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Morphology and cytology of the promycelium and the basidiospore of Puccinia horiana

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Abstract

Teleutospores of *Puccinia horiana* P. Henn. germinate soon after their formation. Promycelia germinating on microscope slides have a tendency to grow in length and become narrow in outline; when germinating in situ, developing promycelia are short and stout, and show a lobed apical cell. The promycelium is usually three-celled, but at times it is one- or two-celled.

Two basidiospores are usually produced by each promycelium (observed range one to three). Basidiospores are uni- or binucleate at first, but later become multinucleate. They germinate rapidly on microscope slides and on host leaves, when sufficiently wet. Three different patterns of basidiospore germination were observed.

Introduction

The white rust of *Chrysanthemum morifolium*, caused by *Puccinia horiana* P. Henn., was extensively studied during recent years (Yamada, 1956; Hiratsuka, 1957; Boerema and Vermeulen, 1964; Punithalingam, 1968; Stahl, 1964; Stark and Stach, 1965; Firman and Martin, 1968; Zadoks and Kodde, 1968; Zandvoort et al., 1968). In these publications little attention was given to the morphology and cytology of the promycelium and the basidiospore of *P. horiana*. Therefore, the development of promycelium and basidiospore, and the nuclear phenomena associated with these structures were studied.

Materials and methods

The rust was grown on the leaves of *C. morifolium* cv. 'Indianapolis White Giant IV' in a conditioned greenhouse at 15 °C and 85% relative humidity. Teleutospores were germinated in situ by floating infected leaves on water, and on microscope slides by smearing a previously prepared teleutospore suspension. When germination in situ was followed, the teleutospores were allowed to germinate in the sori; then they were separated, and transferred to the microscope slides for fixation and staining. Germination took place at room temperature (about 20 to 22 °C).

Germination and nuclear events of basidiospores were studied on microscope slides. *Chrysanthemum* leaves bearing mature teleutosori were placed on a moist cotton wad lining the top of a Petri dish while acid-cleaned slides were placed in the

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bottom, which was flooded with water to maintain high humidity in the system.

The Petri dishes were incubated at room temperature (20 to 22 °C); the teleutospores germinated, and shed basidiospores on the microscope slides. The materials were fixed for 12 to 24 hours in one of the following fixing fluids: F.A.A. (5 ml 40 % formalin, 5 ml glacial acetic acid, and 90 ml 50 % ethanol); Carnoy's fluid A (3:1 mixture of absolute ethanol and glacial acetic acid); Carnoy's fluid B (6:3:1 mixture of absolute ethanol, chloroform and glacial acetic acid); Randolph's modified Navashin fluid (solution A = 1 g chromic acid, 7 ml glacial acetic acid, 92 ml distilled water; solution B = 30 ml neutral formalin and 70 ml distilled water; equal parts of A and B were mixed before use).

Several stains or staining procedures such as crystal violet, Heidenhain's Iron Alum Hematoxylin, Feulgen, Acetocarmine and Acetoorcein were tried for differentiation of the nuclei in the promycelium; usually with little success. Occasionally, 1 % Acetocarmine stained the nuclei distinctly. The same staining techniques were also tried for the basidiospores. Good results were obtained with Iron Alum Hematoxylin and with the modified Feulgen technique described by Maheshwari et al. (1967). Sometimes, basidiospores were stained successfully when slides were first kept in absolute ethanol for 2 to 3 hours at 60 °C, and then stained for 15 to 20 minutes at 60 °C in 1 % Acetocarmine. Photomicrographs and camera lucida drawings were made at a × 1000 magnification.

Observations

The teleutospores of *P. horiana* can germinate immediately. Sometimes, they even germinate in the sori on the leaves that had been removed from the plants. Under laboratory conditions, germination of the teleutospores begins within 6 to 12 hours after bringing them under conditions conducive to germination. Ordinarily, it begins at the apical cell of the teleutospore (Fig. 1–1, 6–1, 6–2). Rarely, promycelium formation starts at the basal cell (Fig. 1–2). Usually, only one promycelium per teleutospore is formed by the apical cell. Sometimes, both the apical and the basal cells produce promycelia (Fig. 1–3).

After germination on microscope slides, the promycelium often is a long and slender tube measuring 70 × 6 μm (Fig. 3); it may be over 150 μm long. During its development two septa are formed; the two distal cells normally produce basidiospores. The sterigma produced on the distal cell is either a short attenuated tube (Fig. 3–4) or curved (Fig. 3–3, 3–5) or coiled outgrowth (Fig. 3–7, 3–8). At times, the promycelium is either branching or forming a basidiospore without visible sterigma (Fig. 3–9, 3–10).

Promycelia developing in situ are short, stout and about 33 × 8 μm in size (Fig. 4). Fig. 1 shows various promycelia grown in situ after 12 hours of germination. The promycelium is either one-celled (Fig. 4–9, 4–10), two-celled (Fig. 4–2 to 4–5), or three-celled (Fig. 4–6 to 4–8) at the time of the formation of the basidiospores. The apical cell of the promycelium often shows a lobed appearance, and the first basidiospore is always produced at the distal part of the promycelium.

Staining of the promycelium was rarely successful though permeability of the promycelial cell wall seemed better than that of the ungerminated teleutospore wall. In certain cases, when nuclei were observed in the uppermost cell of the promycelium,

Fig. 1 (left). *Puccinia horiana.* Promycelia developing from the basal (2), the apical (1), or from both teleutospore cells (3).
Fig. 2 (right). *Puccinia horiana.* Stages of the nuclear division in the apical cell of the promycelium.

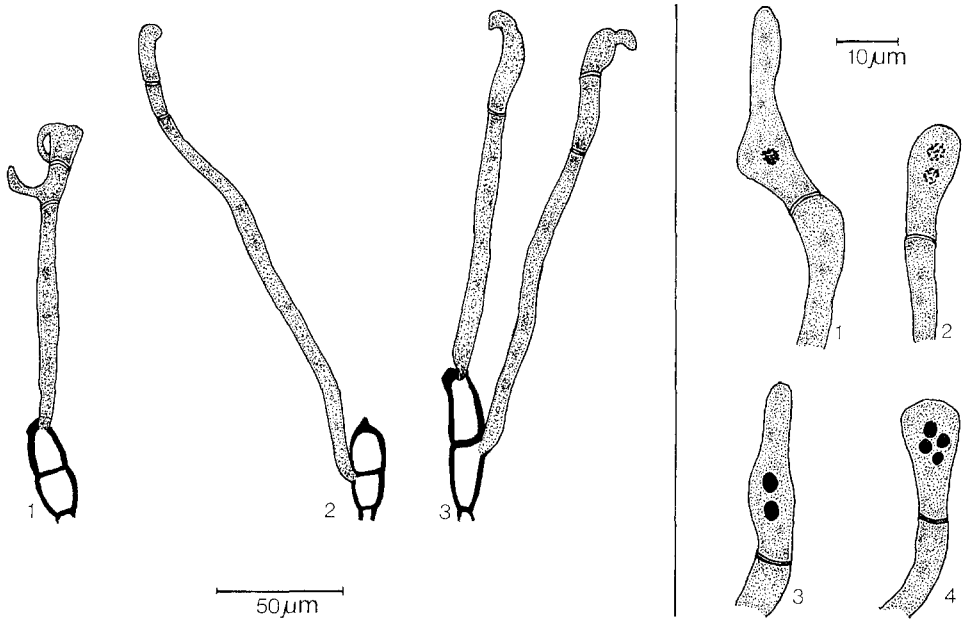

Fig. 1 (links). Puccinia horiana. Promycelia groeiend uit de basale cel (2), de apicale cel (1), of uit beide cellen van de teleutospore (3).
Fig. 2 (rechts). Puccinia horiana. Stadia van de kerndeling in de topcel van het promycelium.

their number ranged from 1 to 4. Apparently, there are 1 or 2 divisions of the diploid nucleus before the uni-nucleate condition in the cells of the promycelium is reached, after which there are 1 or 2 divisions of the daughter nuclei. Thus the mature promycelium may have either two or four nuclei in each cell. Fig. 2–1 to 2–4 show cells with one, two and four nuclei.

Basidiospores are produced within 2 to 6 hours after placing teleutospore bearing leaves in moist Petri dishes. At maturity, the basidiospore is an oval structure with a somewhat rough wall measuring about 12 × 6 μm. When first formed, it has one or two nuclei but later it becomes multinucleate after additional divisions of the nuclei. Of 75 basidiospores examined, 30 were found to be uninucleate, 33 binucleate, whereas 5 had three, and another 5 had four nuclei. One basidiospore showed five and another one six nuclei. The sequence of nuclear divisions in the basidiospores is shown in Fig. 5–1 to 5–10 and in Fig. 6–3 to 6–6.

In one experiment, three substrates for the germination of basidiospores were compared: excised host leaves floated on water in Petri dishes, microscope slides, and 1.5% water-agar. The basidiospores were discharged on healthy *Chrysanthemum* leaves, microscope slides and water-agar, and incubated at about 21 °C under conditions of high humidity. After 12 hours, the germination percentage was 58 on host leaves, 91 on microscope slides, and only 2 on water-agar; after 36 hours the germina-

Fig. 3. *Puccinia horiana.* Sequence of promycelial development on microscope slides.

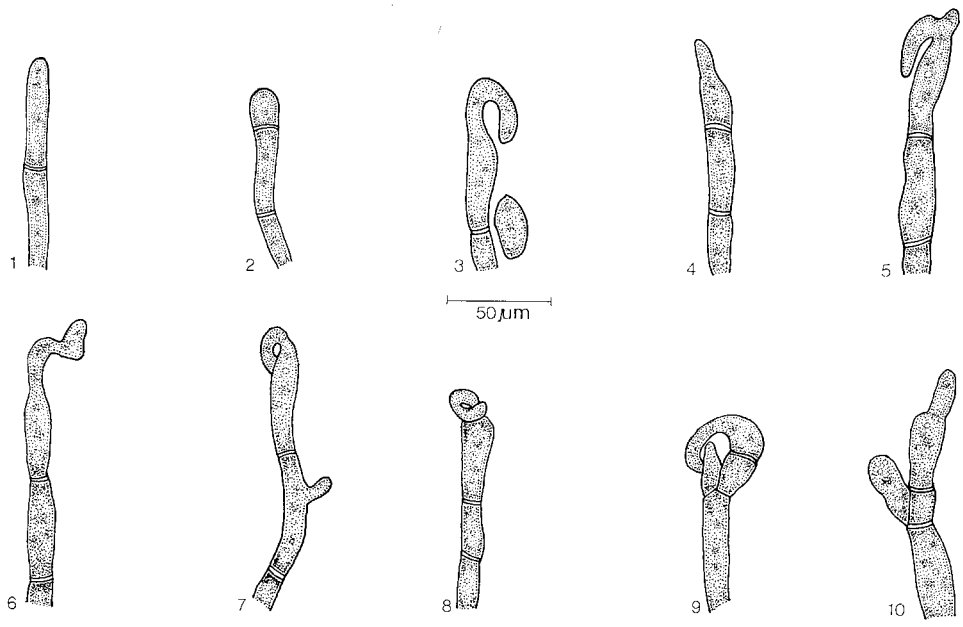

Fig. 3. Puccinia horiana. Reeks van ontwikkelingsstadia van het promycelium op objectglaasjes.

Fig. 4. *Puccinia horiana.* Sequence of promycelial development in situ.

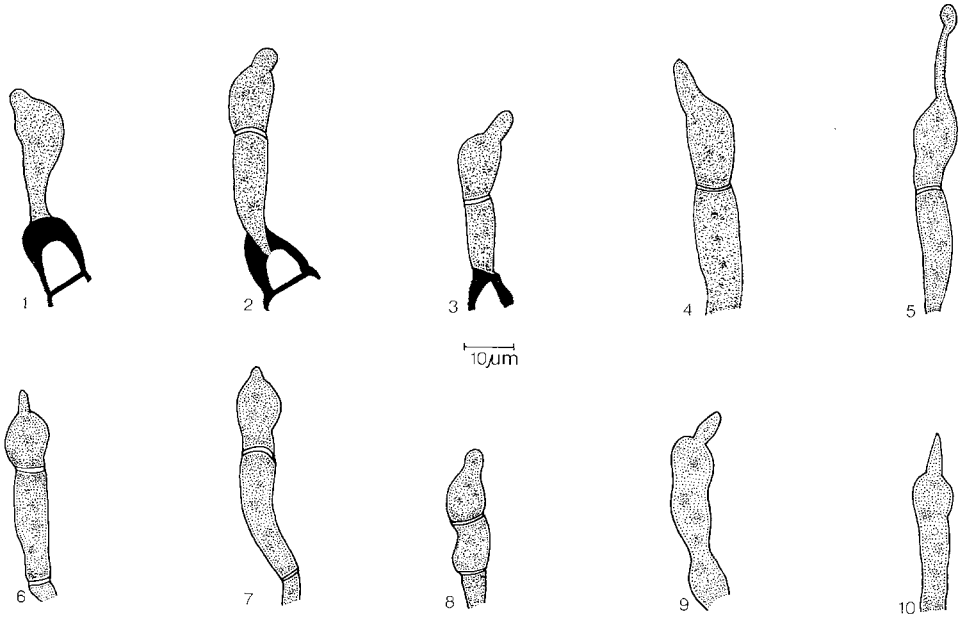

Fig. 4. Puccinia horiana. Reeks van ontwikkelingsstadia van het promycelium in situ.

Fig. 5. *Puccinia horiana.* Stages in the process of nuclear division of the basidiospore.

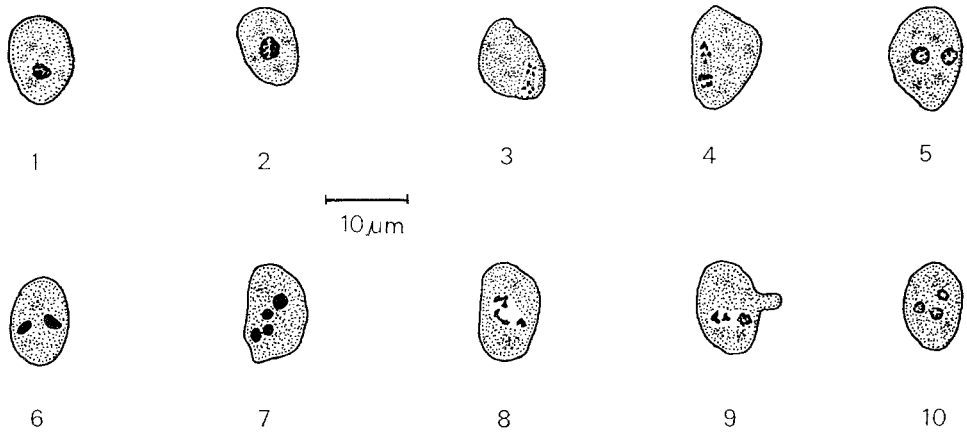

Fig. 5. Puccinia horiana. Stadia in de kerndeling van de basidiospore.

tion percentages were 100, 100, and 4 respectively. On microscope slides, the rate of germination was higher than on the other two substrates.

Basidiospores were allowed to germinate and to develop infection structures on microscope slides for 48 hours to study the germination behaviour of basidiospores on neutral surfaces. Then, the structures were fixed in Carnoy's fluid A, and stained which Iron Alum Hematoxylin. Three patterns of basidiospore germination were distinguished. In the first, the basidiospores developed a germtube of variable length with tapered sharply and gave rise to an infection hypha (Fig. 6–7, 6–8). In the second, the basidiospore germtube developed an appressorium-like vesicle, and eventually produced the infection hypha (Fig. 6–9, 6–10). In the third and rarest pattern, the basidiospore germinated by repetition, and thus produced a secondary basidiospore.

Acknowledgments

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Fig. 6. Photomicrographs of *Puccinia horiana.* (1) A group of germinating teleutospores – Acetocarmine; (2) Teleutospore germinated *in situ*, with promycelium and sterigma – Feulgen; (3) Uninucleate basidiospores – Iron Alum Hematoxylin; (4) Binucleate basidiospore – Feulgen; (5) Basidiospore with 3 nuclei – Feulgen; (6) Basidiospore with 4 nuclei – Feulgen; (7) Basidiospore with short germtube and infection hypha – Iron Alum Hematoxylin; (8) Basidiospore with a long germtube and a short infection hypha – Iron Alum Hematoxylin; (9) Basidiospore; germtube with vesicle at its tip – Iron Alum Hematoxylin; (10) Basidiospore; germtube with vesicle at its tip – Iron Alum Hematoxylin.
Fig. 6. Microfoto's van Puccinia horiana. (1) Een groep kiemende teleutosporen; (2) Teleutospore gekiemd in situ; (3) Eenkernige basidiospore; (4) Tweekernige basidiospore; (5) Driekernige basidiospore; (6) Vierkernige basidiospore; (7) Basidiospore met korte kiembuis en infectiehyfe; (8) Basidiospore met een lange kiembuis en een korte infectiehyfe; (9) Basidiospore; kiembuis met blaasje aan het eind; (10) Basidiospore; kiembuis met blaasje aan het eind.

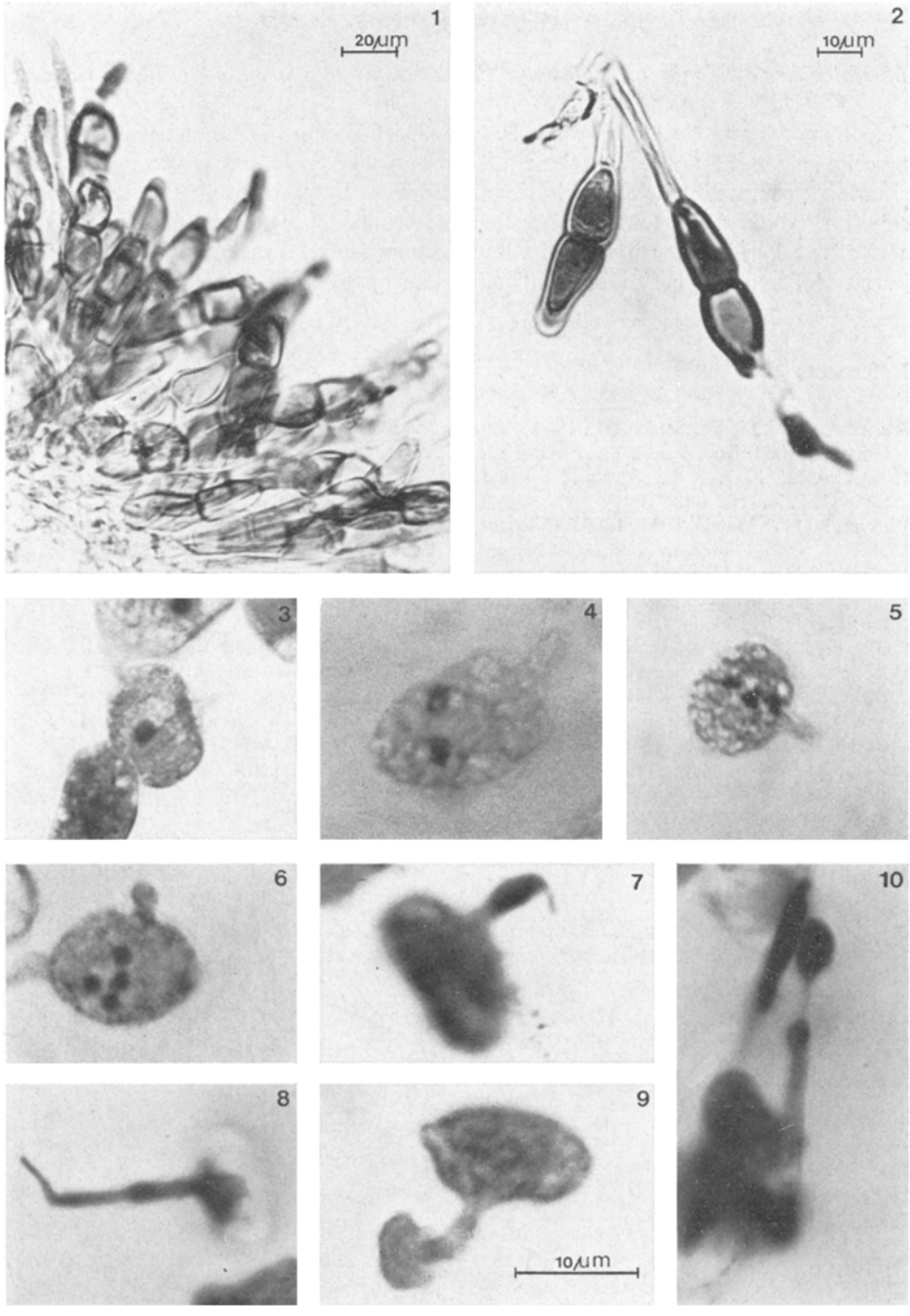

Fig. 6

Samenvatting

Morfologie en cytologie van het promycelium en de basidiospore van Puccinia horiana

Teleutosporen van *Puccinia horiana* P. Henn. kiemen spoedig na hun vorming. Promycelia gekiemd op objectglaasjes zijn langer en smaller dan promycelia gekiemd in situ. Het promycelium is meestal driecellig, soms ook een- of tweecellig. Een promycelium produceert één tot drie, meestal twee basidiosporen. Deze zijn aanvankelijk een- of tweekernig, later meerkernig. Basidiosporen kiemen snel op natte objectglaasjes en bladeren. De basidiosporenkieming kan op drie onderling iets verschillende wijzen verlopen.

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