the structure and behaviour of fungal nuclei, suggests that the typical life history among the Hymenomycetes involves the production by the germinating basidiospores of monokaryotic mycelia which have uninucleate cells and lack clamp connections. Most of these fungi are heterothallic, and the dikaryotic mycelium with clamp connections and binucleate cells is produced by hyphal anastomoses between compatible monokaryotic mycelia. Karyogamy and meiosis occur in the basidia, which develop four uninucleate basidiospores.

Saksena (1961*a*) and Hawn and Vanterpool (1953) state that in *Thanatephorus* the basidiospores each derive only one nucleus from the basidium, and Saksena claims that many mature spores become binucleate prior to germination by mitotic division of the nucleus already in the spore.

All studies of the vegetative cells (Fukano 1932; Hawn and Vanterpool 1953; Boidin 1954; Sanford and Skoropad 1955; Saksena 1961*b*) indicate that they are multinucleate, although there is no indication of the mechanism by which this is achieved. According to Saksena (1961*b*), Boidin (1954), and Sanford and Skoropad (1955) respectively, the number of nuclei per cell varies from 4–25, 6–30, and 2–25, but Sanford and Skoropad state that the numbers are commonly 4–8 in the tip cells and 6–11 in older vegetative hyphae. Hawn and Vanterpool (1953) suggest that eight nuclei are common in tip cells but that the number is reduced to two in older cells. Boidin (1954) found that the number of nuclei differed in successive cells of a hypha.

Saksena (1961*b*) claims to have observed the division of occasional living nuclei implying that individual nuclei divide independently, but Fukano (1932) and Boidin (1954) both observed that the nuclei come together at a common point in the tip cell and divide conjugately, after which a septum divides the cell into two; according to Boidin (1954) there is usually, but not always, an equal number of nuclei in each cell. Saksena (1961*b*) suggests that nuclear division in the vegetative cells is unlike the classic mitotic division of nuclei in higher plants, but is similar to the elongation, constriction, and separation described by Bakerspigel (1959) and Robinow (1957).

Sanford and Skoropad (1955) claim to have observed nuclear migration through septal pores and Saksena (1961*b*) illustrates structures he considers to be nuclei wedged in septal pores of stained cells. Hawn and Vanterpool (1953) found no evidence of nuclear migration through septa.

The hymenium develops on short side branches of the main hyphae but the mechanism by which the nuclear number is reduced to two per cell is unknown, although Hawn and Vanterpool (1953) suggested that septa cut off pairs of nuclei. The formation of the diploid nucleus and subsequent meiosis to give four haploid nuclei in the basidium prior to formation of sterigmata and spores are described in detail by Hawn and Vanterpool (1953) and Saksena (1961*a*). These workers agree that diploidization and subsequent meiosis occur through the formation of chromosomes, and suggest that six is the haploid number.

Boidin (1954) studied the cytology of two species of *Ceratobasidium*. In both species he found the cells generally binucleate, although in *C. anceps* some cells were 3- or 4-nucleate; in both species nuclear division was conjugate. Most spores in *C. cornigerum* were uninucleate and the vegetative cells of single spore cultures were also mostly uninucleate.

## II. Materials

The following isolates were used:

| Species | Isolate No. | Origin |
|---|---|---|
| *Thanatephorus cucumeris* (cf. Flentje and Saksena 1957) | 16 | Wheat roots, west coast, S. Aust. |
| | 80 | Pea stem, Murray Bridge, S. Aust. |
| | 48 | Soil, Waite Institute, Adelaide. |
| *T. praticolus* (cf. Flentje and Saksena 1957) | ST3 | *Sagina* sp., Adelaide. |
| | 42 | Beet stem, Slough, England. |
| *Waitea circinata* (cf. Warcup and Talbot 1962) | | *Pinus radiata* seedling roots, Penola, S. Aust. |
| *Ceratobasidium* sp.* | | *Pinus radiata* seedling roots, Mt. Gambier, S. Aust. |

## III. Methods

### (*a*) *Study of Vegetative Hyphae and Sclerotial Cells*

Isolates were grown on films of 1·5% distilled water agar about 0·5 mm thick. The required amount of molten agar was pipetted on to a sterile microscope slide and spread with a glass rod to make the film. Inoculum plugs approximately 4 by 4 by 1 mm, cut from the growing edge of cultures on potato–"Marmite"–dextrose agar or soil-extract agar, were placed on the centres of the films and placed in a moist chamber in a 25°C incubator.

* This isolate was close in microscopic features to *Ceratobasidium cornigerum* (Bourd.) Rogers, except for a variable number of sterigmata (instead of four) and very much longer sterigmata (20–38–(55) $\mu$ long instead of up to 14 $\mu$ long).

In studies of young living hyphae, common laboratory agar was unsatisfactory because solid particles in it interfered with transmitted light. "Oxoid" ion agar No. 2 was used. The inoculum plug was removed from the film after 24–48 hr, a coverslip placed on the film, and the preparation examined by phase-contrast illumination. Each preparation was studied for several hours; the growth rate slowed considerably after 1–2 hr, particularly with certain isolates. Tip cells and the first three branches back from the tip were the clearest areas for study. Older cells had large oil globules and other inclusions which made it difficult to distinguish nuclei with certainty.

Other preparations were fixed and stained (HCl–Giemsa) to enable nuclei to be counted accurately and their distribution over a wide range of cells to be studied. When required, slides of living material being examined were removed from the microscope, immediately fixed to trap nuclei in particular stages of development, and then stained.

To obtain sclerotial cells, the slide cultures were incubated before fixing and staining for longer periods in moist chambers, the period depending on the isolate. Because of cell inclusions, examination of living sclerotial cells was unsatisfactory.

### (*b*) *Study of Basidia and Basidiospores*

*T. cucumeris* isolate 80, *T. praticolus* isolates 42 and ST3, *Waitea circinata*, and *Ceratobasidium* sp. all developed basidia and spores readily on agar media of low sugar content such as soil-extract agar, cornmeal agar, or distilled water agar. The isolates were grown both on agar-coated microscope slides and in petri dishes and were fixed either shortly after basidial initials formed, or after sporulation, to study the associated nuclear distribution. Stained clumps of basidia and spores were lifted off the agar with fine needles, placed in a drop of buffer on a slide, and the coverslip, when placed over this, was gently tapped with a dissecting needle to spread out the basidia and spores for easy observation.

The other isolates of *T. cucumeris* (16 and 48) which could not be induced to fruit on agar were fruited on the surface of soil or on the bases of seedlings growing in soil as described by Flentje (1956). Small lumps of soil or seedling bases carrying the fructifications were then fixed and stained, the clumps of basidia being readily retained through the various procedures. The material for examination was mounted as described above, except that the basidia were teased apart a little and transferred several times from one drop of buffer to another to free them from adhering grains of sand. Fructifications on the bases of seedlings growing in the soil were stained on the seedlings and consistently stained better than those on soil.

Phase-contrast observation of the movement of nuclei in living basidial cells was unsatisfactory because of the orientation of the basidia, their dense aggregation, and the various cell inclusions, but it was possible to count nuclei in some living preparations.

Spore germination was studied by inverting the fructifications over agar-coated slides for varying periods, incubating the slides and fallen spores for different intervals

from 0–96 hr, and then either observing them directly with phase-contrast illumination or fixing and staining them.

### (c) *Photomicrography*

Photomicrographs were taken on "Adox KB 14" film. A Leitz "Ortholux" microscope fitted with Leitz phase-contrast equipment and either a Leica or a Leitz "Orthomat" automatic camera were used. A blue-green filter was used for stained preparations but for phase-contrast work no filter was used.

## IV. Results

The general pattern of nuclear distribution and behaviour was similar in all isolates of both species of *Thanatephorus* and in *Waitea*. The data for *T. cucumeris* isolate 48 are therefore described in full to indicate this general pattern, and only the differences in detail for the other isolates are included. The isolate of *Ceratobasidium* differed markedly from isolates of the other two genera in nuclear numbers in the vegetative hyphae. No isolate of any of the three genera formed clamp connections. Growth of isolates on different media had no effect on numbers of nuclei in each cell.

### (a) *Vegetative Hyphae and Sclerotial Cells*

(i) T. cucumeris *Isolate* 48

The pattern of hyphal elongation and branching was regular near the growing edge of a colony as illustrated in Figure 1. Cells less than 24 hr old averaged 225–250 $\mu$ in length, usually giving rise to one side branch and forming septa as shown.

The tip cell (*a*,4) carried 3–10 (commonly 5–8) nuclei, which lay scattered in the part between one-third and two-thirds of the distance from the tip to the first septum. The nuclei measured 3·1 by 2·0 $\mu$ and were oval, but readily changed shape as they moved in the cell. A circular dark patch 1·2 by 1·2 $\mu$, possibly the nucleolus, was obvious within the white cloudy mass of each nucleus. The nuclei were readily distinguished from the more sharply defined, brighter oil globules. As the tip cell (*a*,4) elongated, the nuclei moved forward keeping approximately the same distance behind the tip until the cell had reached nearly twice its original length. At this stage the side branch illustrated in (*b*,4) showed as a slight bulge in the cell wall and there was intense activity of the cytoplasm in this area. The nuclei moved up to group so closely around the leading nucleus that their individual outlines could be distinguished only occasionally as active movement continued within the group. The mass of nuclei became less dense, appearing more as strands, and finally became invisible under phase-contrast illumination leaving a clearer area in the cytoplasm. Small circular globules similar to the oil globules in older cells appeared in this clearer area. Shortly afterwards the daughter nuclei began to appear on either side of the clearer area in the reverse sequence of visibility. The original nuclei usually gave rise to twice the number of daughter nuclei, half of which moved forward towards the tip, the other half migrating rapidly back towards the developing side branch. Almost immediately a septum appeared and divided cells 3 and 4. In a freshly mounted slide and at a temperature of 22–25°C the total time for this sequence from the

formation of the septum in (*a*,4) to the new septum in (*b*,4) was approximately 25 min with 3–5 min from the grouping of nuclei to the appearance of daughter nuclei. The latter were approximately 2·0 by 1·5 $\mu$, compared with 3·1 by 2·0 $\mu$ of the original nuclei, and they increased in size until the next division.

The daughter nuclei which migrated back into cell 4 remained scattered but moved continually near the side branch as it elongated. Occasionally one or more nuclei moved into the side branch but during the early growth moved rapidly out

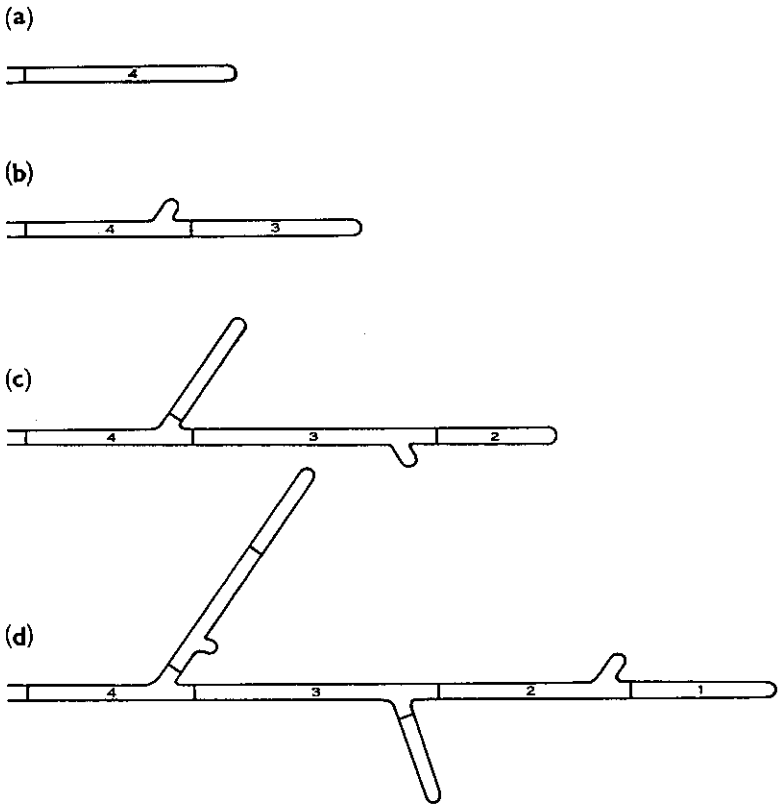


Fig. 1.—Diagrammatic representation, based on camera lucida drawings, of hyphal elongation and branching in *Thanatephorus cucumeris* isolate 48.

again. When the side branch reached a size midway between those shown in (*b*,4) and (*c*,4), the nuclei moved successively into it and usually remained there. Nuclei could be observed more easily in the slight constriction at the junction of the side branch and main hypha than in the tip cell. When they had all moved into the branch they formed a tight clump close to the main hypha and divided simultaneously as described for the tip cell. Half the daughter nuclei migrated back into the main hypha; the other half moved towards the tip of the side branch and a septum formed as shown (*c*,4). Nuclear movement just prior to grouping for division, or immediately following division, was often more rapid and with more obvious direction than at

any other time. This nuclear movement and division in a side branch is illustrated in Plate 1, Figures 1–6.

Stained preparations confirmed the above observation in tip cells and side branches (Plate 2, Figs. 1–4). Living preparations being observed by phase-contrast illumination were removed and fixed to catch nuclei at various stages, particularly during division. The nuclei appeared to break up into chromosomes at division and, although close together, maintained their separate identities. The daughter nuclei moved apart rapidly after division, often while still in the form of chromatin threads, and were already a considerable distance apart before the typical, dense, rounded nuclei were re-formed.

Two aberrant types of division were observed. In living preparations, one nucleus occasionally became separated from the main group at the time of division and the division appeared to be delayed. The separated nucleus in some cases moved

TABLE 1

PERCENTAGE DISTRIBUTION OF CELLS WITH DIFFERENT NUMBERS OF NUCLEI AND APPROXIMATE CELL LENGTH IN VEGETATIVE HYPHAE OF T. CUCUMERIS ISOLATE 48

| Type of Cell | Number of Nuclei per Cell | | | | | | | | | | | | Approx. Cell Size ($\mu$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| Tip cells | | | 12·5 | 4·2 | 25·0 | 16·7 | 20·8 | 8·3 | 8·3 | | | 4·2 | 225–250 |
| Cells other than tip cells and less than 1 day old | | 0·9 | 1·8 | 6·2 | 17·7 | 15·0 | 22·1 | 17·7 | 7·1 | 8·8 | 1·8 | 0·9 | 225–250 |
| Cells 7–10 days old | | 23·2 | 29·0 | 21·7 | 13·0 | 7·2 | 4·3 | 1·4 | | | | | 55–110 |

towards the group of nuclei much more rapidly than was observed under any other circumstances, joined the group, and division immediately took place. In other cases the separated nucleus failed to move up to the group, which divided rather sluggishly without it. The undivided nucleus usually remained behind in the penultimate cell and the number in the tip cell was reduced by one. The undivided nucleus was easily distinguished from the daughter nuclei by its greater size (Plate 2, Fig. 5) despite the increase in size of the daughter nuclei, but it mingled readily with them and took part in the next division in the side branch which grew out of the penultimate cell. After this division, all nuclei appeared to be about the same size.

The second aberration was observed in both living and stained preparations; the daughter nuclei did not separate equally, an additional one migrating into the tip cell leaving the penultimate cell one short or vice versa.

In the studies of living cells, no migration of nuclei from one cell to another through a septum was ever observed, although small oil globules were seen to do so.

It should be pointed out, however, that observation was difficult in cells four or more behind the growing tip because they contained a large number of oil globules.

The number of nuclei in cells 7–10 days old were counted in stained preparations. There were commonly 2–4 compared with 5–8 in cells less than 1 day old. About 200 cells of each category were counted. In most of these older cells one or two secondary septa had formed and reduced the average cell length to approximately one-quarter to one-half that of cells near the growing tips. The secondary septa were easily distinguished from the primary septa, the latter being thicker at the junction with the cell wall, whereas the secondary septa joined the cell walls without increase in thickness and were thinner than the original septa (Plate 2, Fig. 6).

The data on cell size and number of nuclei per cell are set out in Table 1. The numbers of nuclei in consecutive cells from the tips were also counted; those in three typical hyphae are set out in Table 2.

TABLE 2

NUMBER OF NUCLEI IN CONSECUTIVE CELLS FROM THE TIP CELL IN THREE VEGETATIVE HYPHAE OF T. CUCUMERIS, ISOLATE 48

| Hypha No. | Number of Nuclei per Cell | | | | | | |
|---|---|---|---|---|---|---|---|
| | Tip Cell | 2nd | 3rd | 4th | 5th | 6th | 7th |
| 1 | 5 | 8 | 9 | 8 | | | |
| 2 | 5 | 6 | 7 | 7 | 9 | 8 | |
| 3 | 5 | 5 | 7 | 7 | 10 | 5 | 7 |

Frequently in living preparations where several tip cells could be observed simultaneously, nuclear division seemed to be occurring at the same time in most of these cells. Preparations withdrawn and stained while division was occurring confirmed this observation. In other preparations no cells were found with dividing nuclei. Nuclear division appeared to be simultaneous throughout the thallus or at least in certain areas of it.

Sclerotial cells were formed on slide cultures at a late stage of colony development but were not suitable for phase-contrast studies. They stained clearly and the number of nuclei per cell was the same as in the hyphal tip cells.

(ii) *Comparison with Other Isolates*

There were consistently more nuclei in the young cells of *T. cucumeris* isolates 16 and 80 and of *Waitea circinata*, than in *T. cucumeris* isolate 48, but in both isolates of *T. praticolus* there were consistently fewer. The counts based on approximately 200 cells are set out in Table 3.

TABLE 3

PERCENTAGE DISTRIBUTION OF CELLS WITH DIFFERENT NUMBERS OF NUCLEI IN VEGETATIVE HYPHAE OF ISOLATES OF T. CUCUMERIS, T. PRATICOLUS, W. CIRCINATA, AND CERATOBASIDIUM SP.

| Species | Isolate No. | Number of Nuclei per Cell | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| *T. cucumeris* | 48 | | 0·7 | 3·6 | 5·8 | 19·0 | 15·0 | 22·0 | 16·0 | 7·2 | 7·2 | 1·5 | 1·5 | | | | | | |
| | 16 | | | | 0·5 | 1·2 | 6·4 | 8·1 | 35·7 | 9·4 | 28·7 | 1·2 | 4·7 | 1·2 | 1·2 | | 1·8 | | |
| | 80 | | | 0·5 | 0·9 | 2·8 | 5·6 | 5·6 | 22·4 | 14·5 | 15·0 | 6·5 | 11·2 | 4·2 | 6·5 | 1·4 | 2·3 | 0·5 | 0·5 |
| *T. praticolus* | 42 | | 4·5 | 7·2 | 26·1 | 20·0 | 20·6 | 10·3 | 8·2 | 1·0 | 1·4 | 0·7 | | | | | | | |
| | ST3 | | 1·6 | 3·3 | 6·6 | 26·2 | 29·5 | 9·8 | 1·6 | 6·6 | 13·1 | | | | 1·6 | | | | |
| *W. circinata* | | | 0·8 | 3·1 | 19·1 | 20·6 | 18·3 | 19·1 | 11·5 | 2·3 | 5·3 | | | | | | | | |
| *Ceratobasidium* sp. | | | 96·0 | 2·0 | 2·0 | | | | | | | | | | | | | | |

The other isolates of both species of *Thanatephorus* differed from isolate 48 in the manner of formation of side branches, two side branches frequently arising from the one base cell without any septum at first separating them. In such cases the nuclei moved first into the more advanced side branch, which was usually further back from the tip cell, to divide (Plate 2, Fig. 7). Half the resulting daughter nuclei then moved into the second side branch and divided, and after each nuclear division, septa formed cutting off the two side branches and leaving a group of nuclei in the base cell. Aberrations in division, as described above, were more frequent in these isolates than in isolate 48. Subsequent development of secondary septa occurred in the older cells in isolates of both species, but was less common in *T. cucumeris* isolate 16.

The isolate of *Ceratobasidium* contained only two nuclei per cell, throughout the whole range of cells examined (Plate 2, Fig. 8). In the actively growing cells these nuclei invariably became closely associated and divided simultaneously as in *T. cucumeris*.

### (*b*) *Reproductive Hyphae*

To induce fructification, agar and soil cultures were placed in diffuse light on the laboratory bench or in a controlled-environment cabinet and exposed to alternating periods of 12 hr light and 12 hr darkness. The influence of light and temperature was not investigated in detail, but a general response to light was observed. Sterigmatal growth and spore development commenced in the last 2–3 hr of the light period and continued throughout the dark period, falling off in the first 2–3 hr of the next light period. Spores began to be shed freely 1–2 hr after spore development commenced, and continued throughout the dark period and the first few hours of the next light period.

Material for staining was usually obtained during the spore-shedding period. Basidia removed during the middle of the light period showed practically no sterigmata and contained the two parent haploid nuclei or the undivided diploid nucleus.

The basic pattern of nuclear distribution and behaviour was similar in all isolates of both species of *Thanatephorus* and in *Waitea*. The data for *T. cucumeris* isolate 48, which fruited abundantly on the bases of radish seedlings, are described in full to indicate this basic pattern and only the differences in detail for the other isolates are included.

#### (i) T. cucumeris *Isolate* 48

Side branches, four or five cells behind the growing tips, formed distinctively curved prebasidial cells, shorter and more branched than ordinary vegetative cells. The number of nuclei per cell, previously 3–10, was reduced to two, as prebasidial cells developed (Plate 3, Fig. 1). This reduction was preceded by pairing of the nuclei previously scattered at random. The individuals of a pair lay close together but were separated by a considerable distance from the next pair. Septa then formed between the pairs, although in some cases the nuclei divided and new cells were formed at the same time, leading to transition cells with partly reduced numbers of nuclei which complicated the interpretation of the stained preparations. Cells

with 8, 10, or even 12 nuclei were divided by septa into 4, 5, or 6 binucleate cells each of which proliferated by cymose branching to form clumps of binucleate cells (Plate 3, Fig. 3). A similar development occurred where the original cell had an odd number of nuclei except that one cell was left with three nuclei and proliferated to form a clump of trinucleate cells (Plate 3, Fig. 2).

In the terminal binucleate cells the two haploid nuclei fused to form a diploid (Plate 3, Figs. 4 and 5), which then divided by meiosis to give four haploid nuclei. Although the formation of chromosomes was obvious (Plate 3, Figs. 5, 6, and 7), accurate counts were difficult; the chromosomes were clearest during meiosis and there appeared to be six pairs. The details of the stages of karyogamy and meiosis were difficult to distinguish.

In terminal cells whose subterminal cells had three nuclei, it was common to find one large and one small nucleus, or five small nuclei (Plate 3, Fig. 8). This suggested that two of the three nuclei fused to form the diploid and then redivided to produce four haploids while the third remained unchanged, not taking part in karyogamy. There was no evidence of degeneration of the additional nucleus.

Almost without exception the nuclear fusion and redivision occurred before there was any physical sign of sterigmata. Basidial cells with four nuclei almost invariably produced four, and rarely two, sterigmata; cells with five nuclei as regularly produced five sterigmata. The nuclei usually moved into the sterigmata when the latter had reached half or more of their final length and before the small swelling which became the spore had appeared at the distal end. The nuclei moving into the sterigmata (Plate 3, Fig. 9) were invariably elongated, which suggests that they were being drawn into it. The cytoplasm moved out completely from the basidium and in several instances it appeared to divide into four sections as it moved into the sterigmata, suggesting that each nucleus was associated with its own particular unit of cytoplasm. The developing spore had usually enlarged to half or three-quarters of its final size by the time the nucleus moved into it from the sterigma.

Among several hundred basidia examined in these stages, it was rare to find any with more than one nucleus in a sterigma and attached spore; of spores shed on to agar and immediately stained, 98–99% were uninucleate. The remainder were binucleate.

(ii) *Comparison with Other Isolates*

*T. cucumeris* isolate 80 was similar to isolate 48, but isolate 16 differed in the percentage of binucleate spores. Of the spores shed on to agar from isolate 16 and immediately stained, 12% were binucleate; in stained preparations of spores attached to sterigmata, a similar percentage were binucleate. In the latter preparations, observations were made on basidia whose spores were not yet fully formed but whose nuclei had already moved into the sterigmata. In one basidium out of every two or three, one sterigma contained two nuclei and one of the other sterigmata contained no nucleus. In the same preparations, basidia were examined at the stage when nuclei had moved into the spores but the latter had not reached full size. Again in one basidium out of every two or three, one spore contained two nuclei, and of the three or four remaining sterigmata one was without a nucleus and there

was no spore developing (Plate 3, Fig. 10). Next, the development of sterigmata and spores was observed in living material on clumps of soil. Approximately the same percentage of sterigmata failed to develop a spore. This was observed from the very early stage through to maturity to ensure that accidental detachment of spores was not overlooked. This failure was equally common on basidia with either four or five sterigmata.

*T. praticolus* isolates 42 and ST3 were similar to each other; isolate 42, which was investigated in detail, is discussed. In this species most basidia develop only three sterigmata although 5, 4, and 2 are also quite common. The basidia always contained four or five haploid nuclei derived in the manner described for *T. cucumeris* isolate 48. In basidia with four or five sterigmata, the nuclei usually moved into separate sterigmata resulting in uninucleate spores. In basidia with three sterigmata, two nuclei usually moved into one sterigma resulting in a binucleate spore, while on the other two sterigmata, uninucleate spores were formed. In basidia with only two sterigmata, two nuclei moved into each to give two binucleate spores, or occasionally three nuclei moved into one sterigma and only one nucleus into the other. Approximately 35% of the stained spores attached to sterigmata were binucleate and in almost all cases it was clear that this had arisen by migration of two nuclei from the basidium. In a small percentage, however, the binucleate condition had arisen from nuclear division within the spores. Spores were also shed on agar and stained; the percentage of binucleate spores was slightly higher than those still attached to sterigmata and it was assumed this increase was due to nuclear division in the spore.

In *Ceratobasidium* the stained preparations were unsatisfactory and detailed analysis of nuclear behaviour was not obtained although the general behaviour appeared to be similar to *T. praticolus*. In *Waitea circinata* the nuclear behaviour was similar to that of *T. cucumeris* isolate 48, except that mitotic division of nuclei in spores still attached to the sterigmata was more common than in any of the other isolates examined. Many of the mature spores were large; such spores were usually binucleate.

### (c) *Spore Germination*

(i) T. cucumeris *Isolate* 80

Spores were shed from basidia of *T. cucumeris* isolate 80 on to agar-covered microscope slides. Slides were removed after 10 min. Some were fixed and stained immediately; others were fixed and stained at approximately 12-hr intervals over a period of 4 days. Other slides were retained and the germinating spores examined alive under phase-contrast illumination.

Less than 1% of spores stained immediately after being shed from the basidia contained more than one nucleus.

Nuclear division and distribution, during and immediately following spore germination, varied in detail with different spores, but there appeared to be a general basic pattern. The nuclei divided by separation into chromosomes which in a number of instances stained clearly. The spore produced a short germ tube usually before division of the nucleus. After division of the original nucleus, one daughter nucleus

migrated into the germ tube, the other remaining in the spore. Each of these nuclei divided again at about the same time resulting in the formation of two in the spore and two in the germ tube, although a total of three nuclei was often observed, indicating independent division of nuclei during this early stage of spore germination. While the germ tube continued to elongate slightly, the two nuclei in it divided again at about the same time (Plate 3, Fig. 11). These four nuclei now divided simultaneously to give eight in the single germ tube cell and about this time the diameter of the germ tube suddenly doubled or trebled to achieve the diameter of mature hyphae. Thereafter the eight nuclei continued to divide simultaneously. Eight of the daughter nuclei migrated towards, and eight away from, the growing tip and a septum formed rapidly between them.

This pattern was varied by migration of nuclei between the group in the spore and that in the germ tube, or by non-simultaneous division of nuclei which gave rise to a group of 5–9 nuclei in the growing tip. In some instances, also, the group of two or four nuclei in the original germ tube failed to divide further and the germ tube ceased elongation, whereas the group of nuclei in the spore itself continued to divide and a new germ tube emerged from the opposite end of the spore with 5–9 nuclei in the tip cell (Plate 3, Fig. 12). This new germ tube then grew out to form the colony. In yet other instances, the nuclei in both the original germ tube and the spore continued to divide, a second germ tube emerged from the opposite end of the spore, and both germ tubes continued satisfactory growth.

In this isolate, 100% germination of spores was consistently observed; all sporelings continued to make vigorous growth to form colonies.

(ii) *Comparison with Other Isolates*

The pattern of nuclear division described for *T. cucumeris* isolate 80 was similar in *Waitea* and the isolates of both species of *Thanatephorus*. In *T. cucumeris* isolates 80 and 48, however, almost every spore germinated and grew on to produce a vigorous culture. In *T. cucumeris* isolate 16, *T. praticolus* isolates ST3 and 42, and *W. circinata* only about half of the spores produced vigorous colonies. The remainder in each isolate generally produced germ tubes but failed to achieve the stage where four or more nuclei divide simultaneously and the germ tube widens to the mature hyphal diameter.

In *Ceratobasidium* approximately half of the spores produced vigorous colonies comprised of regularly binucleate cells. The remaining spores gave rise to very slow-growing colonies with all cells more like chlamydospores than ordinary vegetative cells and in which nuclear behaviour could not be followed.

## V. Discussion

### (*a*) *Vegetative Phase*

The vegetative cells of *Ceratobasidium* were binucleate and those of *Thanatephorus* and *Waitea* were multinucleate in all isolates studied, whether naturally occurring or derived from single spores, or the result of anastomosis between single-spore cultures. The presence of more than one nucleus per cell in these genera

is apparently not dependent on anastomosis or any other mechanism leading to heterokaryosis and the ultimate production of the sexual stage, as has been suggested by Olive (1953) as typical for many Hymenomycetes. The multinucleate condition of *Thanatephorus* and *Waitea* represents a group behaviour of the nuclei which was demonstrated most clearly in those isolates in which two side branches arose from the penultimate cell of a hypha. The group of nuclei invariably moved first into the more advanced side branch to divide and then half the daughter nuclei moved into the second side branch and divided again. This occasionally resulted in three separate groups of nuclei, one in each side branch and one in the penultimate cell before any septa formed to separate them. No satisfactory explanation can be offered for the multinucleate condition, but the death of sporelings which failed to become multinucleate, the sudden increase in diameter of the germ tube, and the greatly increased rate of formation of new cells of sporelings which did become multinucleate suggest that it is associated with an ability to synthesize substances essential for cell development, rather than with the formation of the sexual stage.

In all the isolates of *Thanatephorus* and *Waitea*, nuclear division was conjugate, usually involving all the nuclei in each cell and was followed by a fairly even segregation of the daughter nuclei. These results agree with those of Fukano (1932) and Boidin (1954); conjugate division was not mentioned by Saksena (1961*b*). This conjugate division and subsequent segregation of daughter nuclei, if regular, should retain in the tip cell the genetically different nuclei of a heterokaryon. However, this conjugate division and segregation of daughter nuclei frequently malfunctioned and we believe that this was the main reason that there were different numbers of nuclei in successive cells of hyphae, which was also reported by Boidin (1954). Nuclear migration through septal pores, which Saksena (1961*b*) and Sanford and Skoropad (1955) claim to have observed, could offer an explanation for this variation. However, we found no evidence of intercellular nuclear migration in any of the living cells examined. Occasionally nuclei were found wedged in septal pores in those stained preparations where the hyphal tips had burst and cytoplasm and nuclei had extruded during bursting. The nuclei wedged in septal pores were most probably caught there in the forced movement of cytoplasm associated with bursting. We think that the variation in number of nuclei in successive cells is not due to nuclear migration, but results both from occasional failure of individual nuclei to take part in simultaneous nuclear division in the cell, and from numerical disparity in segregation of daughter nuclei after division. In addition to numerical disparity it is probable that disparity occurs also in the segregation of the genetically different nuclei which are present in a heterokaryon, but in the absence of any morphological differences between the nuclei this could not be determined. Such malfunction of the conjugate division or segregation of daughter nuclei may have an important bearing on variation in these genera.

The nuclei split into chromosomes at the time of division. We found no evidence to support the suggestion by Saksena (1961*b*) that nuclei in the vegetative hyphae divide by elongation, constriction, and separation as described by both Robinow (1957) and Bakerspigel (1959), although overstained preparations and cells where daughter nuclei were reforming after division occasionally presented an appearance which could

have been interpreted as Saksena (1961*b*) suggested. The close association of nuclei during conjugate division may present opportunity for nuclear exchange as suggested by Weijer and Dowding (1960) for *Neurospora crassa*.

The range in number of nuclei in young cells differed for different isolates of *Waitea* and *Thanatephorus*. It tended to be less in the isolates of *W. circinata* and *T. praticolus* than in the isolates of *T. cucumeris* but a more comprehensive study including the variation in single-spore isolates is required to test the validity of this suggestion.

The range in number of nuclei in cells 30–40 back from the tip did not differ from that in the tip cells, thus indicating that nuclear division did not recur in the older cells. In parts of the thallus 7–10 days old, however, short cells with fewer nuclei were common. In older cells the nuclei lay scattered evenly along the length of the cell, and secondary septation had occurred in these cells without nuclear division. The number of nuclei in these older cells appeared to be characteristic of the isolate, and depended on the original number in the tip cells and the number of secondary septa formed. In *T. cucumeris* isolate 16, cells containing fewer than four nuclei were uncommon; this was associated with one secondary septum and 8–10 nuclei per original cell. In *T. cucumeris* isolate 48, cells with 2–3 nuclei were common; this was associated with more than one secondary septum and 5–8 nuclei per original cell. It is not known whether such secondary septation of cells occurs in hyphae growing in soil, but if so, it could have an important effect on survival of the fungus in soil, by limiting any cell damage to smaller parts of the original cells. It could also have an important bearing on variation of the organism in soil if there were several different types of nuclei present in the one original cell. Regrowth from cells with only two nuclei may not reproduce the original.

Sclerotial cells were found to contain approximately the same number of nuclei as the active hyphal tips; thus regeneration of cultures from sclerotia should reproduce the original culture.

The common occurrence of nuclei at approximately the same stage of division in a large proportion of the actively growing tip cells in some preparations, and the complete absence of dividing nuclei in other preparations, suggest that, at least in a small thallus, development is coordinated throughout, rather than that each cell or each hypha controls its own development.

### (*b*) *Reproductive Phase*

In each isolate, whether it fruited on soil or on agar, there was a marked tendency for the whole thallus to commence fruiting at the same time. This may have been due to the uniformity of the environment. In agar cultures the basidia developed along particular hyphae radiating from the central inoculum plug, whereas other radiating hyphae failed to develop basidia. Basidia developed first near the centre of the culture, then progressively towards the periphery. The fact that development is not haphazard suggests that the initiation of fruiting is governed by internal factors common to the whole thallus, rather than by localized cell conditions.

In *Thanatephorus* and *Waitea* the change from vegetative to reproductive growth apparently involved all nuclei in multinucleate cells. Each pair of nuclei

divided repeatedly in association with the formation of a clump of basidia. In a heterothallic isolate, such development would suggest that there were approximately equal numbers of nuclei of different mating type in the vegetative cells. If more than two types of nuclei were present, the basidial clumps could be of different genetic constitution and give rise to wide variation in the spores.

No clear detail of karyogomy as described by Saksena (1961*a*) was obtained in the stained basidial cells of *Thanatephorus* or *Waitea*; the meiotic divisions were clearly stained but it was difficult to follow the different stages because of the small size of the nuclei. Division took place with normal development of chromosomes and in a few preparations these could be counted. We agree with Hawn and Vanterpool (1953) and Saksena (1961*a*) that there are about six chromosomes in the haploid nuclei of both genera.

Although the basic pattern of nuclear behaviour in the formation of basidiospores was similar to that described for many Basidiomycetes, the inclusion of three nuclei instead of two in the basidial cells and the migration of two nuclei from the basidium into one spore were sufficiently common in certain isolates to complicate any genetic analysis based on single-spore cultures. Such aberrant behaviour was typical of certain isolates, affecting 35% or more of spores in the isolates of *T. praticolus*, and 12% or more of spores in *T. cucumeris* isolate 16. In other isolates of *T. cucumeris* less than 2% of spores were affected. Where three nuclei were included initially in the basidium, the progress of one nucleus through to the spore apparently without taking part in karyogamy means that the spores recover the original parent nuclei in addition to recombinants. Such a mechanism may prove useful in genetical studies.

Kotila's conclusion (1929) that *T. praticolus* is homothallic requires re-examination in the light of these results, especially as Hawn and Vanterpool (1953) failed to get consistent fructifications from single-spore isolates of this organism. Many spores, which they isolated, failed to grow. It is likely that binucleate spores would have a better chance of survival than uninucleate spores and, if so, the percentage of surviving cultures derived from binucleate spores would be much higher than the 30% referred to above. It is also possible in a heterothallic organism that two compatible rather than two incompatible nuclei would be more likely to go into one spore. This may be a mechanism that excluded nuclei from some spores even though they went into one sterigma.

During the daylight period when spores were not formed, a small percentage of basidia carried spores. These remained attached to the sterigmata, but in *Waitea*, in particular, they enlarged considerably beyond the average spore size and were shed at the end of the light period or early in the dark period. Many of these spores when stained before being shed were seen to be binucleate, the original nucleus having already divided in the attached spore.

It would appear that genetic studies on any isolate of *Thanatephorus* should be preceded by cytological studies to determine the type and frequency of aberration in the behaviour of nuclei in the basidia, and their consequent effects on the characteristics of single-spore cultures.

## VI. Acknowledgments

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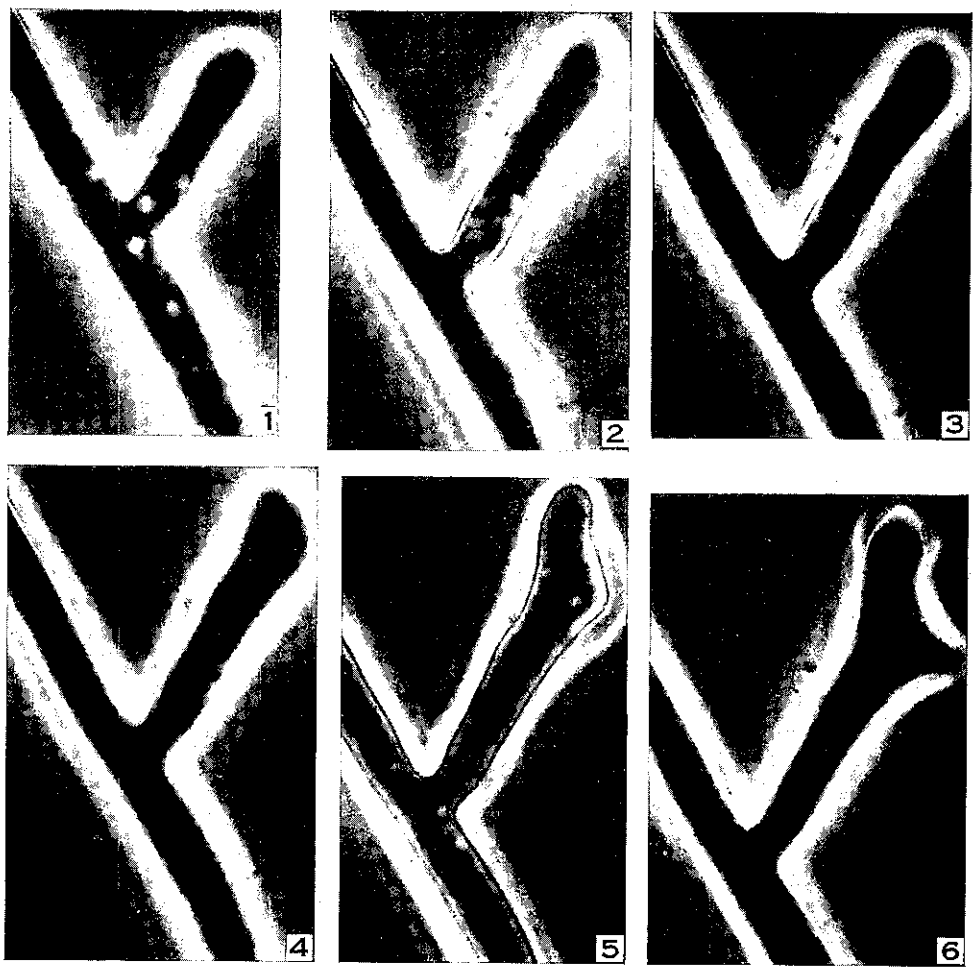
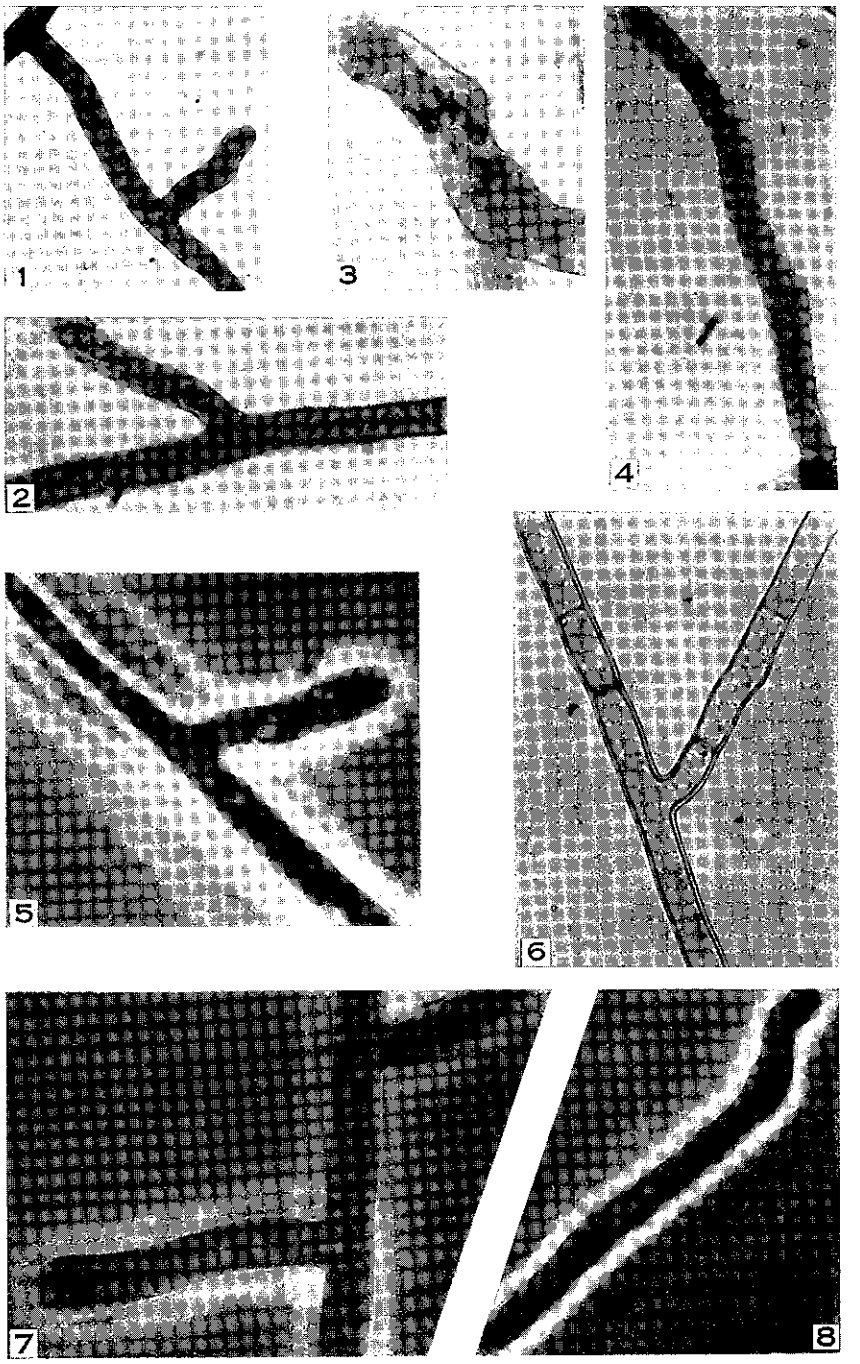
NUCLEAR DISTRIBUTION IN THANATEPHORUS



NUCLEAR DISTRIBUTION IN THANATEPHORUS



NUCLEAR DISTRIBUTION IN THANATEPHORUS

## Explanation of Plates 1–3

### Plate 1

Figures 1–6 are phase-contrast photomicrographs of *T. cucumeris* showing conjugate division of nuclei in a vegetative cell. All × *c.*1000

Fig. 1.—Nuclei moving into side branch prior to division.

Fig. 2.—Nuclei grouping.

Fig. 3.—Nuclei beginning division.

Fig. 4.—Nuclei dividing—not visible under phase-contrast microscope.

Figs. 5 and 6.—Segregation of smaller daughter nuclei.

### Plate 2

Figures 1–4 are stained preparations (HCl–Geimsa); Figures 5, 7, and 8 are phase-contrast photomicrographs

Fig. 1.—Nuclei moving into side branch prior to division. × *c.* 500.

Fig. 2.—Nuclei grouped prior to conjugate division. × *c.* 750.

Fig. 3.—Chromosome separation at division. × *c.* 1500.

Fig. 4.—Formation of daughter nuclei as they segregate after division. × *c.* 750.

Fig. 5.—Showing two daughter nuclei and a larger undivided nucleus. × *c.* 1000.

Fig. 6.—Photograph of living hyphae showing primary and secondary septa in older cells. × *c.* 750.

Fig. 7.—Showing group behaviour of nuclei where two side branches arise from penultimate cell of a hypha. × *c.* 1000.

Fig. 8.—Showing binucleate condition of *Ceratobasidium* sp. × *c.* 1000.

### Plate 3

Figures 1–12 are stained preparations (HCl–Geimsa)

Fig. 1.—Binucleate prebasidial cells. × *c.* 1000.

Fig. 2.—Prebasidial cells, showing a trinucleate one. × *c.* 1000.

Fig. 3.—Cymose branching of binucleate prebasidial cells. × *c.* 1000.

Fig. 4.—Diploid nucleus in basidium. × *c.* 2500.

Figs. 5–7.—Chromosomes of larger diploid nucleus in basidium during meiosis. × *c.* 2500.

Fig. 8.—Basidium showing five daughter nuclei. × *c.* 2500.

Fig. 9.—Nuclei moving into sterigmata. × *c.* 2500.

Fig. 10.—One binucleate spore, and two uninucleate spores from one basidium of *T. cucumeris* isolate 16. × *c.* 750.

Fig. 11.—Germinating basidiospore showing association of increased hyphal diameter with build up of nuclear number. × *c.* 750.

Fig. 12.—Basidiospore showing original abortive germ tube and new active germ tube. × *c.* 750.