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TAXONOMIC STUDIES OF THE LEAF RUSTS OF TEMPERATE CEREALS

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ABSTRACT. The rust fungi (Uredinales) parasitizing the leaves of temperate cereals were studied in their uredinial stage to clarify questions relating to their taxonomy. Five species were recognized, all of them belonging to the genus *Puccinia* (Pucciniaceae). A clear morphological distinction could be made between oat crown rust, barley brown rust, rye brown rust, the yellow rusts of wheat and barley, and wheat brown rust. However, no distinction apart from the host range could be made between wheat and barley yellow rust.

INTRODUCTION

The biotrophic life style of the rust fungi (Uredinales) has led to two lines of thought regarding the systematic classification of these organisms at species level, one emphasizing the host range at species level together with morphological characters, the other relying only on the morphological features irrespective of the host on which the fungus occurs. The species concept in both these approaches is artificial and it is questionable how far they can reflect phylogenetical relationships.

From the beginning of the century until the 1960s, the aim of most researchers was to find taxonomic units in the rust fungi which could be described by their host range as well as their morphology. Thus many new rust species were recognized, although their morphological features were in some cases indistinguishable and only quantitative variation was evidenced. A culmination of these studies was the book, *Die Rostpilze Mitteleuropas,* by Gäumann (1959). In this publication c.2000 rust species were described for Central Europe. Arranging them into morphologically and physiologically similar groups ('Formenkreise'), Gäumann achieved a system of clear differentiation, once the host was identified. This system was based on an assumption of strict host specialization. Where very similar rusts appear on the same host, as in the case of wheat and rye brown rusts on rye, differentiation is, however, difficult. In a more recent book, Wilson & Henderson (1966) approached the British rust fungi from a purely morphological point of view and described only about 250 species, with a number of varieties and formae speciales, for the British Isles. The present investigation was aimed to examine further, from their uredinial stage, the systematic position of the following cereal leaf rusts; *Puccinia coronata* Corda (oat crown rust—OCR); *P. hordei* Otth (barley brown rust—BBR); *P. recondita* Roberge ex Desmellier (rye brown rust—RBR); *P. striiformis* Westendorp (wheat and barley yellow rust—WYR, BYR); *P. triticina* Eriksson (wheat brown rust—WBR).

MATERIALS & METHODS

Ten isolates of *P. striiformis* (WYR), six of *P. triticina* (WBR), four of *P. hordei* (BBR) and one each of *P. recondita* (RBR), *P. striiformis*

(BYR), and *P. coronata* (OCR) were artificially inoculated onto their respective hosts using a set of universally susceptible cereal cultivars. Host plants were grown at 14 ±2°C in Levington's potting compost in 12cm pots for whole plant experiments, or in trays for detached leaf experiments. Host seedling leaves were used when they had fully expanded (10–14 days after sowing). All plants were grown in spore-free cabinets and whole plants were kept under polythene domes with a slight positive air pressure to avoid contamination during the period of the experiment. All true leaves developed were removed at 2-day intervals to avoid excessive foliar growth in the domes. Spores of the rust isolates were brushed onto intact plants with cotton buds or dusted onto detached leaves supported on benzimidazole agar (80ppm benzimidazole in 0·7% agar). Detached leaves were incubated at 14 ±1°C in growth cabinets. Samples from both experiments were taken after various intervals, dried to provide herbarium specimens or prepared for examination using light microscopy (LM), fluorescence microscopy (FM) or scanning electron microscopy (SEM) techniques. Three main features were examined: the sorus structure and spore characteristics; germination characteristics; characteristics of vegetative structures (hyphae, haustorial mother cells, growth patterns). The sorus size was measured after the sori had stopped growing (19–21 days after inoculation). The spore size was determined using vacuum-dried spores. Three to four replicates of 20–100 observations were made for each characteristic.

Light microscopy (LM): specimens were examined directly using a Kyowa stereomicroscope fitted with glassfibre incident light. For the measurement of the spore size the dry spores were dusted onto microscope slides and examined under a Leitz Ortholux microscope.

Fluorescence microscopy (FM): leaf sections of approx. 20mm length were fixed and cleared in lactophenol/ethanol (1:2) for 90 s at 81°C (simmering), left in fresh fixative overnight at room temperature, and processed for fluorescence microscopy using the procedure described by Rohringer *et al.* (1977) and stained in 0·1% Calcofluor White M2R New (Cyanide) in 0·1 M Tris/HCl buffer at pH 8·5 for 5 min. The specimens were examined with a Leitz Ortholux II microscope fitted with epi-fluorescence equipment using a Wotan HBO 100 W/2 bulb and excitation filters at 430 and 515nm and barrier filters at 460 and 490nm.

Scanning electron microscopy (SEM): chilled leaves (3–4°C) were cut to approx. 5 × 5mm using a scalpel. Sections were immersed in liquid nitrogen, transferred into glass vials placed in liquid nitrogen on a brass plate (c.5mm thickness) insulated by polystyrene. This cold stage was transferred into a vacuum chamber containing desiccant (phosphorous pentoxide) and evacuated to 0·05 torr for 10 to 16 hours. Specimens were mounted onto SEM stubs using double-sided adhesive tape and colloid silver, coated with gold for 3 min. at 30 mA in an argon atmosphere (Polaron sputter coater), and examined in a Jeol SEM.

RESULTS

The results of morphological examinations are summarized in Table 1.

TABLE 1
Morphological features of cereal leaf rusts

Structural property	OCR	BBR	RBR	YR**	WBR
	Rust isolates*				
Sorus size (μm)	130 × 790	180 × 401	359 × 1121	158 × 343	200 × 780
S.E.	56 540	60 185	124 605	49 98	120 527
Paraphyses	rare	rare	present	rare	present
Colour of dry spores	light brown	medium brown	dark brown	orange	dark brown
Spore size (μm)	16 × 22	17 × 23	19 × 23	17 × 22	18 × 23
S.E.	1·3 1·8	1·7 1·9	1·9 2·4	2·0 1·9	1·5 2·0
Echinulation spacing (μm)	1·44	1·77	1·83	1·45	1·55
S.E.	0·05	0·10	0·24	0·32	0·24
Number of germ-pores	9–12	6–8	6–8	7–9(–14)	(6–)7–9
Germ-tubes	unbranched	branched	branched	unbranched	unbranched
Appressorium size (μm)	16 × 20	15 × 29	15 × 33	NONE	15 × 27
S.E.	2·4 3·0	2·8 5·7	3·2 1·8	—	2·2 2·3
Substomatal vesicle size (μm)	8 × 45	9 × 41	9 × 58	18 × 27	15 × 21
S.E.	2 5	1 6	2 7	1 4	1 2
Hyphae diameter (μm)	3·15	3·65	4·94	7(12–14)***	3·67
S.E.	1·12	1·22	1·18	1·2 (–)	1·18
Haustorial mother cell size (μm)	3·3 × 11·0	3·6 × 14·7	5·7 × 11·0	7·0 × 8·0	5·7 × 11·7
S.E.	0·1 1·2	0·1 1·0	1·4 2·0	0·6 1·2	1·7 2.2
Mycelium	loose	dense	loose	dense	medium

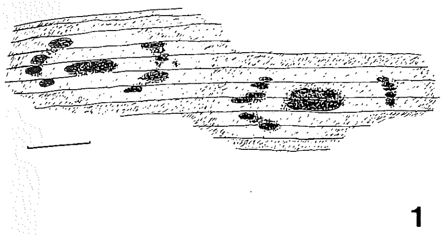
*O = Oat	CR = Crown Rust	**Wheat and Barley isolates
B = Barley	BR = Brown Rust	***Runner hyphae
R = Rye	YR = Yellow Rust	
W = Wheat		

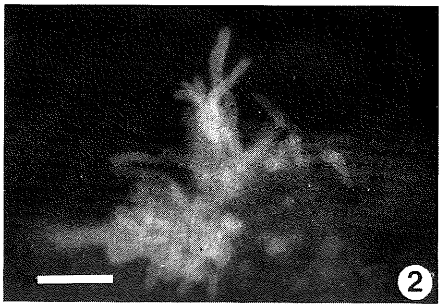
Puccinia coronata (OCR) was distinguished from the other leaf rusts by the light brown colour of its urediniosori (Fig. 1) and the formation of sori which often occurred in small groups. The sori were found to be relatively narrow but could reach a considerable length. No paraphyses could be observed inside the sori, but at their edges hyphae were sometimes extruded (Fig. 2). The spores were relatively small and pear-shaped, the echinulation was very distinct. The spores each possessed 9–12 germ-pores. The germ-tubes were mostly unbranched apart from occasional small branches. The appressoria were the shortest of those of all the leaf rusts producing appressoria, and the substomatal vesicles were long and narrow (Fig. 3). *P. coronata* was found to spread relatively rapidly inside host tissues. At frequent intervals long and narrow haustorial mother cells were formed from the intercellular hyphae which formed a loose mycelium; the hyphae were the narrowest of the rusts examined. Shortly after the eruption of each initial sorus secondary sori were formed close alongside.

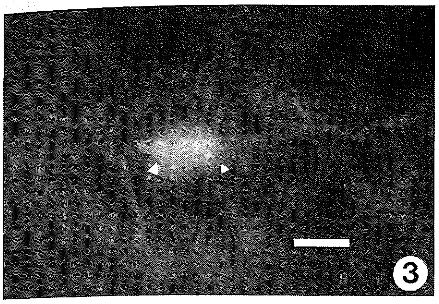
Puccinia hordei (BBR) produced scattered 'rust' brown sori (Fig. 4) of small to medium size. A few simple paraphyses could be observed and, at the edge of each sorus, hyphae protruded occasionally from the mycelium (Fig. 5) as was the case with *P. coronata*. The spores were relatively small in size and oval or round in shape, each with six to eight germ-pores. The echinulation spacings on the spores were relatively wide. The germ-tubes were relatively short and always branched, with the main branch normally leading to the formation of a large appressorium on the host's stoma (Fig. 6). Substomatal vesicles were longitudinal and septate, with normally three to four cells (Fig. 7). *P. hordei* grew slowly to form a dense mycelium. Hyphae had a medium diameter and produced many haustorial mother cells. Only occasionally did secondary sori arise beside the initial sorus. 'Green island' formation was a very common phenomenon on barley hosts (Fig. 8).

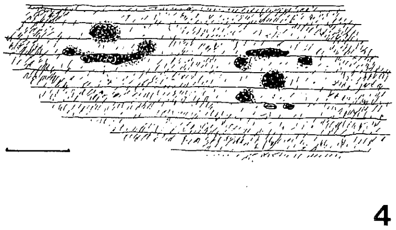
Puccinia recondita (RBR) produced scattered dark brown pustules on rye (Fig. 9). The sori reached 0·8 × 2·7mm and were the biggest within the cereal leaf rusts. In some combinations many simple paraphyses could be observed (Fig. 10). The spores were relatively large, had six to eight germ-pores, and their echinulation spacing was the widest within the rusts observed in this study. The germ-tubes were found to be of medium length, and they sometimes branched dichotomously (Fig. 11). Appressoria developed on stomata, and long, septate, usually two-celled, substomatal vesicles were present (Fig. 12). In rye leaves a loose mycelium grew at a medium speed. Intercellular hyphae were relatively thick. The density of haustorial mother cells was low, partly because rye tissue possessed larger intercellular cavities than the other cereal hosts. Haustorial mother cells were short and often showed a dark spot when stained with calcofluor, presumably where the haustorial neck was formed. The initial sorus was often surrounded by a ring of secondary sori a short time after its eruption.

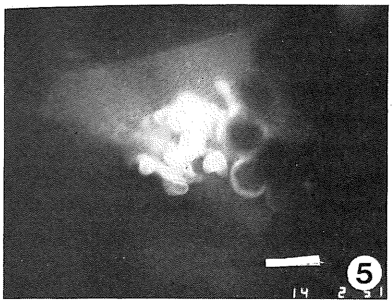
Puccinia striiformis on barley (BYR) and wheat (WYR) developed large colonies in host leaves. The sori were small but joined together longi-

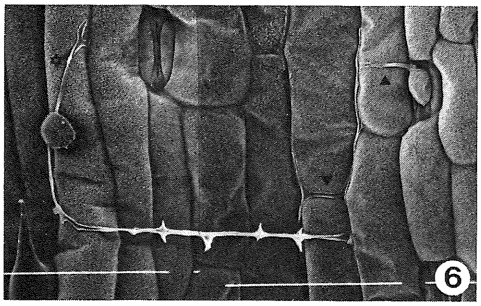


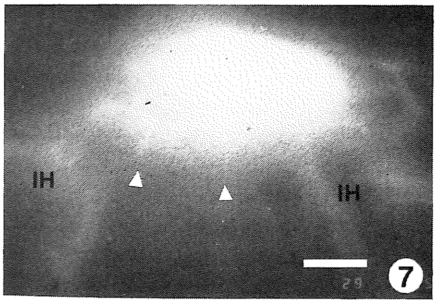


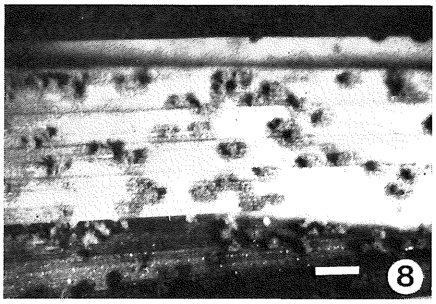






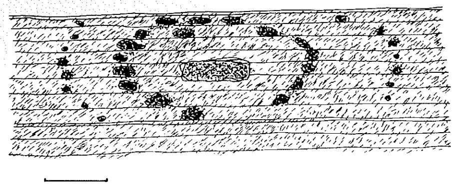






FIGS 1–3, *P. coronata*. 1, urediniosori of *P. coronta* on oats, scale = 1mm; 2, peripheral paraphyses and spores in urediniosorus (FM), scale = 50μm; 3, substomatal vesicle with septa (▲) and infection hyphae (FM), scale = 20μm.

FIGS 4–8, *P. hordei*. 4, urediniosori of *P. hordei* on barley, scale = 1mm; 5, sterile hyphae protruding at sorus edge (FM), scale = 20μm; 6, germinating urediniospore on barley leaf showing initial secondary germ-tube (*) and germ-tube branches (▲), (SEM), scale = 100μm; 7, substomatal vesicle showing septa (▲) and infection hyphae (IH), (FM), scale = 10μm; 8, 'green island formation' on fully susceptible barley (LM), scale = 1mm.



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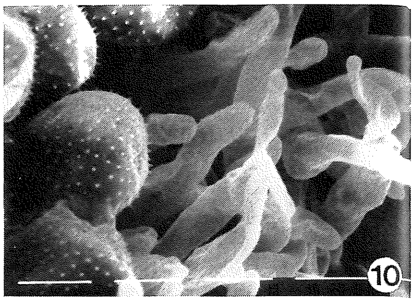

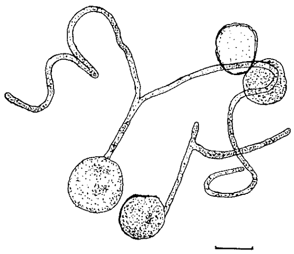

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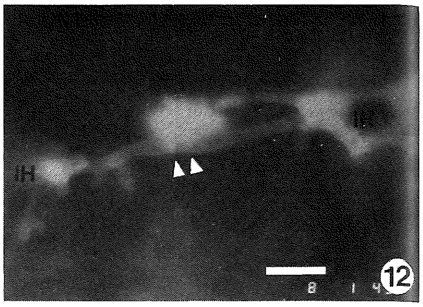

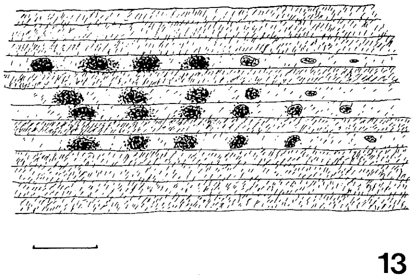

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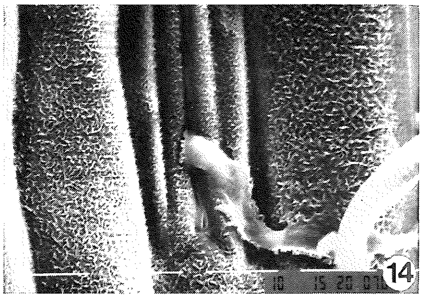
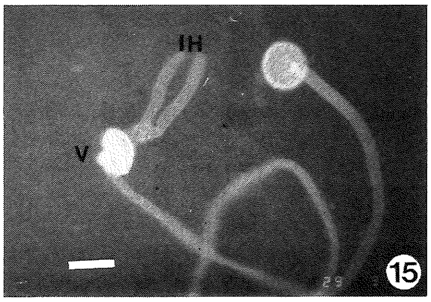
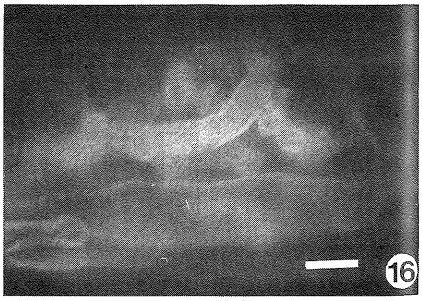
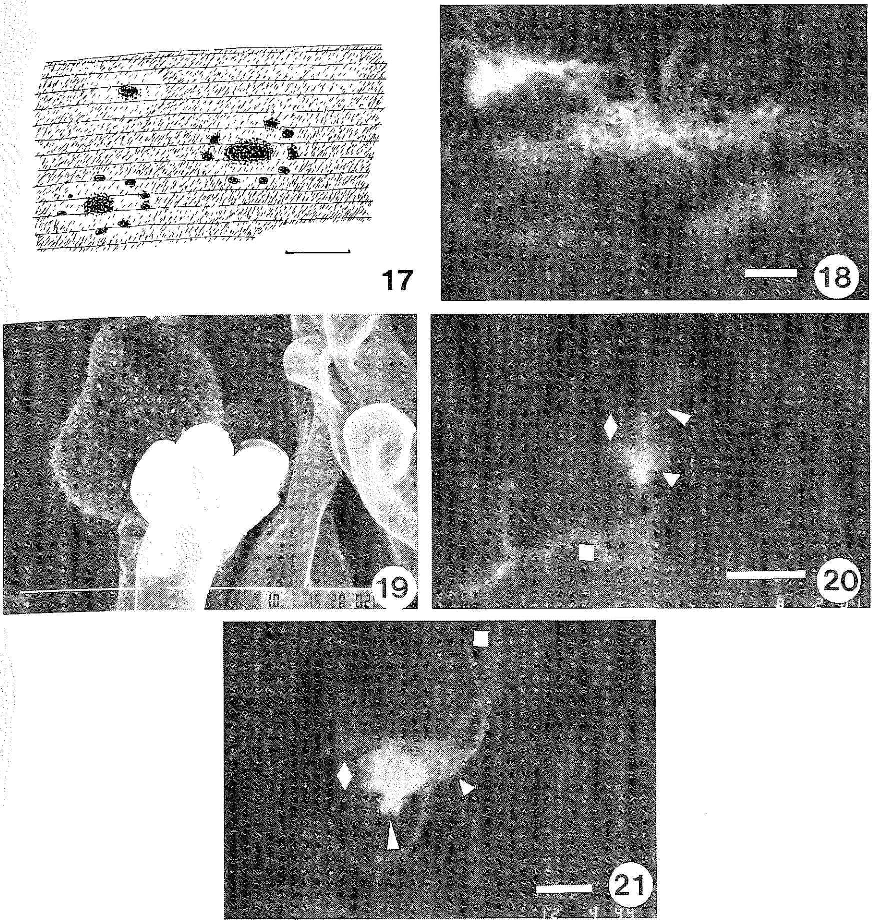






FIGS 9–12, *P. recondita*. 9, urediniosori of *P. recondita* on rye, scale = 1mm; 10, paraphyses at the sorus edge with young, immature spores (SEM), scale = 10μm; 11, germinating urediniospores with germ-tube branching dichotomously, scale = 10μm; 12, substomatal vesicle showing septa (▲) and infection hyphae (IH), (FM), scale = 20μm.

FIGS 13–16, *P. striiformis*. 13, urediniosori of *P. striiformis* on wheat, scale = 1mm; 14, stomatal penetration of BYR on barley without appressorium formation (SEM), scale = 10μm; 15, formation of substomatal vesicle (V) and infection hyphae (IH) in WYR, note also germ-pores in spore (FM), scale = 20μm; 16, runner hypha between host cells in BYR (FM), scale = 20μm.



FIGS 17–21, *P. triticina*. 17, urediniosori of *P. triticina* on wheat, scale = 1mm; 18, simple paraphyses at the sorus edge (FM), scale = 20μm; 19, lobed paraphyses together with simple ones at the sorus edge (SEM), scale = 10μm; 20, germ-tube (■), appressorium (▲), substomatal vesicle (◆) and infection hypha (▼) formed inside host leaf (FM), scale = 50μm; 21, as fig. 20 but formed on surface of host leaf (FM), scale = 20μm.

tudinally when the infection matured, forming long and narrow stripes of a yellow to orange colour (Fig. 13). Barley and wheat isolates showed no observable morphological differences but were not compatible with the respective other host. No paraphyses could be observed. The urediniospores were of medium size and often pear-shaped. The spore wall was hyaline, so that the cytoplasm determined the colour. Echinulation spacing was relatively dense. The number of germ-pores was 7–9(–14). The germ-tubes were very long and unbranched and entered the stomata of hosts directly without forming appressoria (Fig. 14). Inside the substomatal cavity a globular vesicle was formed with normally two or three infection hyphae branching off (Fig. 15). In the host leaves, *P. striiformis* grew very fast and developed a dense mycelium. The intercellular hyphae were large in diameter, and runner hyphae of even bigger dimensions grew for a long stretch along the leaf veins (Fig. 16). Haustorial mother cells were short and thick.

Puccinia triticina (WBR) produced dispersed, chestnut-brown pustules of medium size (Fig. 17). Paraphyses and hyphae often protruded from the sori, especially at the sorus edges (Fig. 18) and sometimes paraphyses showed special shapes (Fig. 19). The urediniospores were relatively large and the distance between neighbouring spines (echinulation spacing) was small. The number of germ-pores per spore varied from (6–)7–9. The germ-tubes were of medium length, unbranched when germinated on agar, but formed small branches on wheat leaves (Fig. 20). Appressoria were formed on host stomata, and globular substomatal vesicles with 4–5 infection hyphae were produced (Figs 20, 21). The rust developed at a medium speed, and colonization of the host tissue took place by sending infection hyphae deep into the mesophyll, rather than spreading mainly close under the epidermis as the other leaf rusts did, and a dense mycelium grew through the whole depth of the infected leaves. Infection hyphae were relatively narrow with haustorial mother cells of a medium size.

DISCUSSION

The present results show clear morphological differences over a wide range of morphological characters between the investigated cereal leaf rusts in their uredinial spore stage. In the past only one worker, Pole Evans (1907), brought into consideration features such as the morphology of appressoria or substomatal vesicles for taxonomic purposes. Other workers (Gäumann, 1959; Wilson & Henderson, 1966; Urban, 1967; Cummins, 1971; Bartos, 1984; and Savile, 1984) mainly emphasized features like spore-size or host range to establish the taxonomy of cereal rusts.

The sorus size is mentioned by some workers (Pole Evans, 1907; Gäumann, 1959; Wilson & Henderson, 1966), but it seems that this feature is partly determined by the host plant tissue and environmental conditions. The variation within the species is relatively great, and only parts of any interspecific differences may be attributed to the fungal genotype. In yellow rust (*P. striiformis*) the sorus size is limited by the relative proximity of neighbouring sori which is a result of the semi-systemic colonization and the rapid growth of this fungus.

In parasites such as the brown rusts, which produce scattered pustules and grow at a slow or medium speed, relatively large pustule sizes are characteristic: the large size giving large numbers of spores may be regarded as compensating for the fewer sori in comparison with yellow rust where large numbers of spores arise by the production of fast growing colonies of unlimited size with many sori from single infections.

Although the presence of paraphyses in urediniosori has been considered unimportant taxonomically (Grove, 1913) this feature was included in the present study. Possibly only one of the earlier descriptions of cereal rusts (Arthur, 1934) mentioned paraphyses in the sori of *P. recondita* and *P. triticina,* while variable observations appear in the literature on other rusts. Cummins (1971) and Savile (1984) report few or no paraphyses for *P. coronata, P. hordei* and *P. striiformis,* whereas Gäumann (1959), and Wilson & Henderson (1966) report the presence of paraphyses in *P. coronata* only. From the present study simple paraphyses, which were observed within sori of all species, probably represent non-functional spore pedicels which occur in certain environmental conditions only. Together with the sterile hyphae protruding at the periphery of the sorus they can separate the spore mass and promote spore detachment in damp conditions. However, more distinctive paraphyses, as in *P. triticina,* seem to represent features of greater importance. Hiratsuka & Sato (1982) described several types of urediniosori in relation to their taxonomic position and the cereal leaf rusts can be classed into two of these categories: aparaphysate and capitate paraphysate. Their form of classification, however, would seem to be too rigid and, from the evidence of the present work, all cereal rusts were found to develop simple paraphysis-like structures, at least in certain conditions, and *P. triticina* was found to approach a capitate paraphysate form.

Another character of minor taxonomic importance is the spore colour. It varies from isolate to isolate and colour mutants of brown and yellow rusts have been reported (Watson & Luig, 1968; Nayer *et al.,* 1981; Newton *et al.*, 1986); thus some caution is needed in its interpretation. However, as a first differential character, the spore colour can be useful in the field. In yellow rust the spore wall is completely devoid of pigment and the orange colour originates from the cytoplasm. The cytoplasm itself is thus unprotected against damage through radiation. This may be one of the reasons why, in single spore inoculations, yellow rust performed less successfully than the other leaf rusts. In the other rusts the brown pigment is located in the spore wall, and the cytoplasm has a light orange colour. This could provide protection against radiation damage.

The spore size depends much on the water content of the spores and the methods of examination. The present investigations showed that spores on water agar were up to 50% larger in diameter than vacuum dried spores. Also, the method of preparation and observation influence the spore size greatly (Beckett *et al.,* 1984), although the method of preparation and examination is not always mentioned in the literature; observations in lactophenol have been carried out by a number of investigators (Cummins, 1971; Savile, 1984; Pole Evans, 1907). The spore sizes reported by these and other workers were generally larger than the results from this study, but results of individual workers differ. However, the relative sizes of spores of the different rusts follow roughly the same trend, although slightly different rankings are evidenced (Table 2). The importance of spore size for the survival of the fungus can be viewed in two ways: larger spores contain more reserve material and thus may give the fungus an advantage in longevity and in the first stages of infection. On the other hand, more material is invested into fewer propagules, and the transportation in air currents and the epidemic spread of larger spores is less extensive.

In this study the echinulation pattern of the rusts was found to be closely linked to the spore size: the number of spines on every spore is

TABLE 2

Comparison of spore size measurements in the literature

	Rust isolates* (Length × breadth or diameter in μm)				
	OCR	BBR	RBR	YR**	WBR
Literature source					
Fischer (1904)	14–18 × 16–21	19–22 × 22–27	20–28	15–20 × 17–30	19·2–27·2
Sydow (1904)	16–24 × 20–30	15–20 × 22–27	22–28[1]	18–26 × 25–30	22–28[1]
Pole-Evans (1907)	20–22	17–22 × 20–30	19–20	25–30	19–20 × 24–25
Liro (1908)	17–24	19–24	20–28 × 28–34	20–30	19–27
Grove (1913)	14–20 × 16–25	16–28[1]	16–28[1]	18–26 × 25–30	16–28[1]
Fragoso (1924)	14–18 × 16–21	18–22 × 18–27	22–26 × 22–28	15–20 × 17–30	20–27
Arthur (1934)	16–20 × 18–24	18–24 × 22–28	13–24 × 16–32[1]	16–26 × 19–30	13–24 × 16–32[1]
Săvulesco (1953)	14–20 × 15–25	18–24 × 20–30	17–23 × 20–28	15–26 × 17–30	18–24 × 20–28
Gäumann (1959)	17–21 × 24–27	17–22 × 20–30	17–22 × 20–28	13–23 × 14–36	17–22 × 18–29
Wilson & Henderson (1966)	10–35 × 13–39	15–20 × 18–26	13–24 × 16–34[1]	12–24 × 25–30	13–24 × 16–34[1]
Cummins (1971)	20–24 × 25–30	18–25 × 21–30	20–25 × 24–32[1]	20–24 × 25–30	20–25 × 24–32[1]
Savile (1984)	17–23 × 22–29	18–25 × 23–30	21–27 × 25–29	18–24·5 × 26–30	19–24·5 × 22–30
Present results	16 × 22	17 × 23	19 × 23	17 × 22	18 × 23

*O = Oat
B = Barley
R = Rye
W = Wheat

CR = Crown Rust
BR = Brown Rust
YR = Yellow Rust

**Wheat and Barley isolates

[1] Considered as the same species in publication.

roughly the same and the distance between spines becomes a variable of the spore size. Only since the development of the electron microscope can precise measurements be made, although Fischer (1904) and more recent workers (Gäumann, 1959; Wilson & Henderson, 1966; Savile, 1984) tentatively described this feature from LM studies. The function of the urediniospore echinulation is to facilitate the spore's attachment to host leaves and possibly the attachment of spores to one another, as in *P. striiformis*. Some advantage may lie in increased friction when air-borne.

Apart from one exception (OCR), where the reported number of germ-pores varies considerably in the literature, this feature is reported consistently by all workers. During germination all the germ-pores swell, but the germ-tube is finally formed only from one of them. Occasionally two germ-tube initials protrude from the spore during the first hours of germination, but when the main germ-tube growth began only one tube remained.

The branching characteristics of rust germ-tubes represent an adaptation to the surface typography of their hosts' leaves (Dickinson, 1970, 1971, 1972). From the presented data it can be seen that two strategies are followed to obtain maximum efficiency in finding stomata. Firstly germ-tubes can grow for a long way across the leaf blade without branching. Sooner or later they will come across one of the host's stomata which are arranged in parallel rows along the leaf; this strategy was found in *P. coronata, P. striiformis* and *P. triticina*. Secondly, the germ-tubes can branch in the proximity of the germinating spore to find the nearest stoma—as could be observed with *P. hordei* and *P. recondita*. According to the literature cereal rust germ-tubes do react to chemotropic stimuli (Grambow & Reisener, 1976) but the host leaf cuticle also provides a thigmotactic stimulus for the growth across the leaf (Johnson, 1934; Dickinson, 1949; Lewis & Day, 1972; Staples & Macko, 1984). The germ-tubes of all cereal leaf rust fungi examined in this study grew across the leaf blade, some short and branching (BBR and RBR), others long and unbranched (OCR, YR and WBR).

CONCLUSIONS

In attempting to clarify the taxonomic position of the cereal leaf rusts, classical taxonomic features as well as developmental characteristics of the urediniospore stage were taken into account. Most questions concerning the taxonomy of these fungi are now resolved in the literature and, from urediniospore stage features together with the partial use of other developmental stages, a clear distinction of the yellow rusts from the brown rusts and crown rust, as well as barley brown rust from the brown rusts of wheat and rye, can be made. On the other hand, the position with wheat and rye brown rust has been less well defined. The present study, where special attention was paid to differences which were independent of the plants the parasites were growing on, showed clear morphological as well as physiological differences between wheat and rye brown rust, and provides evidence in keeping with the view that these two organisms should be regarded as two distinct species, i.e. *Puccinia triticina* and *P. recondita* respectively.

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REFERENCES

ARTHUR, J. C. (1934). *Manual of the Rusts in United States and Canada.* Lafayette, Indiana.

BARTOS, P. (1984). Genes from stem rust resistance in European wheats. *Proceedings of the VIth European and Mediterranean Cereal Rusts Conference,* 29–32.

BECKETT, A., READ, N. D. & PORTER, R. (1984). Variations in fungal spore dimensions in relation to preparatory techniques for light microscopy and scanning electron microscopy. *J. Microsc.* (*London*) 136: 87–95.

CUMMINS, G. B. (1971). *The Rust Fungi of Cereals, Grasses and Bamboos.* New York.

DICKINSON, S. (1949). Studies in the physiology of obligate parasitism: I. The stimuli determining the direction of growth of the germ-tubes of rust and mildew spores. *Ann. Bot.* (*London*), n. ser. 13:89–104.

—— (1970). *Op. cit.*: VII. The effects of a curved thigmotropic stimulus. *Phytopathol. Z.* 69:115–124.

—— (1971). *Op. cit.*: VIII. An analysis of fungal responses to thigmotropic stimuli. *Ibid.* 70:62–70.

—— (1972). *Op. cit.*: IX. The measurement of a thigmotropic stimulus. *Ibid.* 73:347–358.

FISCHER, E. (1904). *Die Uredineen der Schweiz.* Bern.

FRAGOSO, R. J. (1924). *Uredales I. Género Puccinia.* Madrid.

GÄUMANN, E. (1959). *Die Rostpilze Mitteleuropas.* [*Beiträge zur Kryptogamenflora der Schweiz* XII]. Bern.

GRAMBOW, H. J. & REISENER, H. J. (1976). Differenzierung und Wachstum von Puccinia graminis f. sp. tritici in vitro. *Ber. Deutsch. Bot. Ges.* 89:555–561.

GROVE, W. B. (1913). *The British Rust Fungi.* Cambridge.

HIRATSUKA, Y. & SATO, S. (1982). Morphology and taxonomy of rust fungi. In SCOTT, K. J. & CHAKRAVORTY, A. K. (eds) *The Rust Fungi,* 1–36. New York.

JOHNSON, T. (1934). A tropic response of germ-tubes of urediniospores of Puccinia graminis tritici. *Phytopathology* 24:80–82.

LEWIS, B. G. & DAY, J. R. (1972). Behaviour of urediospore germ-tubes of Puccinia graminis tritici in relation to the fine structure of wheat leaf surfaces. *Trans. Brit. Myc. Soc.* 58:139–145.

LIRO, J. I. (1908). Uredineae fennicae. *Bidrag Kannedom Finlands Natur Folk* 65.

NAYAR, S. K., SRIVASTAVA, M., GOEL, L. B., SHARMA, S. K., BAHADUR, P. & MEENA, K. L. (1981). Colour mutation in race 10 of Puccinia recondita Rob. ex. Desm. *Cereal Rusts Bulletin* 9 (1):14–15.

NEWTON, A. C., JOHNSON, R. & CATEN, C. E. (1986). Attempted somatic hybridisation of Puccinia striiformis f. sp. tritici and P. striiformis f. sp. hordei. *Plant Path.* 35:108–113.

POLE-EVANS, B. (1907). The cereal rusts. I. The development of their uredo mycelia. *Ann. Bot.* (*London*) 21:441–466.

ROHRINGER, R., KIM, W. K., SAMBORSKI, D. J. & HOWES, N. K. (1977). Calcofluor: an optical brightener for fluorescence microscopy of fungal plant parasites in leaves. *Phytopathology* 67:808–810.

SAVILE, D. B. O. (1984). Taxonomy of the cereal rust fungi. In BUSHNELL, W. R. & ROELFS, A. P. (eds), *The Cereal Rusts* Vol. I, 79–112. London etc.

SĂVULESCU, T. (1953). *Monografia Uredinalelor din Republica Populară Română.* 2 vols. Bukarest.

STAPLES, R. C. & MACKO, V. (1984). Germination of urediospores and differentiation of infection structures. In BUSHNELL, W. R. & ROELFS, A. P. (eds), *The Cereal Rusts* Vol. I, 255–289. London etc.

SYDOW, P. (1904). *Monographia Uredinarum. I: Genus Puccinia.* Lipsiae.

URBAN, Z. (1967). The taxonomy of some European graminicolous rusts. *Česká Mycol.* 21:12–16.

WATSON, I. A. & LUIG, N. H. (1968). Progressive increase in virulence in Puccinia graminis f. sp. tritici. *Phytopathology* 48:358–361.

WILSON, M. & HENDERSON, D. M. (1966). *British Rust Fungi.* Cambridge.