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JOHNKARLINGIA, A NEW GENUS OF THE SYNCHYTRIACEAE

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Abstract

A chytridiaceous fungous species was found parasitic in the roots of cauliflower (*Brassica oleracea* L. var. *botrytis*) and cabbage (*Brassica oleracea* L. var. *capitata*) in the heavy soil fields of Varanasi, U.P. The morphology of resting sporangia and life cycle of the pathogen revealed that no fungus species has hitherto been described befitting its developmental pattern. A new genus *Johnkarlingia* Pavgi & Singh has been proposed to accommodate the fungus with *Johnkarlingia brassicae* Singh & Pavgi as its type species. The taxonomy and affinities of the genus are discussed.

Introduction

During the survey, a root rot of cauliflower (*Brassica oleracea* L. var. *botrytis*) and cabbage (*B. oleracea* L. var. *capitata*) appeared entirely distinct from the previously known ones and proved to be incited by a chytridiaceous fungus. The disease was noticed in severe form in scattered pockets in the intensively cultivated, heavy soil fields of Varanasi, U.P., India, during December–January after the winter rains. Sporadic incidence was observed in the early transplantations during August–September. The fields retaining moisture over longer periods were showing the maximum disease incidence, and loss in the stand ranged between 8–15 %. The plants appeared susceptible to infection in all growth stages from seedling to the stage of flower/head formation. A detailed study of the chytrid parasitic in the roots of cauliflower (*Brassica oleracea* L. var. *botrytis*) was made and observations are presented here.

Materials and methods

Germination of resting sporangia: The rootlets and mesocotyls loaded with resting sporangia were immersed in sterile water (2 parts pond water + 1 part distilled water) at room temperature (18–20 °C) for 3–4 days allowing the root tissues to rot and disintegrate. The resting sporangia were teased out on a slide with small fragments of the host tissue, collected and kept immersed in water in a petri plate for 8–12 days, until the resting sporangia germinated. The sequence of germination was observed on slides, plain or with depression, as necessary. The resting sporangia germinated and released motile zoospores in water after 7–9 days.

Staining of zoospores: The morphology of the zoospores and flagella was studied following the methods suggested by Couch (6) and others (7, 8, 9, 14).

Inoculation of hosts with zoospores: Fifteen to 20 day old seedlings of cauliflower (*Brassica oleracea* L. var. *botrytis*) raised in sterile sand from surface-sterilized seed were kept in 1 : 10 sodium hypochlorite soln. for 10–15 min, washed several times in sterile water and immersed in a fresh zoospore suspension for 20–30 min. Several such inoculated seedlings were transplanted in sterile sand in pots and grown for over a month for observations on the progressive stages of the fungus in the roots. The observations were taken at 2 – day intervals for 25–30 days over several seedlings raised in successive lots. The infected (inoculated) seedling was uprooted, gently washed in sterile water and examined under a light microscope (19). Several such observations were made and the typical ones illustrated (22).

Observations

Development of symptoms

The disease usually made its appearance during the rainy season (August–September) in early plantation, but the incidence was greater in December–January after the

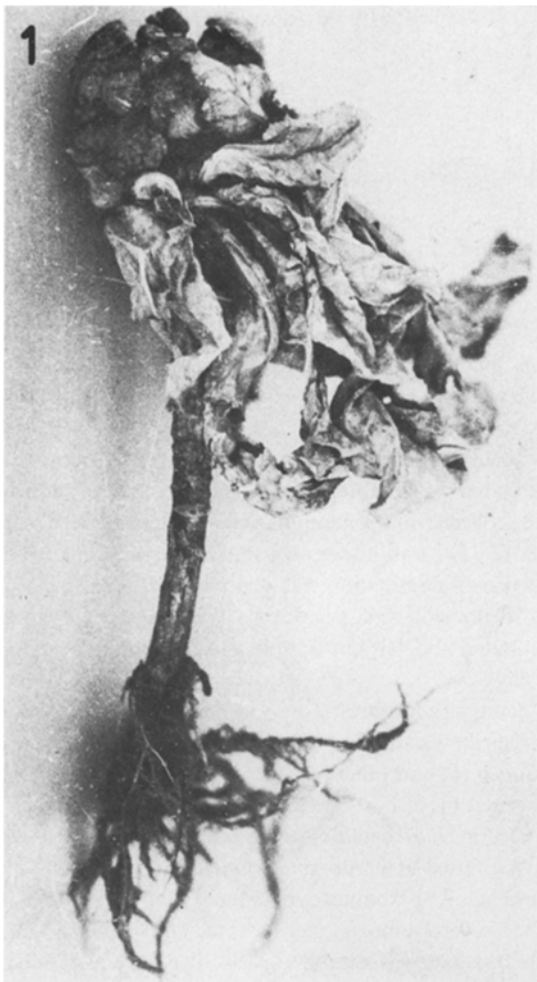


Plate 1. Disease symptoms incited by *Johnkarlingia brassicae* in cauliflower plant; wilting of aerial parts and hypertrophy of lateral and finer roots. (1/3 Nat. size).

winter rains. Young rootlets, secondary and lateral roots and tips of the main roots were attacked by the pathogen, but infected plants could not be recognized in the field in the primary stage of disease development. As the disease progressed, the fine rootlets became softened and were killed; their color changed to creamy/bright white due to softening of the internal cell layers (Fig. 1). Incipiently infected seedlings developed normally, their roots seemed apparently healthy, but in severe infections the lateral and secondary roots showed reddish brown discoloration at the tips and slight unthriftyness or sickness was the only sign of the disease noticeable in these plants. Infected cabbage seedlings developed short, stunted lateral roots in contrast to a bunch of fine, long lateral roots of an uninfected plant. Typical symptoms of the disease were exhibited on the rootlets and fine roots which remained stunted and were reddish brown in color. These symptoms were mainly governed by the environment, and typical symptoms developed only under low temperature with abundant moisture enhanced by the winter rains. The conditions incited partial wilting of the host plants, which might recover but with smaller head formation, if the atmospheric conditions such as moderately high temperature, low relative humidity and low soil moisture prevailed over a longer period.

Severely infected plants soon withered probably due to clogging of the conducting tissues and interference thereby with the supply of water and nutrients after the formation of abundant resting sporangia in the fine rootlets, secondary and lateral roots which resulted in starvation of the crown foliage and younger flower heads. Affected plants, however, recovered overnight when transpiration was reduced, but lost turgidity and became limp again during the morning. Wilting of the infected plants started in the lower leaves and progressed upward. As the disease advanced, the entire foliage of the infected plant yellowed, shrivelled and dropped off. Sometimes the infected plants showed no pronounced wilting because of lesser resting sporangial formation in the conducting tissues of the rootlets. The pathogen induced hypertrophy and hyperplasia in the infected root cells (Fig. 1), which expanded about twice their normal size and formed large cavities in the cells, which caused a constriction of the surrounding cells. Finally, the resting sporangia were formed in the cavities. They developed in rows in the rootlets (Fig. 2) and were also found embedded in the cortical cell layers (bark) of the mesocotyl of the infected plants (Figs. 3, 4).

Morphology and germination of resting sporangia

Morphology of resting sporangium: Resting sporangia developed in the rootlets, lateral roots or embedded in the bark of mesocotyl of the stem, and were mostly acentric, spherical to subspherical, pale yellow, spiny and 3 – walled. The wall layers were differentiated as:

a. Perisporium – The outermost layer which was rough and spiny; b. Episporium – The intermediate thick coat/layer which immediately surrounded the essential elements of the sporangium; c. Endosporium – That which is the most internal surface structure of the sporangium. The

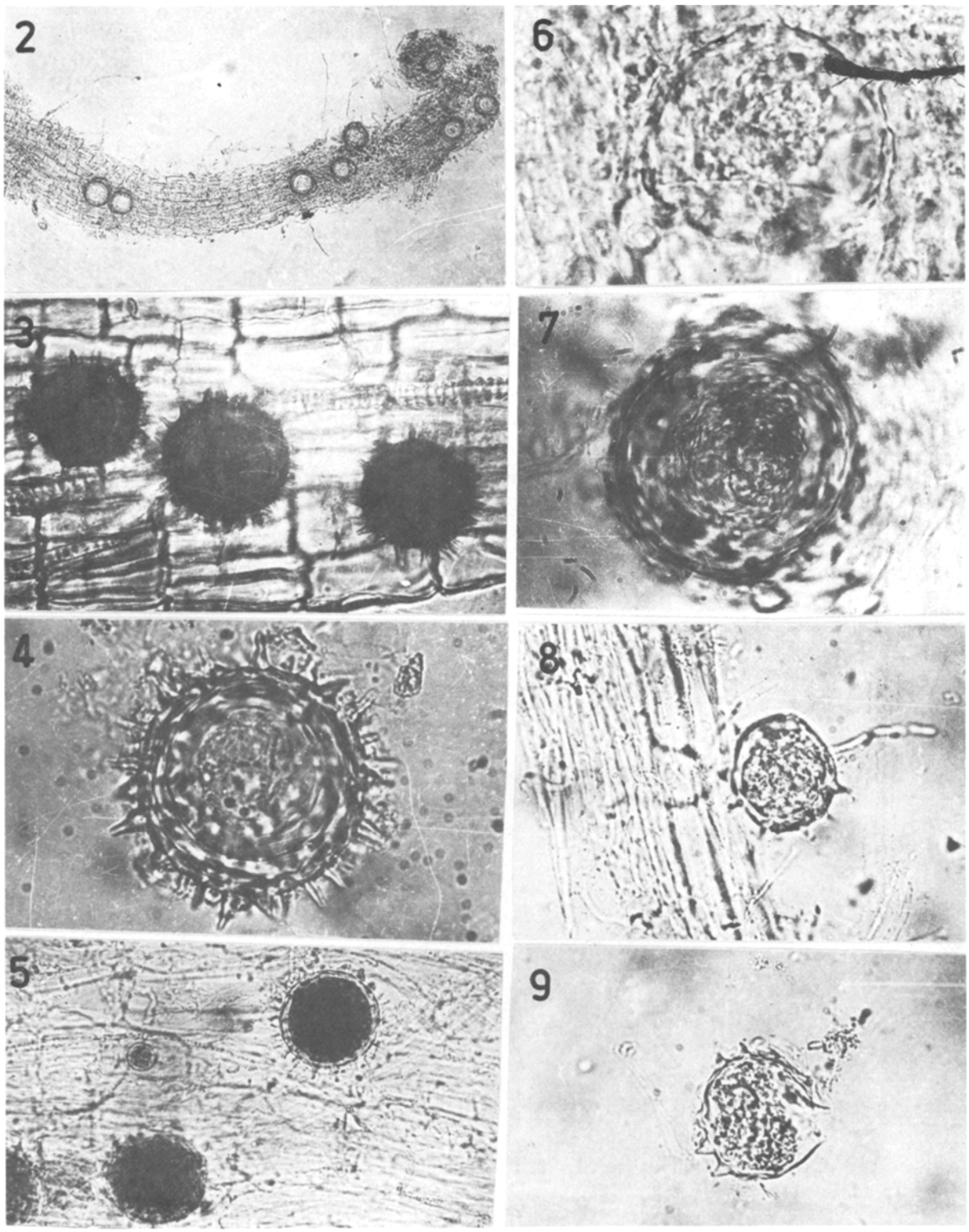


Plate 2–9. Development of *Johnkarlingia brassicae* in the cauliflower roots. 2, 3. Resting sporangia embedded in lateral roots and cortical layer. 4. A mature resting sporangium showing the spiny wall. 5. Protoplasm cleaving in a resting sporangium. 6. Protoplasm accumulating at the center, epi- and endosporic walls are dissolving. 7, 8. Stages in zoospore formation. 9. Zoospore liberation after a crack in perisporium.(Figs. 2 ×100; 3, 5, 8, 9 ×300; 4, 6, 7 ×600).

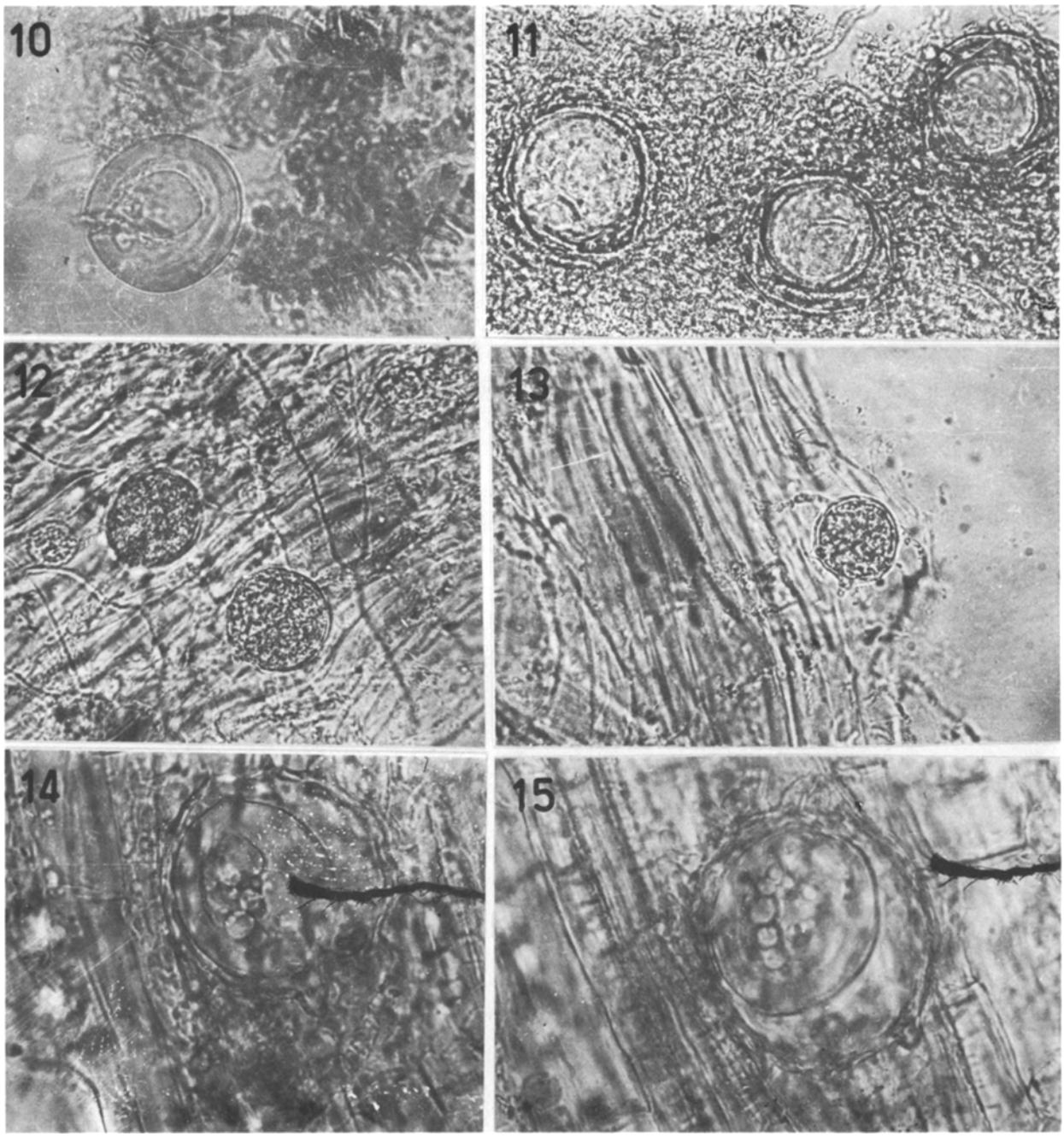


Plate 10–15. Development of *J. brassicae* in the cauliflower roots (contd.). 10. Resting spore escaping out by cracking the perisporium. 11. Resting spore showing gradual dissolution of thickening material on the episporium. 12. Protoplasm cleaving into zoospore initials. 13. Zoospores enclosed by a thinned episporium being released. 14, 15. Protoplasm cleaving into zoosporangial initials. (Figs 10 ×400; 11 × 350; 12, 13 ×300; 14, 15 ×600).

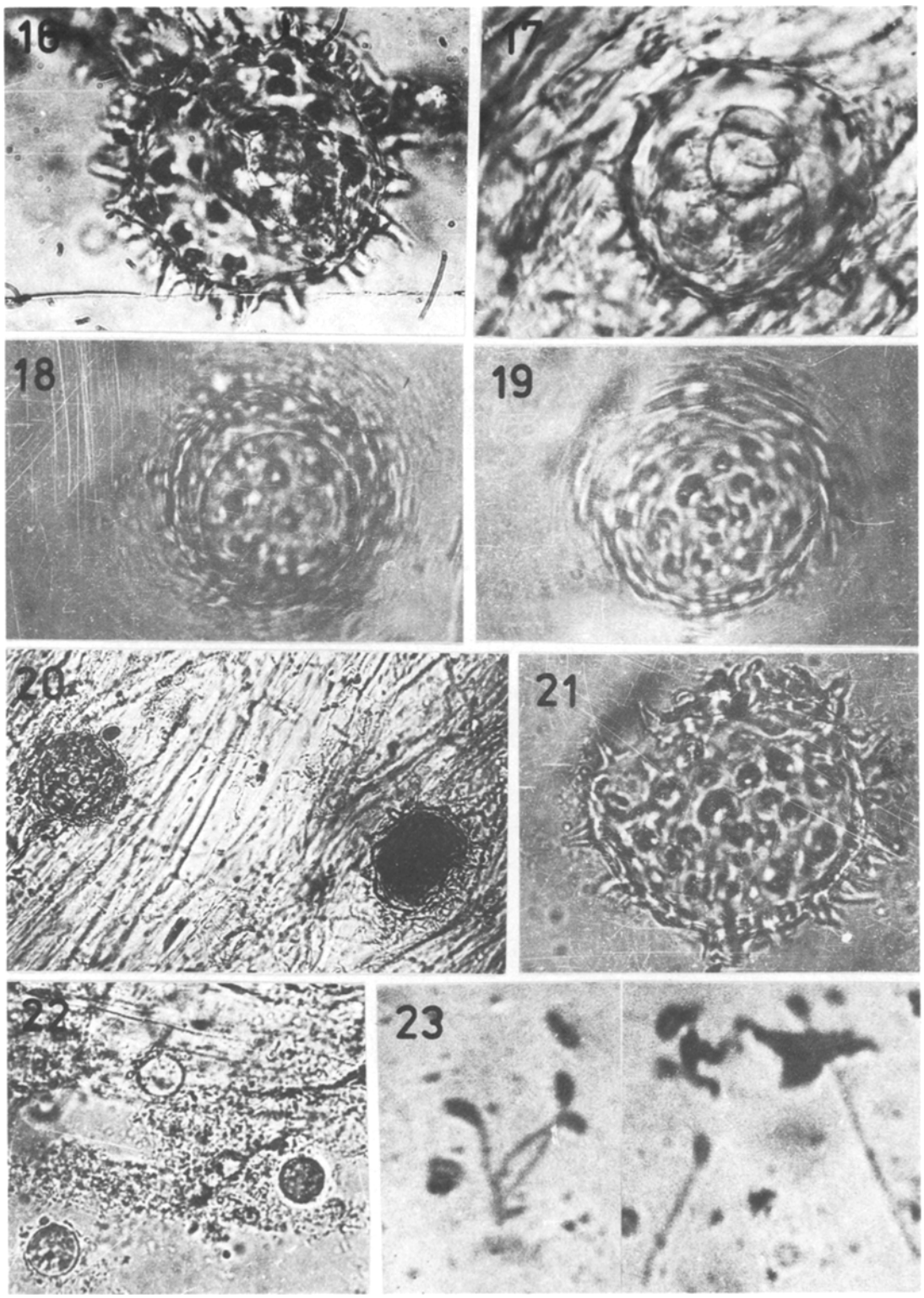


Plate 16–23. Development of *J. brassicae* in the cauliflower roots (contd.). 16–20. Progressive stages in development of zoosporangia. 21. Release of zoosporangia through a crack in perisporium. 22. Thinwalled zoosporangia containg zoospores. 23a. Zoospore with a short flagellum; b. Zoospore with a long flagellum. (Figs 16–19, 21 ×600; 20 ×300; 22 ×200; 23a, b ×3000).

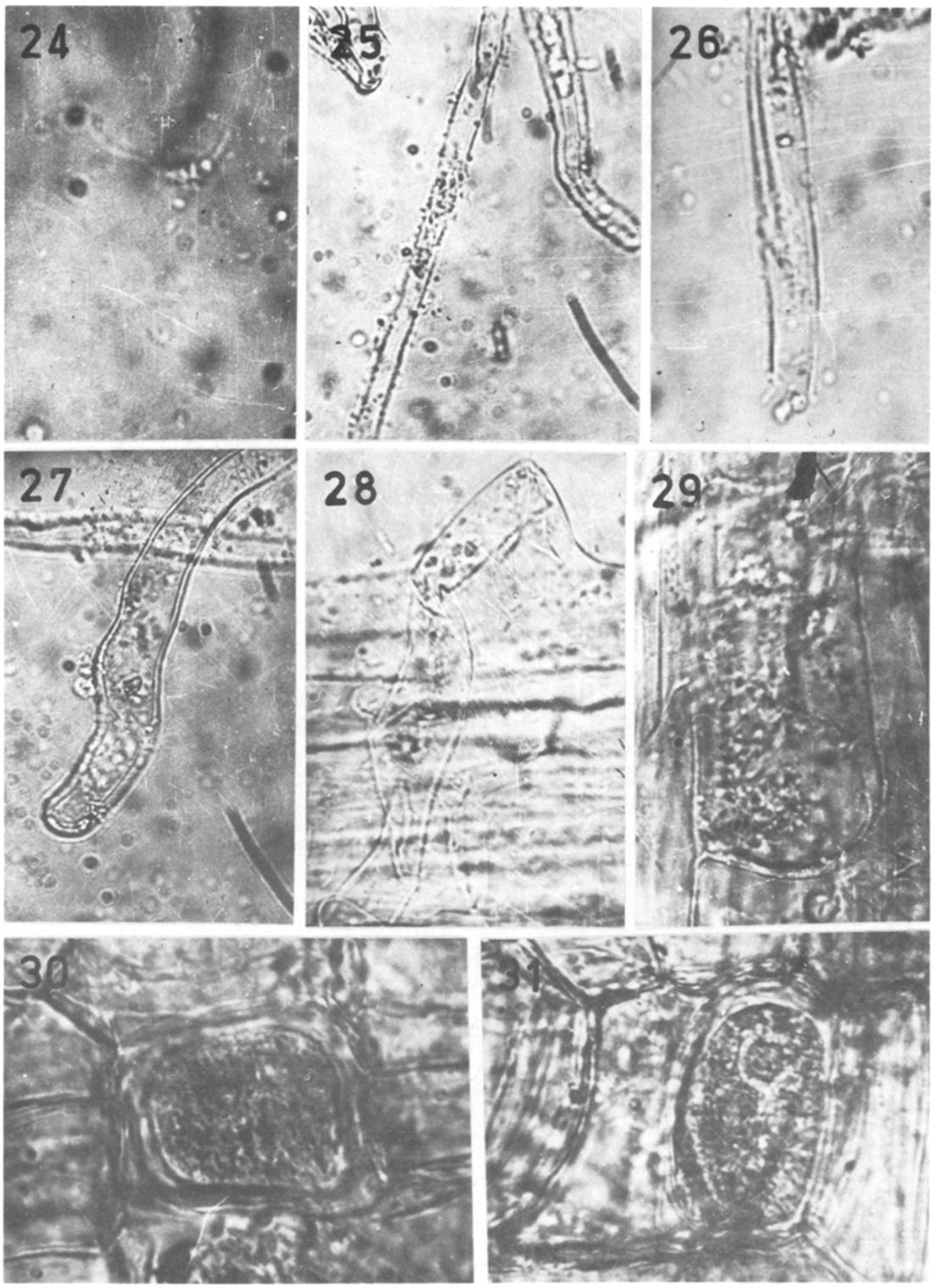


Plate 24–31. Development of *J. brassicae* in the cauliflower roots (contd.). 24. Fusion between zoospores (planogametes). 25. Zoospores encysting on surface of a root hair. 26. Fusion between zoospores at the tip and inside of a root hair. 27. Zygote encysting on the surface of a root hair. 28, 29. Englargement (growth) of parasitic protoplasm within a root hair and formation of initial thallus in the outer cell of epiblema. 30. Mature vegetative thallus. 31. Thallus containing 3–4 refractive bodies. (Figs. 24, 26, 27 ×1500; 25 ×750; 28 ×150; 29 ×1000; 30, 31 ×600).

epi- and endosporium walls provided support and rigidity to the spore and maintained its shape.

The resting sporangia measured 45–62.5 μm (avg. 52.8 μm) in diam., the thickness of the perisporium (outer wall) measuring 3–7.5 μm the episporium being 2.5–5 μm thick and the endosporium 0.75–1.25 μm. The outer wall (perisporium) was ornamented with 22–35 short, bluntly tapering, conical spiny processes, measuring 4.5–6 μm at the base and 9.5–15 μm in height (Fig. 4).

Mode(s) of germination: Several attempts to secure germination of the resting sporangia were unsuccessful, but germination was induced in water culture after disintegration and rotting of the infected host tissues; apparently this helped break down dormancy and/or stimulated their germination. The rootlets and mesocotyls loaded with resting sporangia were submerged in sterile water and incubated at room temperature (18–20 °C) for 3–4 days, which helped the root tissues to rot and disintegrate. The submerged rootlets, bearing resting sporangia were mounted on a slide, the sporangia teased out along with a small portion of the host tissues under a dissecting microscope, finally collected and kept immersed in water in another petri plate and retained for 8–12 days to observe the germination process. Care was taken that the pieces of infected rootlets always remained submerged under water. Germination of the resting sporangia occurred after 7–9 days by one of the following methods:

i. The homogeneous protoplasmic content of the sporangium accumulated in the center (Fig. 5). The thickening material of the episporium and the endosporium had disappeared/dissolved leaving a thin bounding wall surrounding the protoplasm, and the entire protoplasm broke into minute granules (Fig. 6). These were the zoosporic primordia which underwent progressive physiological maturation (Figs. 7, 8) and finally uniflagellate zoospores were released in water through a rupture in the bounding wall and the perisporium (Fig. 9). The resting sporangium thus behaved on germination as a zoosporangium as in the germination pattern of the subgenus *Mesochytrium* under the genus *Synchytrium* De Bary and Woronin (13).

ii. The resting sporangium bounded by the thick, smooth wall (episporium and endosporium) shot out as a spherical body rupturing the perisporium (outer spiny wall) 6–8 days after submersion in water (Fig. 10). The endosporium and the thickening material of the episporium began to dissolve, becoming homogeneous with the protoplasmic mass, and the whole mass participated in cleavage to form minute granular bodies. These underwent progressive physiological maturation (Figs. 11, 12) and ultimately minute zoospores escaped in the water after cracking open the thin enclosing wall layer of the episporium (Fig. 13).

iii. In this germination pattern, the endosporium and thickening material of the episporium dissolved and became homogeneously mixed with the protoplasm, and the entire protoplasmic contents segmented into several merons after 7–9 days, which subsequently developed in 8–24 spherical zoosporangia (Figs. 14–20). This was followed by cleavage of the sporangial protoplasm into zoospore initials. The zoosporangia, which were spherical to subspherical, hyaline and thin-walled, measuring 11.5–17.5 μm in diam., later escaped through the cracks/tears in the perisporium (Fig. 21). The zoospores from the zoosporangia were released after maturation through a tear in the thin, hyaline zoosporangial walls (Fig. 22). Thus, the resting sporangia behaved as prosori on germination as described for the subgenus *Pycnochytrium* under *Synchytrium* (13). The zoospores were minute, hyaline, pear-shaped, measuring 0.5–1 μm, posteriorly uniflagellate; the flagella were long, trailing slender, whiplash type, tapering at the end and 5.5–10.5 μm in length (Fig. 23a, b).

Infection of the host and development of the pathogen

Infection in the roots of cauliflower (*Brassica oleracea* L. var. *botrytis*) took place during the rainy season (August–September), when the resting sporangia germinated after release from the rotting debris and behaved as prosori or sporangia. The zoospores swam in a film of water in the soil and encysted on the moist exterior surface of the root hair of the host (cauliflower) 20–25 min after release (Fig. 25). The flagella were withdrawn into the body or disappeared before encystment. A minute penetration tube grew out 10–15 min after encystment and the protoplasm of the encysted zoospore passed into the root hair within 35–40 min (Fig. 25), similarly as described for the species of *Olpidium* (16, 20, 23, 24) and *Synchytrium* (13). The time period taken in the initiation of germination and complete migration of protoplasm from the encysted zoospore in the root hair was about 5 min. Fusion between 2 naked protoplasmic masses of zoospores now took place inside the root hair, which steadily increased in size after consummation, and the zygote moved further into inner cells of the root, probably along the protoplasmic stream. Usually several zoospores penetrated the root hairs of the host, fused therein, and the resulting zygote(s) further migrated in deeper cell layers of the root.

It appeared that under certain environmental condi-

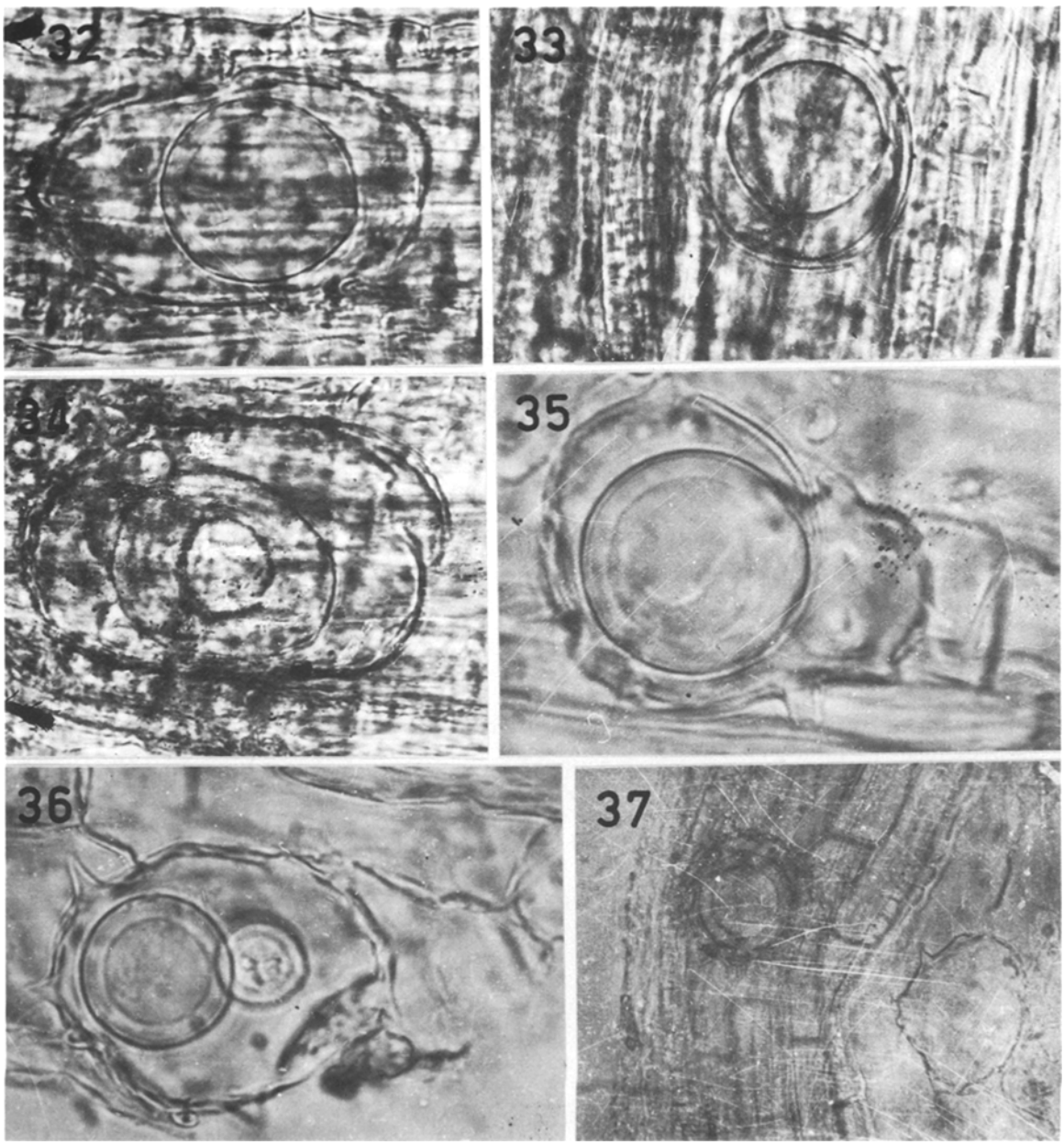


Plate 32–37. Development of *J. brassicae* in the cauliflower roots (contd.). 32. Young resting spore showing peri- and episporial layers. 33. Initial protrusions of outer spines on the perisporium. 34. Peri-, epi- and endosporium developing and outer spines initiated. 35. Formation of outer spines initiates and peri-, epi- and endosporium fully developed. 36. 2 spores enclosed by a common perisporium, one spore remaining immature. 37a, b. Mature and aborted resting sporangia. (Figs. 32 ×800; 33 ×500; 34–36 ×600; 37 ×300).

tions, segments of resting sporangia developed into gametangia, indistinguishable from the zoosporangia except that the former gave rise to planogametes which copulated in pairs (Fig. 24). Copulation between the gametes took place in the soil in a film of water either on the surface of the root hair (Figs. 25, 26) or inside the root hair after penetration as described. Also, fusion between 2 planogametes outside the host resulted in the formation of a zygote, which became amoeboid and swam about for some time before it finally came to rest on the exterior surface of the root hairs (Fig. 27). Its protoplasm migrated into the root hairs by perforation of the root hair wall through a slender penetration tube (Fig. 27), as described earlier for the penetration by zoospores. The zygotic protoplasm moved further into the epidermal or an inner cell (in the second or third layer) of the root which gradually enlarged in size (Figs. 28, 29). The naked protoplasm increased in size inducing enlargement of the host cell, 7–10 days after infection, completely filling the cell and finally developing into a thallus measuring 60.5–67.5 X 43.5–54.5 μm filled with a dense granular protoplasm surrounded by a thin, hyaline membrane (Figs. 29, 30) Soon several refractive bodies intermingled with the dense granular protoplasm appeared in the mature thallus, which took an elliptical shape now (Fig. 31). After attaining a moderate growth, the protoplasm accumulated in the center and walls developed around it in 16–25 days after entrance (infection). The perisporium developed first, followed by the episporium, and the endosporium developed just 2–3 days prior to sporangial maturation (Figs. 32–36). Sometimes the walls of the perisporium and episporium developed at the same time (Figs. 32, 35). The resting sporangium became mature 20–25 days after infection (Fig. 37). The wall layers of the pathogen under study resembled those of the ascospores of *Neurospora tetrasperma* Shear and Dodge described by Lowrey and Sussman (18). In brief, the wall structures of the resting sporangium may be described as:

i. Perisporium: The outermost rough and spiny layer; this includes all the layers or all the outer elements of the spore and has no role in the functioning of the spore except probably in helping survival of the spore under adverse environment. The perisporium structures include the subperisporic layer or other cytoplasmic deposition on the surface, which is often so narrow as to be inconspicuous on the surface of the perisporium (Fig. 4).

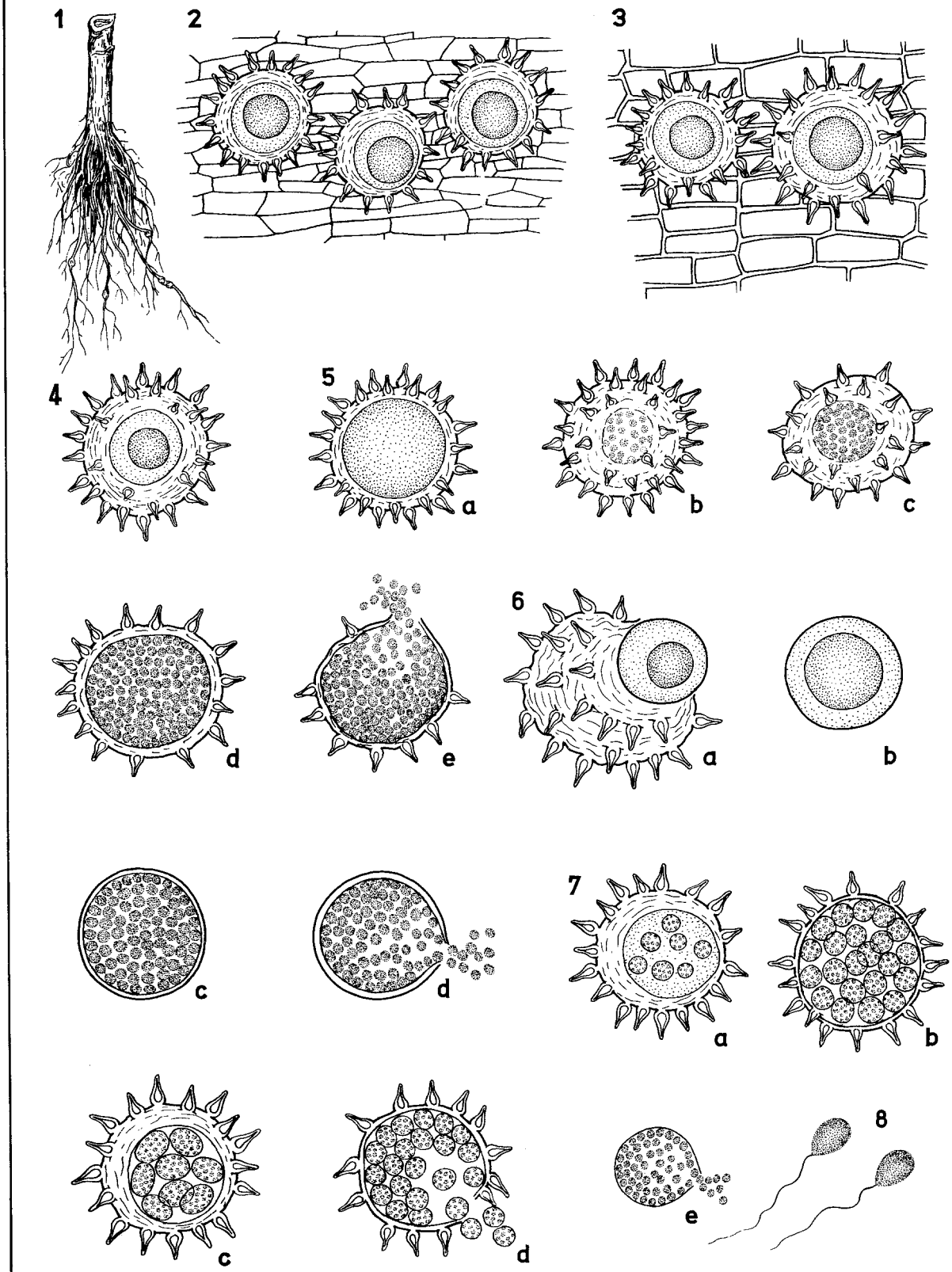
ii. Episporium: An intermediate thick coat/layer which immediately surrounds the essential elements of the spore. The epi- and endosporium walls provide support and rigidity to the spore and maintain its shape.

iii. Endosporium: A thin layer which is the most internal wall surface and in which protoplasmic contents are condensed.

The development of spines started either just after formation of the peri- and endosporium (Fig. 33) or after maturation of the resting sporangium (Fig. 36). These were 22–35 in number, short, bluntly tapering, conical processes measuring 4.5–6 μm at the base and 9.5–15 μm in height. Sometimes aborted cells were also noticed (Fig. 37), which were probably due to a functionless nucleus. Sometimes 2 spores cleaved within one perisporium, and one of them developed, while the sister cell remained empty and abortive. This was either due to the formation of one mature resting sporangium and another small and immature one. Possibly the nucleus might be suppressed in divisions resulting in the formation of one mature resting sporangium and another small and immature (Fig. 36). The infection and direct development of a resting sporangium resembled the development of *Synchytrium fulgens* Schröter of the subgenus *Microsynchytrium* under the genus *Synchytrium*, in which such motile cells function as gametes and fuse (5, 12, 15, 17). The resulting zygote has been assumed to develop into a diploid resting spore, awaiting a direct cytological proof. The resting sporangia of the pathogen under study similarly appeared to be diploid and developed from a zygote which was formed from the fusion of zoospores (planogametes).

Discussion

The parasitic nature of the chytridiaceous fungi was first established by Braun (3) with the erection of a new genus *Chytridium* Braun and *C. olla* Braun was found to be parasitic in the oogonia of *Oedogonium* species. Later De Bary and Woronin in 1864 recognized the chytrids and grouped them into a family and named the genera *Chytridium* Braun, *Rhizidium* Braun and *Synchytrium* De Bary and Woronin (23). Since then very few reports are available in literature regarding a chytridiaceous fungus species parasitizing the roots of phanerogamic seedlings. Few species of *Olpidium* (Braun) Rabenhorst and *Rhizidium* Schenk have been recorded to cause injury to several economic crop plants (1, 4, 20, 21, 23). The species of *Synchytrium* are obligate parasites and many are known to induce gall formation on the leaves of flowering plants. *Synchytrium endobioticum* (Schilb.) Percival has been reported in the underground portions also of the crop host, but it does not invade the root system (13). *Syn-*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chytrium fragariae Zeller and Campbell has been found responsible to induce gall formation in the roots of strawberry (*Fragaria chiloensis* Duchesne var. *amanassa* Bailey) (25). The present chytrid species, parasitic in the roots of cauliflower and cabbage makes an addition to the series.

The resting sporangia of the present chytridiaceous root parasite directly develop in a similar manner as described by Couch (5), Karling (12), Kusano (15) and Lingappa (17), for *Synchytrium fulgens* Schröter of the subgenus *Microsynchytrium* under *Synchytrium*, but they differ in the morphology of the wall structure possessing i) perisporium, an outermost layer, ii) episporium, an intermediate layer and iii) endosporium, an innermost membraneous wall. Resting sporangia of the aquatic (underground) members of the subgenus *Microsynchytrium* (*Micromyces* Dangeard) behave as prosori on germination, the contents of which migrate out and develop into an attached incipient sorus in which zoospores are formed later; whereas in the present fungus an incipient sorus never develops, the perisporium cracks open and the entire protoplasmic mass shoots out and undergoes progressive cleavage and releases zoospores later. The resting sporangia also behave as prosori as described for the members of the subgenus *Pycnochytrium* under *Synchytrium*, which give rise to an attached sorus of sporangia, whereas in the present case an attached sorus is not developed, but the entire contents become segmented into zoosporangia within the resting sporangium and escape through a tear in the perisporium. Its life cycle parallels that of *Pycnochytrium* in which only the resting sporangia are developed, but differs in the methods of their germination. Thin-walled zoosporangial thalli are not formed as in the Olpidiaceae and the life cycle of the fungus consists of only the resting sporangia giving rise to zoospores or gametes by various modes of germination.

The morphology and developmental cycle of the present fungous species are not duplicated in any fungus hitherto described and represents a taxon of a genus new to science. A new genus is, therefore, being proposed to accommodate the fungus as *Johnkarlingia* in honor of Professor Dr. John S. Karling, Wright Distinguished Research Professor, Purdue University, Indiana, who has contributed so much to our present day knowledge of this interesting group of fungi. A formal diagnosis is provided below:

Johnkarlingia Pavgi and Singh Gen. Nov.

Thallus monocentric, endobiotic, holocarpic; consisting of spherical to subspherical resting sporangia, with a 3-layered wall made up of an outer perisporium, intermediate episporium and inner endosporium; functioning either as sporangium or a prosorus or a sorus of zoosporangia in germination. Zoospores minute, posteriorly uniflagellate.

Type species: *Johnkarlingia brassicae* Singh and Pavgi.

Thallus monocentricus, endobioticus, holocarpicus; e sporangiis residibus sphaericis vel subsphaericis ab tunica trillamellata perisporic externo, episporio medio, et endosporio intimo contenta circumdatis, ut sporangium vel prosorus vel sporangiorum sorus constans. Zoosporae minutae, flagellum unicum postice exserentes.

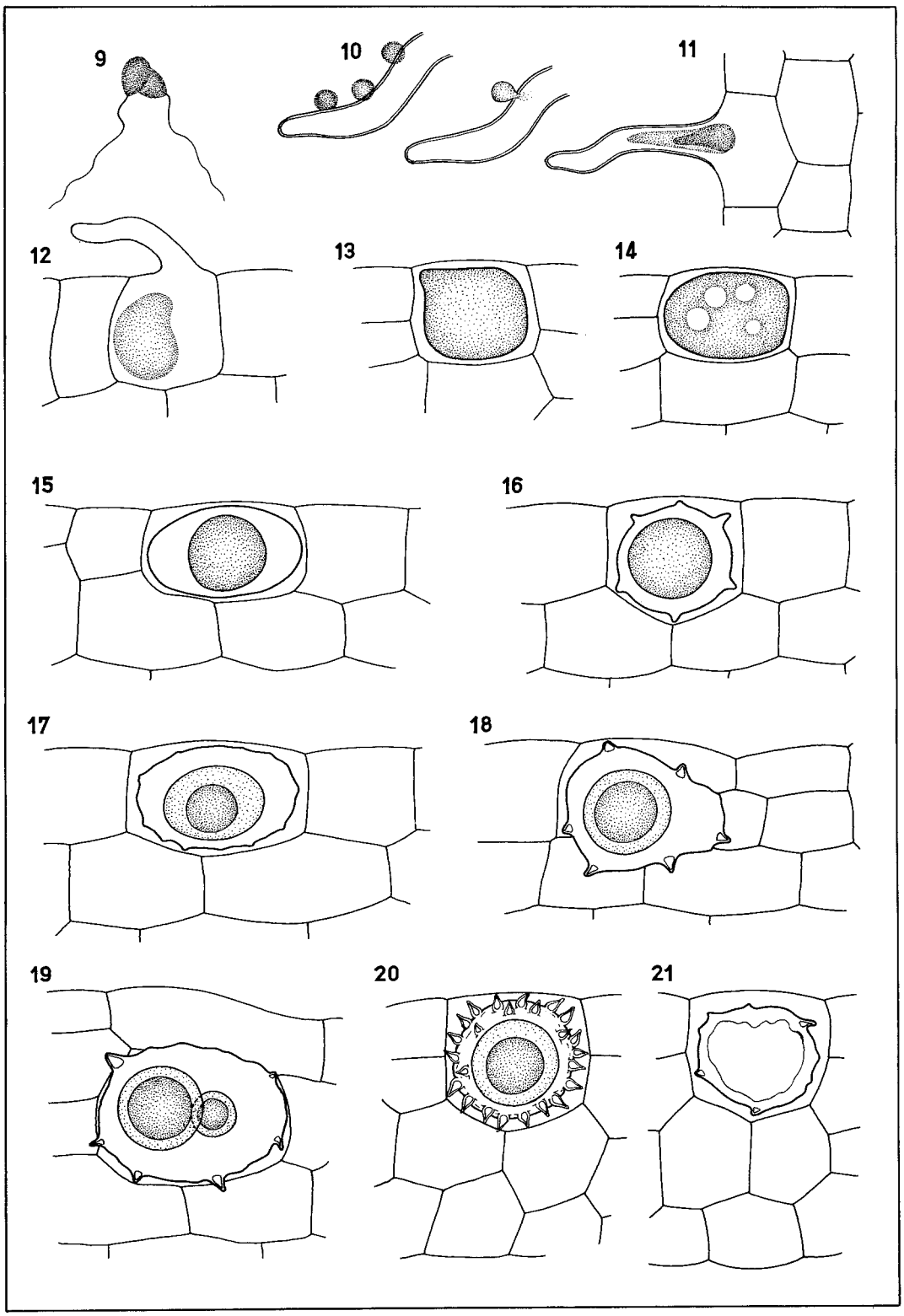
Johnkarlingia brassicae Singh and Pavgi sp. nov.

Resting spores occurring singly or in rows, spherical to subspherical, 45–62.5 μm in diam. (avg. 52.75 μm), pale yellow, with an outer spiny 3–7.5 μm thick perisporium, an intermediate 2.5–5 μm thick episporium, and an inner thin, 0.75–1.25 μm thick endosporium; blunt tapering conical spines on perisporium, 9.5–15 μm high by 4.5–6 μm diam. at the base. In germination contents cleaving directly into zoospores, which escape through a crack in the perisporium, or contents emerging in entirity as a globular body, whose protoplasm cleaves directly into zoospores, or contents cleaving into 8–24 thin-walled, hyaline zoosporangia, 11.5–17.5 μm in diam., which emerge through the periplasm and later give rise to zoospores. Zoospores minute, 0.5–1 μm in diam., pear-shaped, hyaline; flagellum trailing, slender, 5.5–10.5 μm long and tapering.

Obligately parasitic in living roots of *Brassica oleracea* L. var. *botrytis* at Varanasi, U.P. causing medium-sized, simple galls. Leg. S. L. Singh on 16 December, 1970. (TYPE). (IMI 199663; HCIO 32095).

Sporae resides singillatim vel in seriebus positae, sphaericae vel subsphaericae, 45–62.5 μm (medio 52.75 μm) diametro, pallide luteae, perisporio spinoso, 3–7.5 μm

Figs 1–8. Semidiagrammatic representation of the development of *Johnkarlingia brassicae* in the cauliflower roots. 1. Wilting aerial parts and hypertrophy of lateral and finer roots. 2, 3. Resting sporangia embedded in lateral root and cortical tissue. 4. A mature resting sporangium. 5a–e. Cleavage of protoplasm, its accumulation in the center, dissolution of epi- and endosporic wall layers in the resting sporangium, formation of zoospores and their liberation after cracking the perisporium. 6a–d. Resting sporangium escaping out after cracking the perisporium, resting spore outside, cleavage of protoplasm into zoospores and their liberation through the episporium. 7a–e. Protoplasm cleaving into zoosporangia, their release outside through a crack in perisporium, and release of zoospores through thin-walled, hyaline zoosporangium. 8. Posteriorly uniflagellate zoospores.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crasso, episporio 2.5–5 μm crasso, et endosporio tenui, 0.75–1.25 μm crasso circumdatae; episporii spinae obtusae, attenuatae, conicae 9.5–15 μm altae, ad basim 4.5–6 μm diametro. Plasma internum sub germinatione in zoosporas per episporii rimam emissas fissum vel ut massa globularis demum in zoosporas fissa extrusum, vel in zoosporangia 8–24 tenuiter tunicata, hyalina, 11.5–17.5 μm diametro per perisporium emergentia et demum zoosporas gigentia fissum. Zoosporae minutae 0.5–1 μm diametro, pyriformes, hyalinae, flagellum posticum gracile, attenuatum 5.5–10.5 μm longum exserens.

Taxonomy and affinities:

The absence of a true mycelium, posteriorly uniflagellate zoospores and their minute size (0.5–1 μm) and regular alternation of generations suggest the pathogen to be a member of the family Synchytriaceae in the class Chytridiomycetes (2). Karling (13) recognized that morphology (size shape and structure) of the zoospore and gametes should be considered as prime diagnostic characters, since they relate to single cells. He further suggests that zoospores of the underground species of *Synchytrium* such as *Synchytrium endobioticum* and *S. fragariae* (1–2 μm) are relatively smaller in size in contrast to zoospores of the terrestrial species (3.5–6 μm). It is interesting to note that the present fungus possesses still smaller -sized zoospores (0.5–1 μm) than those in the aquatic (underground) species of *Synchytrium*. This fact suggests that the present fungus takes an earlier place in the evolutionary line than the aquatic species of *Synchytrium*.

The mode of development of the resting sporangium indicates resemblance with *S. fulgens* under the subgenus *Microsynchytrium*, but the diagnostic wall morphology and germination pattern of the present fungus distinctly differ from *S. fulgens*. During the course of evolution, the species of *Synchytrium* appear to have lost one of the spore walls, as it (perisporium) merged with the episporium resulting in the formation of epi- and endosporium. But the present fungus has 3 walls in the resting sporangium and it appears logical to retain it prior to or ahead of the genus *Synchytrium* in the evolutionary line (2, 10).

The fungus shows some affinity to the fam. Olpidiaceae. The zoospore of this fungus after infection develops into a thallus, which never gets segmented into merons and zoosporangia as in the members of Olpidiaceae. The thallus directly develops into a resting sporangium and on germination produces and releases numerous zoospores. No exit canals/tubes are formed by the germinating sporangia as in the Olpidiaceae. The mode of sporangial germination nevertheless appears reminiscent of the Olpidiaceae.

The fungus cannot be placed in the genus *Synchytrium* as it does not exhibit the developmental morphology of the summer sori, resting sporangia, wall structure, and germination pattern as in many of the subgenera of *Synchytrium*, viz. *Microsynchytrium*, *Mesochytrium*, *Pycnochytrium* or *Woroninella*. Therefore, the genus occupies a position earlier to *Synchytrium* in the family Synchytriaceae with an advancement over the Olpidiaceae, bypassing the family Achlyogetonaceae during the course of evolution.

Figs 9–21. Semidiagrammatic representation of the development of *Johnkarlingia brassicae* in the cauliflower roots (contd.). 9. Fusion between planogametes. 10a, b. Migration of zygote in the root hair through a penetration tube. 11–14. Progressive development of vegetative thallus in the outer epiblema cell and mature thallus containing 3–4 refractive bodies. 15–18. Progressive development of sporangial walls and its spiny ornamentation. 19. Common perisporium enclosing 2 spores, one remaining immature. 20. A mature resting sporangium. 21. Aborted resting sporangium.

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