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MORPHOLOGY OF GERMLINGS OF UREDINIOSPORES AND ITS VALUE FOR THE IDENTIFICATION AND CLASSIFICATION OF GRASS RUST FUNGI

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Proefschrift
ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen
op gezag van de rector magnificus,
dr. C. M. Karssen,
in het openbaar te verdedigen
op donderdag 16 juni 1994
des namiddags te half twee in de Aula
van de Landbouwuniversiteit te Wageningen

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Stellingen

1. De overeenkomsten in morfologie van de infectiestructuren en in isozym-bandenpatronen tussen roestschimmels voorkomend op tarwes (*Triticum* spp.) en *Aegilops squarrosa* en op kweekgras (*Agropyron repens*) en *Hordeum jubatum* suggereren dat beide kenmerken specifiek zijn voor een roestsoort en niet voor de waard waarop de roest gevonden wordt.

2. Kenmerken van uredinia en urediniosporen worden sterker door milieu-omstandigheden beïnvloed dan de kenmerken van de infectiestructuren ontstaan uit de urediniosporen.

3. Gezien de morfologische, biologische en biochemische overeenkomsten tussen *Puccinia hordei* en *Uromyces*-roesten voorkomend op gerst, is het juister deze roesten in één soort onder te brengen.

4. Een volledige referentie-collectie van roestschimmels bestaat uit een preparatendoos met daarin stukjes gekleurd blad.

5. Een bijzondere roestschimmel vind je pas als je de moed hebt opgegeven.

6. De hoge populariteit van biotechnologisch onderzoek mag niet ten koste gaan van klassiek taxonomisch onderzoek.
   L. Fresco, NRC-Handelsblad, 10-3-94.

7. Vanuit een botanisch standpunt kan de aanleg van de Betuwelijn aantrekkelijk zijn.
   Koster, A. 1987. Notitie no. 14. Adviesgroep Vegetatiebeheer Wageningen.

8. Militaire ingrepen van buitenaf kunnen geen oplossingen brengen in burgeroorlogen.

9. Het is juister het Nationale Ballet het Amsterdams Ballet te noemen.

10. Positieve discriminatie in sollicitatie-procedures is een belediging voor de vrouw.

11. De mededeling op pakken Hagelslag: 'doordat nu ook het tuitje van karton is, kun je het pak bij het oud papier zetten' wekt de indruk dat de producent niet vermoedt dat de consument in staat is een metalen tuitje te verwijderen.

12. Een roestende vogel is beter af dan een roestende plant.

Stellingen behorende bij het proefschrift 'Morphology of germlings of urediniospores and its value for the identification and classification of grass rust fungi'

Charlotte Swertz

Wageningen, 16 juni 1994

ADDENDUM

De publicatie van dit proefschrift is gedeeltelijk gefinancieerd door het LEB-fonds (Stichting 'Fonds Landbouw Export Bureau 1916/1918').

Aan mijn ouders

VOORWOORD

Het is al weer bijna 5 jaar geleden dat ik op de vakgroep Plantenveredeling van de Landbouwuniversiteit Wageningen ben begonnen aan een onderzoek aan roestschimmels van grassen en granen. Gedurende deze vijf jaar heb ik niet voortdurend op Plantenveredeling achter een microscoop, computer of ziek plantje gezeten, maar ik heb ook vele verzamelreizen en werkbezoeken aan andere landen en instituten gebracht.

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De gemaakte reizen waren niet alleen wetenschappelijk maar ook cultureel interessant, omdat ik bezoeken heb gebracht aan o.a. Tsjechië (voor èn na de Fluwelen Revolutie), de Verenigde Staten en Canada, Schotland, Duitsland en Israël. Het ontmoeten van mensen uit zoveel verschillende culturen en landen heb ik als een unieke en belangrijke levenservaring opgevat. Vooral het werkbezoek aan Noord Amerika in de zomer van 1991, een busrondreis om roest te verzamelen door Tsjechië met Dr. Marková in september 1990 en een roest-verzameltrip over de Golan hoogte, Israël, in mei 1992 met Yehoshua Anikster hebben grote indruk op mij gemaakt.

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TERMINOLOGY AND ABBREVIATIONS

Germling morphology

SSV = substomatal vesicle
PIH = primary infection hypha
HMC = haustorial mother cell
SIH = secondary infection hyphae arising from the substomatal vesicle
SH = secondary hyphae arising at the SSV-side of the HMC-septum
SH-ADD = secondary hyphae arising along the primary infection hypha

Orientation:
longitudinal = parallel to the long axis of the leaf
transverse = perpendicular to the long axis of the leaf
horizontal = in the same plane as the epidermis
vertical = growing deeply into mesophyll layers

Molecular and biochemical techniques

DAPI = 4',6-diamidino-2-phenylindole
DIA = diaphorase
EP = electrophoretic phenotype
GDH = glutamate dehydrogenase
LAP= leucine amino peptidase
PGI = phospho glucoisomerase
6-PGDH = 6-phosphogluconate dehydrogenase
PCR = polymerase chain reaction
PI = propidium iodide
RAPD = random amplified polymorphic DNA
RFLP = restriction fragment length polymorphism

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Abstract

The identification and classification of grass rust fungi is often difficult since most traditionally used morphological characters are quantitative and subjective. Besides, when using the host range as a taxonomic criterion, it is important to realize that a rust fungus may have jumped to a new host species and that host range may also be affected by the variability and age of the host plant, and inoculation conditions. The present study was initiated to assess the taxonomic value of the germling morphology of the urediniospores of grass rust fungi. The germling morphology of grass rust fungi was observed after inoculation on the barley line L94. Since the rate of development and the morphology of the germlings was similar in host and non-host plants until formation of the first haustorium, rust fungi collected from barleys were studied at about 20 h after inoculation and from other grasses at about 40 h.

Germling morphology was proven to be a reliable and useful criterion for identification and classification of grass rust fungi. It enabled an easy discrimination of species complexes that are very similar in traditionally used morphological characters, e.g. *Puccinia hordei* and *P. recondita*. Species complexes which are distinct on the basis of these traditionally used morphological characters were also distinct in germling morphology, viz. *Puccinia coronata*, *P. graminis* and *P. brachypodii*. Besides, germling morphology differed greatly between taxa subsumed under the *P. recondita* and *P. brachypodii* complexes. In other taxa the differences were mostly quantitative.

The differences and similarities in germling morphology observed within and between species complexes were in general correlated with differences in isozyme banding patterns, nuclear DNA contents, and literature data on several other molecular, biochemical, and hybridization experiments.

The results from the studies on germling morphology and isozyme banding patterns suggest to treat the species included in the *P. brachypodii* and *P. recondita* complexes as separate species, to recognize varieties within *P. coronata* and *P. striiformis* (including the newly described var. *poae*), to unite *P. hordei* and *Uromyces* rusts from barleys in one species, and not to assign any taxonomic rank yet to taxa subsumed under *P. graminis*.

1 GENERAL INTRODUCTION

The rust fungi or rusts (Uredinales, Basidiomycotina) are biotrophic parasites. They grow on a wide range of green plants varying from ferns and conifers to the more advanced families of monocotyledons and dicotyledons (Cummins & Hiratsuka, 1983). In total, about 6000 species distributed among 150 genera have been described worldwide (Hawksworth *et al.*, 1983). A few species have now been successfully cultured on artificial media (Maclean, 1982; Fasters *et al.*, 1993).

On the Gramineae about 400 rust species distributed over six genera have been recognized worldwide (Cummins, 1971). In Europe on cereals and grasses two genera prevail: about 23 to 35 *Puccinia* species and about 6 *Uromyces* species have been identified (Urban, 1969; Cummins, 1971).

Economically, rust fungi are major pathogens as they are the causal agents of several important plant diseases in cereals and other crops all over the world. Examples of cereal rusts and their causal organisms are: Wheat stem rust, *Puccinia graminis* Pers. : Pers. (also named *P. graminis* subsp. *graminis* f.sp. *tritici* Erikss. & E. Henn.); Wheat leaf rust, *P. triticina* Erikss. (also named *P. recondita* f.sp. *tritici* Erikss. & E. Henn.), and corn leaf rusts, *P. sorghi* Schw. and *P. polysora* Underw. Examples of other economically significant rusts are Coffee leaf rust caused by *Hemileia vastatrix* Berk. & Br. and White pine blister rust caused by *Cronartium ribicola* J.C. Fischer.

Life cycle

In the life cycle of a rust fungus, up to five morphologically, cytologically and functionally different spore stages may occur, viz. pycnia, aecia, uredinia, telia and basidia, generally numbered 0–IV (Table 1). Depending on the number of spore stages present, the life cycle is called macrocyclic (having all spore stages), demicyclic (lacking the uredinial stage) or microcyclic (lacking both the aecial and the uredinial stage). In the macrocyclic and demicyclic life cycles either all spore stages can be produced on the same host species, an autoecious life cycle, or the fungus may need two unrelated host species, which generally live in the same plant association, to complete its heteroecious life cycle (Laundon, 1973; Cummins & Hiratsuka, 1983).

Many rust fungi causing diseases on cereals and grasses (further named grass rusts) have a heteroecious macrocyclic life cycle. *Puccinia graminis*, for example, forms its aecia on *Berberis* (the aecial host) and its uredinia and telia on several cereals and grasses (the telial hosts). However, several grass rust species are facultatively heteroecious and can survive in the absence of the aecial host. The rust fungus overwinters as urediniospores or as mycelium on (voluntary) host plants or winter cereals. When the urediniospores do not survive during overwintering, the spores may come into the region by long-range annual dispersal. Examples of rusts

Table 1. Spore stages present in the complete life cycle of rust fungi.

spore-bearing structure	spores	numerical designation	nuclear condition
pycnium[a]	pycniospores[a]	0	monokaryotic
aecium	aeciospores	I	dikaryotic
uredinium	urediniospores	II	dikaryotic
telium	teliospores	III	dikaryotic[b]
basidium	basidiospores	IV	monokaryotic

[a] Also named 'spermogonium' and 'spermatia'.
[b] Nuclear fusion and meiosis accompany germination of the teliospores.

which may survive in absence of the aecial host are rusts belonging to *P. graminis*, the *P. brachypodii* Otth complex and the *P. recondita* Rob. ex Desm. complex. Besides, for some grass rusts the aecial host is – still – unknown, e.g. *P. striiformis* Westend. and *P. holcina* Erikss. (also named *P. recondita* f.sp. *holcina* Erikss.) (Wilson & Henderson, 1966; Littlefield, 1981).

Host specialization

Grass rust fungi vary widely in the width of their host range. In the aecial stage most heteroecious grass rust species can occur only on plants belonging to one family. Aecia of *Puccinia coronata* Corda, for example, are always formed on species of the Rhamnaceae (*Rhamnus* and *Frangula*) and aecia of *P. hordei* Otth on species of the Liliaceae, commonly *Ornithogalum*. Only in *P. recondita* s.l. a very wide aecial host range has been reported (Anikster & Wahl, 1979).

In the telial stage some grass rust fungi have a very wide host range that includes many grass species belonging to different genera, e.g. *P. coronata* (Eshed & Dinoor, 1981), whereas other rusts can occur only on plants belonging to one genus, e.g. *P. hordei* and *P. sorghi*.

Grass rust fungi may also show host specialization at the infraspecific level. Examples are *P. graminis* subsp. *graminis* which is restricted to cereals and subsp. *graminicola* Urban which is restricted to various grasses, and *P. striiformis*, of which var. *striiformis* is restricted to cereals and var. *dactylidis* Manners to *Dactylis glomerata* (Manners, 1960; Laundon, 1973; Savile, 1984).

Host specialization is helpful for the identification of grass rust fungi. When the identity of the grass infected with a rust fungus is known, a host index for the uredinial and telial stages as prepared by Cummins (1956) will only give a few names which have to be checked. Though helpful, this index should be used with caution, since identification of, particularly vegetative, grasses can pose severe problems. Besides, the host range should not be given full reliance. A rust fungus may attack normally resistant or non-host species under certain conditions, like

under a heavy spore attack (Savile, 1984), or the rust may have jumped to a new host species (Savile, 1979). When using the host range it is also important to take into account the variability of the host plant, whether the plants have been naturally infected or artificially inoculated, and the age of the host plant (Anikster, 1984).

Taxonomy

In the beginning of this century some authors (i.a. Arthur, 1929) considered the life cycle an important criterion in rust taxonomy. Many genera were erected or resurrected on the basis of the life cycle alone. However, the recognized genera were too artificial to gain wide acceptance (*Puccinia* species can be macrocyclic or microcyclic and autoecious or heteroecious) and the approach was abandoned even by its initiators. Nowadays, generic delimitation is based on morphological characters of telia and pycnia (Cummins & Hiratsuka, 1983).

At the infrageneric level, taxonomic concepts in rust fungi differ greatly between authors. Some authors emphasized the value of the morphological characters together with the host range (i.a. Gäumann, 1959; Urban, 1969; Savile, 1984). They recognized many species (sometimes subspecies or varieties) which often are morphologically identical, but differ in telial host range. With this narrow species concept a reliable identification of rust fungi that occasionally occur on the same plant species may be difficult when morphological characters are very similar (e.g. Wheat leaf rust fungus and Rye leaf rust fungus which both may infect rye).

Other authors used a broad species concept. They preferred to use the morphological characters, irrespective of the host on which the fungus occurs (i.a. Wilson & Henderson, 1966; Cummins, 1971). They recognized some large (morpho-)species complexes, of which the included taxa show a large overlap in morphological characters (e.g. subspecies or varieties recognized in *P. graminis* or *P. brachypodii*) or of which the included taxa are (nearly) identical in morphology (e.g. *formae speciales* of *P. recondita*).

However, a *forma specialis* is a taxonomic category not officially recognized in the International Code of Botanical Nomenclature. Hiratsuka (pers. comm.) advised to use only sanctioned categories, like subspecies, variety, sub-variety, *forma* or *sub-forma*, instead of *forma specialis*. In the present study the *forma specialis* designation is only occasionally used to quote an author's opinion about a specific rust fungus.

Both narrow and broad species concepts are being used when naming rust fungi, which results in the occurrence of many different and confusing scientific names. Wheat leaf rust, for example, is either called *P. triticina* (Eriksson, 1899), *P. perplexans* var. *triticina* (Erikss.) Urban f.sp. *triticina* (Urban, 1969), *P. persistens* subsp. *triticina* (Erikss.) Urban & Marková (Urban & Marková, 1992) or *P. recondita* f.sp. *tritici* (Wilson & Henderson, 1966).

Each species concept has its constraints. In the narrow species concept much value is given to the identity of the host. Most host genera have their own rust

fungus, e.g. *Puccinia triticina* and *P. aegilopis* R. Maire, on *Triticum* and *Aegilops*, respectively, but when rust fungi collected from one of these hosts may also infect the other host, conspecificity would be more appropriate. In the broad species concept much value is given to morphological similarities between taxa, whereas taxa included may differ considerably in host specialization in the aecial and telial stages and may also show small but constant differences in morphological characters of the aeciospores, urediniospores or teliospores, e.g. the *P. recondita* species complex.

The various names and species concepts in rust fungi are caused mainly by the paucity of differences in the few available spore characters between taxa and the difficulties encountered when using the host range as a taxonomic criterion. One or more additional criteria, enabling a more reliable distinction of rust taxa might be very valuable not only for the identification but also for the classification of rust fungi. Mycelium morphology of urediniospore germlings (Pole Evans, 1907; Niks, 1986, 1987, 1989; Helfer, 1987), biochemical (Burdon & Marshall, 1981; Micales *et al.*, 1986; Chen *et al.*, 1992) or molecular techniques (Michelmore & Hulbert, 1987; Durán & Gray, 1989; Vogler & Bruns, 1993) might prove useful.

Research objectives

This research was initiated to assess the value of mycelium morphology (germling morphology) of the urediniospores as a criterion for identification and classification of rust fungi of cereals and grasses. This value was determined by relating differences in germling morphology of selected grass rust taxa to morphological characters of the sori and spores, the host range, and to the nuclear DNA content and isozyme banding patterns of the urediniospores.

Taxa to be studied were selected on the basis of (i) economical importance, (ii) differences in taxonomic concepts between authors and (iii) availability. Although in grass rust fungi both narrow and broad species concepts are still used, the broad species concept was followed to refer to the selected species, as this concept facilitated the comparison of morphologically similar taxa occurring on various host plants. The following species complexes were studied:

- *Puccinia coronata* Corda
- *Puccinia graminis* Pers. : Pers.
- *Puccinia brachypodii* Otth
- *Puccinia recondita* Rob. ex Desm.
- *Puccinia hordei* Otth and *Uromyces* species occurring on barleys
- *Puccinia striiformis* Westend.

Outline of this study

In Chapter 2 the value of the traditionally used morphological characters of uredinia, urediniospores, telia and teliospores for identification and classification are reviewed.

To study the germling morphology of the urediniospores, the spores need to germinate on a suitable substrate. The development of infection structures in host and non-host plants is described and compared in Chapter 3.

Chapter 4 describes the germling morphology of five of the rust complexes studied. In addition, for each species complex the main differences in sorus and spore characters, the host range and the taxonomic concepts of some authors are given.

Chapter 5 discusses the value of the morphological characters of urediniospore germlings for identification and classification and the suitability of several methods to observe the germling morphology.

Chapter 6 explores the use of some modern techniques in rust taxonomy, viz. the nuclear DNA content and isozyme banding patterns.

In the general discussion, Chapter 7, the value of the germling morphology as a criterion for taxonomic studies of grass rust fungi is discussed and compared to morphological characters of sori and spores, host range, isozyme banding patterns, nuclear DNA content and literature data.

2 MORPHOLOGICAL CHARACTERS OF UREDINIA AND TELIA

For the taxonomic classification of rust fungi morphological characters of all spore stages are used. The identification of genera and species, however, relies mainly on the morphology of uredinia and telia.

Observation of morphological characters

For the observation of the morphological characters of sori and spores a stereomicroscope or a magnifying glass and a microscope with both bright field and phase contrast optics are required. Microscopic slides are prepared by mounting spores or sections of sori in a mounting medium followed by gentle heating to achieve full turgor and clearing of the spores (Savile, 1984). Mounts are best made in lactophenol (Cummins & Hiratsuka, 1983; Savile, 1984) or a similar non- or slow-drying medium with some clearing action, like polyvinyl alcohol (Omar *et al.*, 1978). In general, no stain is needed to improve the visibility of spore characteristics.

Value of morphological characters of uredinia and telia for taxonomic purposes

Uredinia, telia and spores have many morphological characters that are used to classify the grass rust taxa at the generic or (infra)specific levels. The value for identification of many of these characters can be questioned. Firstly, because environmental conditions may bring about morphological changes (Stakman & Levine, 1919; Levine, 1923; Littlefield & Schimming, 1989; Jacobs & Buurlage, 1990); secondly, because quantitative characters may show a large overlap between taxa (Cummins & Greene, 1966; Cummins, 1971), and thirdly, because many characters are difficult to observe or name accurately (e.g. colour of the spore wall). In the following sections some characters used to delimit rust taxa will be discussed, with emphasis on the taxa investigated in this study.

Uredinia

The uredinia (Fig. 1A) are sori bearing one-celled urediniospores singly on pedicels. The position on the plant, the arrangement, size and colour of the uredinia, and the presence of paraphyses in the uredinia are characters which are to some extent used for identification and classification.

The position of the uredinia on the plant may be amphigenous (on both sides of the leaves), abaxial (lower side), adaxial (upper side) or on the leaf sheaths. Often the uredinia are not restricted to one particular position but may be found on several parts of the plant. Uredinia of *Puccinia graminis* and *P. coronata*, for example, can be found both on the leaves and the leaf sheaths.

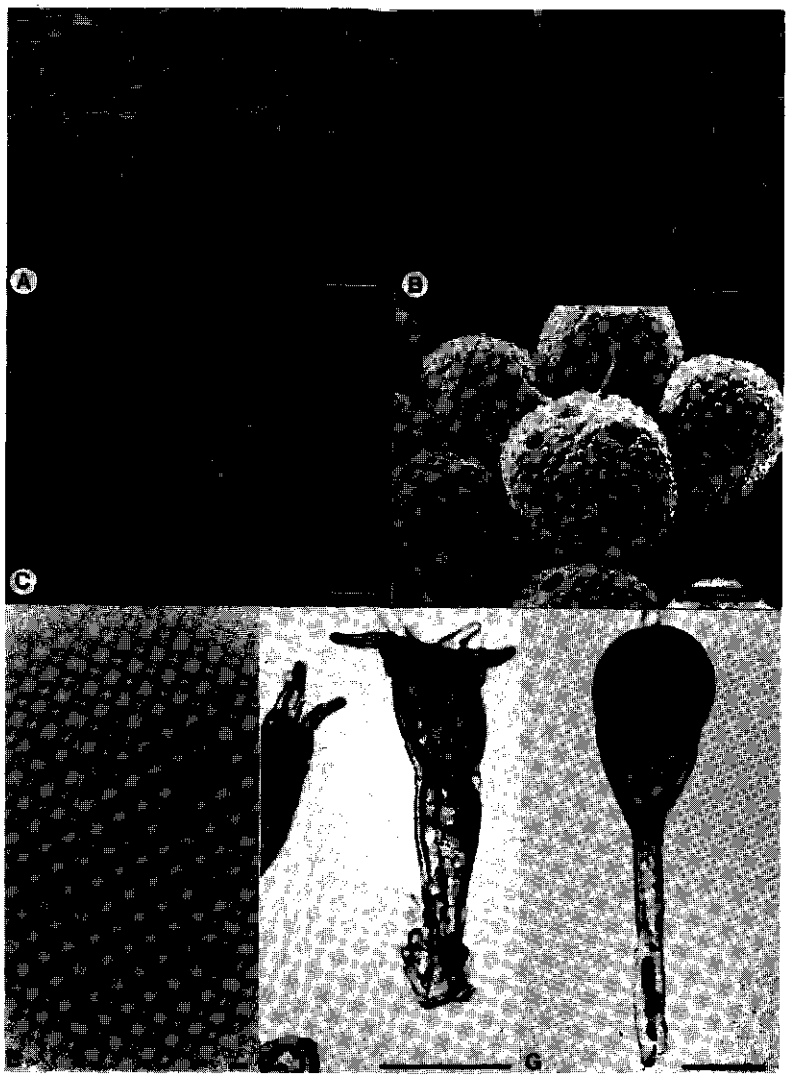


Figure 1. **A–D.** Scanning electron micrographs. **A–C.** Leaves were gold-coated; **D.** after acetolysis (Reitsma, 1969) which removed the outer spore wall layer, the spores were critical point-dried and gold-coated. Observations were made with a Cambridge Stereoscan at the section Electron-Microscopical Structure Analysis of the Department of Biology, University of Utrecht. **E–G**: Bright-field micrographs, scale bar = 20 μm. **A.** Ruptured urediniun with urediniospores of *Puccinia hordei* from *Hordeum vulgare* (isolate 1-2-1). Note the presence of a pedicel (arrow). **B.** Thick-walled paraphyses and urediniospores of *P. brachypodii* from *Arrhenatherum elatius* (C.S. 90.070). **C.** Detail of spine spacing (echinulation) of *P. brachypodii* from *Arrhenatherum elatius* (C.S. 90.070). **D–E.** *Puccinia hordei* from *Hordeum vulgare*: **D.** Germ pores (isolate 1-2-1). **E.** One- and two-celled teliospores (isolate 28). **F.** Coronate teliospore of *P. coronata* from *Holcus lanatus*. **G.** Persistent pedicel of teliospore of *P. graminis* from *Secale cereale*.

The arrangement of the uredinia is either linear or scattered. Stripe rust or yellow rust (caused by *P. striiformis*) derives its name from the arrangement of the sori (uredinia and telia) in chlorotic stripes, although the sori may be scattered on seedling or emergent leaves (Savile, 1984). Also some other leaf rust fungi have linearly arranged uredinia. Examples are: *P. brachypodii* on *Brachypodium* spp.; *P. pygmaea* Erikss. on *Calamagrostis* spp.; *P. elymi* Westend. on *Elymus arenarius*; and *P. recondita* s.l. on *Agropyron junceiforme*. On the latter three mentioned hosts, rust fungi must develop their sori in lines because of the longitudinal rows of sclerenchyma (Guyot, 1939; Wilson & Henderson, 1966). Other grass rusts (treated in the present study) have scattered uredinia. In many instances a primary uredinium will be surrounded by secondary uredinia arranged in a circle in later stages of fungus growth.

The size of the uredinia is influenced by host plant tissue and environmental conditions. Uredinia which are produced on resistant genotypes or uncongenial hosts are smaller than on highly susceptible or congenial hosts (Levine, 1923; Jacobs & Buurlage, 1990). This was also confirmed in experiments conducted during this study, in which urediniospores of Barley leaf rust (*P. hordei*) were inoculated on three barley accessions which differed in susceptibility to this rust fungus. Uredinial size was significantly larger on a highly susceptible line (L94), than on a partially resistant cultivar (Vada) and an incomplete hypersensitive line (L94 + Pa7)[1]. This was found in the seedling stage and, to a lesser extent, in the adult plant stage (Table 2). Adverse environmental conditions (like excessive heat, deficient light and drought) decrease the size of the uredinia too (Levine, 1923). The size of sori can also vary considerably between sori produced on the same leaf.

The colour of the uredinia is often used as a first differential character for identification in the field. Several common names of grass rust fungi even refer to this colour (yellow rust, brown rust). However, adverse environmental conditions can affect it. For example, at high temperature, low humidity or high illumination the uredinia are darker in colour than under normal greenhouse conditions (Stakman & Levine, 1919). Even in the greenhouse it was observed that pale- and dark-coloured uredinia could be present on the same leaf. Examination of spore characteristics and germling morphology of urediniospores collected from these uredinia indicated that they belonged to the same species. The occurrence of aberrantly coloured urediniospore populations in the cereal rusts (i.a. Watson & Luig, 1968; Green, 1971; Martens, 1985; Newton *et al.*, 1986), the effect of environmental conditions on spore colour, and the fact that the colour of the uredinia has only a few (often intergrading) character states — yellow, yellowish brown, cinnamon-brown, brown, orange-yellow and orange — affect the merits of uredinial colour as a diagnostic character.

[1] A near-isogenic line of barley line L94, in which the recessive pa7 gene is substituted by the dominant Pa7 gene. The hypersensitive reaction conferred by this gene is expressed incompletely in the L94 background (Niks & Kuiper, 1983).

Table 2. Average length and width of uredinia and urediniospores produced on leaves in seedling and adult plant stage of three barley accessions differing in susceptibility to Barley leaf rust, *Puccinia hordei* (see text for explanation). Measurements were made three weeks after inoculation. Uredinia were observed with a stereomicroscope (30 ×); urediniospores were mounted in polyvinyl alcohol and observed with bright field microscopy (1000 ×).

accession	observation on seedling		observation on adult plant	
	length	width	length	width
uredinia[a]				
L94	8.32a[c]	3.04a	6.98a	2.18a
Vada	5.48b	2.12b	3.96b	1.63a
L94+Pa7	1.34c	0.84c	3.76b	1.64a
urediniospores[b]				
L94	27.6a[c]	22.0a	26.6a	21.2a
Vada	26.0b	21.0a	25.6a	19.8a
L94+Pa7	26.4b	20.6b	26.8a	20.8a

[a] Means (mm) are based on measurements of 20 uredinia from 5 leaves per cultivar.
[b] Means (μm) are based on measurements of 5 urediniospores from 5 uredinia from 5 leaves per cultivar.
[c] Values in each column followed by different letters differ significantly (P = 0.05) according to the LSD test.

The presence of paraphyses in the uredinia has been considered taxonomically important by Hiratsuka & Sato (1982). A distinction should be made between thin-walled and thick-walled paraphyses. Thin-walled (wall *c.* 1 μm thick) paraphyses, which resemble spore pedicels but differ from these by their slightly clavate shape, are mostly situated at the margins of the uredinium. (Only in *P. pygmaea* they may be intra-uredinial). Often several cross-sections of uredinia have to be made to observe thin-walled paraphyses and to distinguish them from the pedicels. Besides, thin-walled paraphyses are not necessarily present in all uredinial collections of a taxon (Ullrich, 1977). Therefore, in his group system key for grass rusts Cummins (1971) had to include some rusts twice: in the group with and in that without paraphyses (e.g. *P. coronata* and *P. striiformis*). Moreover, Helfer (1987) found that all cereal rusts, including *P. hordei* and *P. recondita* which are reported in most literature as having no paraphyses (Cummins, 1971; Savile, 1984), could develop simple (thin-walled) paraphyses, at least under certain conditions. The

occurrence of these structures renders the presence of thin-walled paraphyses unsuitable for the identification of rust fungi.

Thick-walled paraphyses (Fig. 1B), however, which are intra-uredinial in the *P. brachypodii* complex, are present in each uredinium studied and easily discerned from spore pedicels due to the thick wall (2–4(–9) μm). The shape of the upper part (head) of the paraphysis may be clavate to capitate, depending on the presence of a constricted 'neck'. The shape of the head of the paraphyses was rather variable within a collection and therefore not useful for the recognition of taxa within the *P. brachypodii* complex. Still, due to their easy observation the presence of thick-walled paraphyses is very useful for the recognition of *P. brachypodii* s.l.

From the above-mentioned reports and observations it is clear that only few uredinial characters have the potentials to discriminate between taxa. The arrangement and position of the uredinia and the presence of thick-walled paraphyses are useful features, though arrangement of the uredinia is often not constant in a taxon and position has only a few character states (linear or scattered). Size and colour of the uredinia can be suitable characters, but they are influenced by various factors, subjective, and can show overlap between taxa.

Urediniospores

Several characteristics of the urediniospores, viz. visibility, arrangement and number of the germ pores, size and echinulation of the spores, and thickness and colour of the spore wall, are frequently used for identification and classification.

Every urediniospore of the Pucciniaceae has germ pores (Fig. 1D). The germ pores are (often) obscure, unless (i) the wall is appreciably pigmented, (ii) there is a visible annular wall thickening around the inner side of the pore (internal ring), or (iii) a pale blister-like cap covers the pore (papilla) (Savile, 1984). Staining the spores with congo red in caustic potash (Urban, 1963), with aniline blue in lactic acid (Jennings *et al.*, 1989) or with trypan blue in lactophenol/ethanol (method of Chapter 4) may improve the visibility of the pores. Spores also become visible after swelling in chloral hydrate. The urediniospores of *P. coronata* are recognized by their obscure germ pores, since they have no internal ring or papilla, whereas the other rust fungi studied have rather clearly visible pores due to the presence of a distinct internal ring or papilla.

Arrangement of the germ pores may be equatorial, scattered or bizonate (Cummins, 1936). In many cases the distinction between bizonate and scattered arrangement is rather vague (Ullrich, 1977), and it would be more convenient to make only a distinction between scattered (including bizonate) and equatorial arrangements. The equatorial arrangement of the germ pores readily delimits the stem rusts, *P. graminis*, from all other species studied in the present study, which have germ pores with a scattered arrangement.

The number of pores can be a valuable diagnostic character as was shown by Urban (1967), who recognized two varieties of the brown rust on *Bromus* spp. which

Table 3. Distinction of three grass rust taxa (*Puccinia* spp.) according to four authors (ref) and four frequently used characters of urediniospores. The typical size is given, occasional extremes are in parentheses. nd = values not determined.

character	ref[a]	rust taxon		
		P. hordei	*P. triticina*[b]	*P. recondita*[b]
number of germ pores	Ur	8–10(–11)	(7–)8–9(–11)	(6–)7–9(–10)
	WH	8–10	6–8	6–8
	Cu	7–9	6–10	6–10
	Sa	(6–)7–11(–13)	(6–)7–9(–10)	(6–)7–9(–10)
length × width (μm)	Ur	25–30(–37) × (17–)21–25	(20–)25–30(–32.5) × (17–)20–25	22–31 × 22–27
	WH	22–27 × 15–20	13–24 × 16–34	13–24 × 16–34
	Cu	(18–)21–30(–32) × (15–)18–25(–28)	(20–)24–32(–36) × (17–)20–25(–28)	(20–)24–32(–36) × (17–)20–25(–28)
	Sa	23–30(–33) × 18–25	(20–)22–30(–33) × (18–)19–24.5(–26)	(22–)25–29(–35) × (19–)21–27(–29)
spine spacing (μm)	Ur	1.7–2.5(–3)	(1.7–)2–2.5(–3)	2.5–3
	WH	nd	nd	nd
	Cu	nd	nd	nd
	Sa	1–1.7(–2)	(1–)1.2–2.2(–2.5)	1.2–3
wall thickness (μm)	Ur	2(–2.5)	2	2
	WH	nd	1–2	1–2
	Cu	(1–)1.5–2(–2.5)	1–2	1–2
	Sa	1–1.7(–2)	1–1.5	(0.7–)1–1.7

[a] References Ur — Urban, 1969; WH — Wilson & Henderson, 1966; Cu — Cummins, 1971; Sa — Savile, 1984.

[b] Considered as the same species, *Puccinia recondita* s.l., by Wilson & Henderson (1966) and Cummins (1971).

differed in germ pore numbers. Since a considerable variation and overlap in pore numbers may exist between taxa and between observations of different authors (Table 3) some caution is needed. Accurate counting of the pores is usually difficult, especially when the germ pores are obscure.

For a given taxon spore size measurements vary considerably between authors (Table 3). Besides, a wide overlap in spore size measurements exists between taxa. This makes spore size a poor feature in the identification of (morphologically similar) taxa. The size of the urediniospores, like the size of the

uredinia, is negatively influenced by uncongenial or resistant hosts and adverse environmental conditions (Stakman & Levine, 1919; Levine, 1923). In Table 2 average sizes of urediniospores produced in uredinia on barley accessions with different levels of susceptibility to Barley leaf rust are presented. The table shows that in the seedling stage the spore size was larger on the highly susceptible line L94 than on the other accessions. In the adult plant stage the differences between accessions were less pronounced.

Spine spacing (echinulation, Fig. 1C) may provide useful additional information for the identification of species. Spine spacing of spores of *P. recondita* from rye, 2.5–3 μm (measurements from Urban, 1969; Table 3), is somewhat larger than that of *P. hordei*, 1.7–2.5(–3) μm, and *P. recondita* from wheat, (1.7–)2–2.5(–3) μm, and clearly larger than that of *P. recondita* from *Holcus* spp., 1.5–2(–2.5) μm. Unexperienced workers, however, may find it difficult to measure the distances between spines accurately.

Thickness and colour of the spore wall are commonly used for identification. Wall thickness can vary from 1–2(–2.5) μm. Differences between taxa in thickness of the spore wall are too small to be of value for identification (Table 3). The colour of the spore wall is described in several shades of brown (pale brown, yellowish brown, cinnamon-brown, dark brown). Defining the colour of the thin spore wall is subjective, and intergrading colours are not easy to name (Chesters, 1968). Therefore, the colour of the spore wall has limited value for identification of rust taxa.

Urediniospores have several characters that might be useful for the identification and classification of rust taxa. Of these the visibility, the arrangement and the number of germ pores appear useful. The other characters are less suitable because they either show a large overlap between taxa, they vary considerably within taxa or are difficult to assess accurately. Still, it is recommended to consider the whole complex of uredinial features in identification.

Telia

Observations of characters of telia and teliospores are important for the identification and classification of rust specimens. Telia, however, are not always present on the specimens studied. Most rusts form telia only at the end of the growing season. Specimens collected in spring or early summer often do not contain telia. *P. poarum* Nielsen is one of the few grass rust species that forms telia shortly after the uredinia (Urban & Marková, 1987). Observations of the uredinia of this rust can even be difficult as telia mostly prevail. Other rusts rarely form telia, e.g. *P. brachypodii* on *Poa annua* and *P. striiformis* on *Poa* spp., which can greatly hamper the identification of rust specimens.

Frequently used telial characters for identification are the position, size, covering by the epidermis, and the presence of paraphyses in the telium.

The position of the telia, like the position of the uredinia, may be amphigenous, abaxial, adaxial or on the leaf sheaths. Telia, too, are not restricted to one particular part of the plant and therefore their position has limited value.

Telia differ considerably in size between taxa. On most wild grasses telia tend to be very small, while on the cereals telia may be larger. Also telia may unite and form large coalescent groups (e.g. *P. recondita* s.l. on *Bromus* spp.). Telia of *P. graminis*, on cereals and wild grasses, are extremely large (up to a few centimetres).

Another character is the covering of the telium by the epidermis. Telia can be long-covered by the epidermis, not showing the teliospores, or they can be erumpent, early naked and showing the individual spores under a stereomicroscope. In the field this feature distinguishes for instance the stem rusts with long, blackish, erumpent telia clearly from the other rusts with shorter, brownish, long-covered telia. However, in some rusts with long-covered telia (*P. coronata*, *P. recondita* s.l.) the epidermis may rupture eventually.

Finally, many grass rusts have paraphyses in the telia which divide them into locules. Stem rusts do not have such paraphyses. The presence of paraphyses in the telia is valuable for identification and classification, though this character is not easily observed due to difficulties in the sectioning of firm telia and in the recognition of locules.

The most reliable characters for identification and classification are the size and the telial covering by the epidermis. The position of the telia may be suitable but can vary on a plant. Paraphyses and locules often are not easily observable.

Teliospores

The teliospores provide several characters useful as diagnostic taxonomic characters. Of these the number of cells in the teliospores is considered a major taxonomic character (Hiratsuka & Sato, 1982), as it serves to discriminate between the genera *Puccinia* with two-celled teliospores and *Uromyces* with one-celled teliospores. However, it is questionable whether the number of cells deserves this taxonomic value, as in some species, e.g. *P. hordei* (Fig. 1E), *P. recondita* and *P. allii* high proportions (up to 95%) of one-celled teliospores may be observed (Wilson & Henderson, 1966; Savile, 1984). During this study at least a few one-celled teliospores were found in most of the telia of the species studied. In addition, occasionally some three- or four-celled teliospores were present. Until now, the exclusively one-celled genus *Uromyces* is kept as a separate genus for convenience (Hawksworth *et al.*, 1983; Savile, 1990).

A second readily observable character is the shape of the apical part of the upper cell. In Crown rust, the teliospores bear conspicuous digitate appendages (Fig. 1F), whilst in the other taxa these appendages are reduced to shallow bumps or even absent (Savile, 1984). Occasionally, in Crown rust isolates collected from *Calamagrostis epigeios*, *Agrostis tenuis* and *Phalaris arundinacea* the appendages were

very short or lacking (Urban, 1967), whereas in collections from *Hordeum*, *Agropyron repens* and *Secale cereale* they could be rather long (Peturson, 1954; Schwinghamer, 1955; Jin & Steffenson, 1992). Length of the appendages could vary within a telium (own observations), so the taxonomic value of the shape of the apex of the teliospores is limited within the crown rusts. It is only valuable for the delimitation of crown rusts from the other rusts studied.

Small differences in the size of the teliospores and in thickness of the teliospore wall were reported (Urban, 1969; Savile, 1984). Teliospores of *P. coronata* for example are distinguished from *P. gibberosa* by their longer length (Urban & Marková, 1994). As with urediniospores, size measurements of teliospores show a considerable overlap between taxa and differ between authors. Therefore, the size of the teliospores and the thickness of the teliospore wall are considered not very valuable for the identification of taxa.

Teliospores may vary in shape. By calculating the L/W ratio (the arithmetical mean of all length : width ratios of individual spores), two types of teliospores were recognized: brachysporous types have a L/W ratio of 2–3 and dolichosporous types a L/W ratio of 3–4 (Guyot, 1937 and Urban *et al.*, 1989, who used a slightly different calculation). A difficulty using this character may be that some collections are difficult to place as their L/W ratio can be intermediate between the brachysporous and dolichosporous type.

A sixth character was introduced by Urban (1966). He differentiated three groups of grass rust taxa on the basis of pedicel morphology. The value of this character is doubtful as it seems to vary within collections (Urban, pers. comm.) and it only serves to separate three pedicel groups.

In addition, the length and persistence of the pedicel may be helpful for identification. *P. graminis* mostly has long persistent pedicels (Fig. 1G), whereas the other rusts studied have short deciduous pedicels.

Finally, the numbers of basidiospores formed after germination of the teliospores may be useful for identification of some *Uromyces* rusts on barleys (Anikster & Wahl, 1979). Knowledge of the identity of the alternate host is important for identification of *Uromyces* rusts as well. Both characters require the presence of viable teliospores and a living plant stock of alternate hosts.

Useful and easily applicable characters for rust identification and classification are the shape of the apical part of the upper cell and the persistence of the teliospore pedicel. The numbers of teliospore cells and the shape of the teliospores should be used with caution.

Conclusions

In most identification keys characters of the telia are required for the differentiation of genera, in particular to distinguish the genera *Puccinia* and *Uromyces*; the distinction of these genera is, however, debatable since it is only based on teliospore

septation. Furthermore, characters of telia and teliospores are useful to recognize the crown and stem rusts. Unfortunately, telia are often lacking, which implies that identification has to rely mainly on uredinial features (and host range).

Of all reliable uredinial features only the arrangement of the sori and the presence of thick-walled paraphyses in the sori identifies the stripe rusts and *P. brachypodii* rusts, respectively. The crown rusts are recognized by obscure germ pores and the stem rusts by the equatorial arrangement of the germ pores. Identification of all other uredinial collections of grass rusts with scattered uredinia without thick-walled paraphyses, and urediniospores with numerous scattered germ pores poses severe problems due to the overlap in size measurements of the available morphological characters. New characters may be very valuable for the identification of these rust fungi and for the infraspecific delimitation of large species complexes, like *P. brachypodii* and *P. recondita*.

3 THE DEVELOPMENT OF INFECTION STRUCTURES IN HOST AND NON-HOST PLANTS

To initiate infection, urediniospores must germinate on a plant cuticle. Upon germination, urediniospore germlings develop a series of highly specialized structures in order to establish a parasitic relationship with the plant tissue. A prepenetration and a postpenetration stage can be recognized. This chapter reviews the factors affecting germination and development of early infection structures and describes the morphological differences between infection structures formed in a host plant (a plant in which an invading rust can successfully reproduce) and a non-host plant (a plant in which reproduction cannot be completed) and the effect of time on the development.

The prepenetration stage

Germination

To germinate, urediniospores usually need a dark period of several hours at an optimal temperature range and high humidity (Knights & Lucas, 1981; Staples & Macko, 1984). Optimal conditions may differ considerably between rust taxa (cp. Rowell *et al.*, 1958; Sharp *et al.*, 1958; Pavgi & Dickson, 1961; Sharp, 1965). Quality of the air may also influence germination as has been shown for urediniospores of *Puccinia striiformis*; air pollution with large ions decreased the germination rate of the urediniospores (Sharp, 1967).

Under favourable environmental conditions germination occurs equally well on host and non-host plants, but certain characteristics of a non-host plant may create unfavourable conditions (Heath, 1977). On plants with waxy surfaces (e.g. corn, cabbage or pea), the uneven distribution of surface moisture during the germination period may result in the occurrence of patches of non-germinated or poorly germinated spores, often adjacent to patches of high germination (Heath, 1974, 1977). Secondly, on pubescent leaves, urediniospores cannot make contact with the leaf surface and, because of insufficient moisture, show low germination (Heath, 1974; Zaiter *et al.*, 1993). Close adherence (Wynn, 1981) of the spores to the leaf surface appears to be essential for successful germination of urediniospores (Zaiter *et al.*, 1993).

Formation of the germ tube and the appressorium

After germination on a host plant, the protruded germ tube elongates closely appressed to the leaf cuticular surface (Wynn, 1981) and grows towards the stomata (directional growth, Johnson, 1934; Dickinson, 1969; Wynn, 1976). Several physical or chemical features of the leaf surface may influence directional growth. These features may include the orientation of epidermal cells (Staples & Macko,

1980), cuticular ridges (Wynn, 1976) and patterns of epicuticular wax crystals (Lewis & Day, 1972), or the pH gradients at the leaf surface (Edwards & Bowling, 1986).

When a stoma is sensed and recognized, an appressorium is formed. Stimuli that may induce appressorium formation are the shape of the stomatal lip (Wynn, 1976), the height of cuticular ridges (Dickinson, 1970; Allen *et al.*, 1991), various chemical factors at the stoma, like acrolein or mineral oil (Maheshwari *et al.*, 1967; Macko & Fuchs, 1970; Emmett & Parberry, 1975; Grambow & Riedel, 1977; Macko *et al.*, 1978), the CO_2 level in the substomatal cavity (Yirgou & Caldwell, 1968) or specific light and/or temperature regimes (Emge, 1958; Sharp *et al.*, 1958; Emmett & Parberry, 1975; Kochman & Brown, 1976; Hoch & Staples, 1987). However, even under favourable environmental conditions a low percentage of germ tubes fails to recognize stomata and to form appressoria on a host plant (Heath, 1974, 1977).

On non-host plants the germ tube frequently grows over the leaf surface without orientation (Heath, 1974, 1977; Elmhirst & Heath, 1989), does not locate stomata (Wynn, 1976) and is unable to form appressoria (Heath, 1974, 1977; Wynn, 1981); occasionally appressoria are formed on interstomatal areas (Heath, 1974, 1977; Wynn, 1976). Physical or chemical differences between the leaf surfaces of a host and a non-host plant may explain the poorly induced directional growth and subsequent appressorium formation. Elmhirst & Heath (1989) found that germ tubes of urediniospores of Cowpea rust fungus and Bean rust fungus did not locate stomata on leaves of the non-host *Vigna monophylla* which has an unusual venation pattern and a waxy leaf surface. The same phenomenon has been reported for Cowpea rust germ tubes on the waxy leaves of cabbage, pea and corn (Heath, 1974, 1977) and for Barley leaf rust germ tubes on lettuce leaves, which have an epidermis of the dicotyledoneous type (Niks, 1981). Germ tubes of Barley leaf rust urediniospores (Niks, 1981, 1983) and of many other grass rusts (Tani *et al.*, 1978; Niks, 1986; Luke *et al.*, 1987; Jacobs, 1989), however, were able to find and recognize stomata and form appressoria (and infection structures) on leaves of virtually any host or non-host plant species with an epidermis of the gramineous type.

Experiments conducted during this study reconfirm that most grass rust fungi germinate, find stomata and form appressoria on seedling leaves of the gramineous non-host barley (*Hordeum*) line L94. However, germ tubes of urediniospores of *Uromyces graminis* (Niessl) Dietel from *Melica ciliata* and *Puccinia phragmitis* (Schum.) Körnicke from *Phragmites australis* failed to find the stomata of 'L94'; germ tubes occasionally formed an appressorium on the leaf. Neither did most germ tubes of *P. magnusiana* Körnicke, also collected from *Phragmites australis*, find the stomata of 'L94', and if they did they failed to enter successfully the substomatal cavity. Differences in stomatal features between *Melica* and *Phragmites* on one hand,

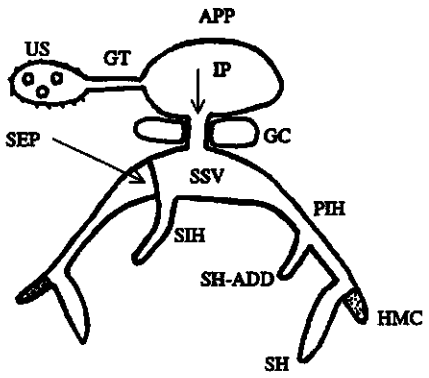


Figure 2. Schematical representation of the urediniospore germling morphology of grass rust fungi in vertical section. US = urediniospore, GT = germ tube, APP = appressorium, IP = infection peg, SSV = substomatal vesicle, PIH = primary infection hypha, HMC = haustorial mother cell, SH = secondary hypha, SH-ADD = additional secondary hypha, SIH = secondary infection hypha, SEP = septum, GC = guard cell.

and *Hordeum* on the other hand, may account for the non-recognition of stomata and the subsequent poor stomatal penetration (Brown & Johnson, 1962).

The postpenetration stage

When an appressorium is successfully formed on a stoma, an infection peg develops and penetrates the stomatal aperture. Within the substomatal cavity the infection peg swells to form a substomatal vesicle (SSV) into which the contents of the appressorium are transferred. From the SSV one or more primary infection hyphae (PIH) arise, depending on the taxon. Upon contact with a mesophyll cell, a septum is formed between the tip of the PIH and the SSV, which delimits a haustorial mother cell (HMC). Close contact between the tip of the PIH and the host cell wall stimulates the formation of the haustorial mother cell in a host (Staples & Macko, 1980). From the HMC a penetration peg develops, which penetrates the mesophyll cell wall and forms a haustorium. At the SSV side of the HMC septum secondary hyphae (SH) may emerge, which in turn may delimit HMC, form haustoria and give rise to a highly branched septate intercellular mycelium (Heath, 1974, 1977; Staples & Macko, 1984; Hughes & Rijkenberg, 1985; Mims *et al.*, 1989). In some rusts secondary infection hyphae may arise from the SSV (SIH; e.g. *Puccinia hordei*) or along the PIH (additional secondary hyphae — SH-ADD; e.g. *P. graminis*) as well (Fig. 2).

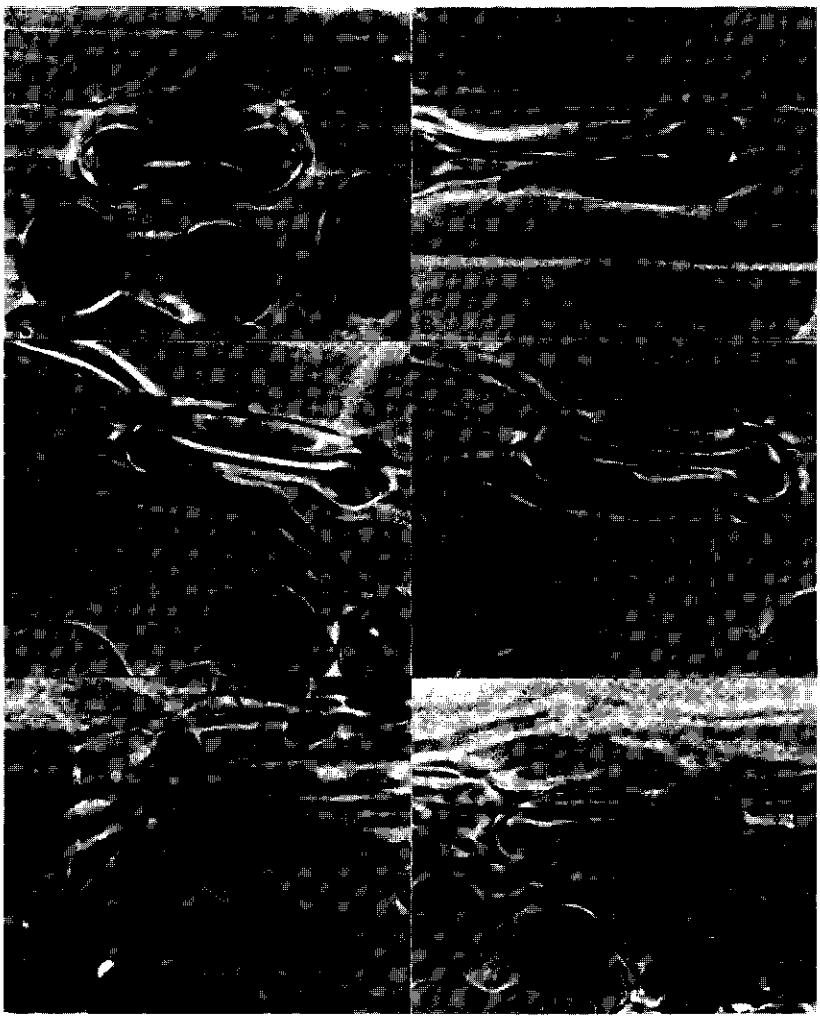


Figure 3. Development of *Puccinia hordei* urediniospore germlings. Phase contrast micrographs, scale bar = 20 μm.
A. Ellipsoid substomatal vesicle, 12 h after inoculation on wheat cv. Morocco. **B.** Fusiform substomatal vesicle, 5 h after inoculation on wheat cv. Morocco. **C.** Fusiform substomatal vesicle with two primary infection hyphae, 12 h after inoculation on barley cv. Akka. **D.** Central septum present in the substomatal vesicle (arrow), 12 h after inoculation on barley cv. Akka. **E.** Haustorial mother cell, 18 h after inoculation on wheat cv. Morocco. **F.** Additional septa (arrowheads) and secondary infection hyphae (arrows), 40 h after inoculation on barley cv. Akka.

In non-hosts infection structures develop in a similar sequence to those in host species, but fungal growth frequently stops at or before the formation of the first haustorium (Heath, 1974, 1977; Niks, 1983; Luke *et al.*, 1987; Elmhirst & Heath, 1989; Lennox & Rijkenberg, 1989; Moerschbacher *et al.*, 1990). The inhibition of functional haustorium formation results in the death of the invading parasite.

However, there is a considerable variation in the stage at which development stops on a leaf of a resistant host genotype or a non-host plant. Germling development may stop anywhere between the onset of penetration of the stoma up to the formation of several haustorial mother cells and secondary (infection) hyphae (Gibson, 1904; Heath, 1974, 1977; Niks & Kuiper, 1983; Lennox & Rijkenberg, 1989; Mims *et al.*, 1989; this study).

To use the morphology of urediniospore germlings for identification and classification, it is important to understand the morphological differences in the development of the infection structures in a (susceptible) host plant and a (resistant) non-host plant and the effect of time on the development. For these purposes and to assess the period after inoculation properly fit to observe urediniospore germlings an experiment was carried out with urediniospores of Barley leaf rust fungus (*P. hordei*).

Urediniospores were inoculated on seedling leaves of barley cv. Akka (a cultivar highly susceptible to Barley leaf rust fungus; referred to as host) and of wheat cv. Morocco (a cultivar highly resistant to Barley leaf rust fungus; referred to as non-host). The seedlings were incubated in a dark moist greenhouse compartment for 8 h and subsequently transferred to another greenhouse compartment at an 8-h night/16-h day regime. After start of the incubation (referred to as inoculation) two leaves of the host and the non-host, respectively, were harvested at intervals, fixed, stained with 0.03% trypan blue in lactophenol/ethanol, destained in chloral hydrate, and embedded in glycerol (after Niks, 1986; Chapter 4). The morphological characters of the infection structures were described for 30 appressoria of each leaf. At each period of incubation the percentage of germlings at a specific developmental stage was recorded.

Results confirm the experiments of Heath (1974, 1977) that the sequential development of infection structures was similar in host and non-host plants. Moreover, the overall morphology of the germlings of Barley leaf rust was similar in host and non-host: upon entering the stomatal slit, the infection peg swelled to form an ellipsoid SSV (Fig. 3A), which gradually elongated to become fusiform (Fig. 3B) and gave rise to two PIH, one at each end of the SSV (Fig. 3C). Upon formation of a central septum in the SSV (arrow in Fig. 3D), each PIH differentiated an HMC (Fig. 3E) and SH could arise at the SSV side of the HMC. Subsequently, haustoria, additional septa and SIH were formed (Fig. 3F). Quantitative differences were visible between infection structure formation in host and non-host plants: in the host a higher percentage of germlings had formed haustoria, SIH and septa at both

T = 21 h and T = 40 h (Table 4). Similar observations were made for Wheat leaf rust and Couch grass leaf rust (*P. agropyrina* Erikss.) (Swertz, unpublished data).

The rate of development of Barley leaf rust fungus infection structures was similar in host and non-host until formation of the first haustorium (Fig. 4). During formation of the first haustorium the development of the germling slowed down in the non-host: from 20 h after inoculation fewer haustoria, septa and SIH (Fig. 4) were formed. On the other hand, the presence of SH was similar in host and non-host, as was the increase over time (Fig. 4). This supports the suggestions of Heath (1974, 1977) that SH could arise with and without formation of a haustorium.

For observations of the germling morphology for identification and classification the developmental stage at about 20 h after start of the incubation is suitable in a susceptible (host) plant. At later moments, the observation of germling

Table 4. Percentage[a] of germlings at two developmental stages of Barley leaf rust fungus (*Puccinia hordei*) after inoculation of urediniospores on the host barley cv. Akka and the non-host wheat cv. Morocco.

developmental stage	T = 21 h		T = 40 h	
	Akka	Morocco	Akka	Morocco
substomatal vesicle	100	100	100	100
primary infection hypha	94	100	100	100
central septum	94	97	98	100
one haustorial mother cell	91	90	97	100
two haustorial mother cells	83	83	90	98
secondary hyphae	69	71	97	100
at least one haustorium[b]	67	36	90	75
secondary infection hyphae	41	21	85	38
two septa	39	23	79	51
three septa	36	12	74	22

[a] Data are means of two leaves and 30 germlings per leaf.

[b] In cv. Morocco haustoria were young and often associated with necrosis of the invaded mesophyll cell.

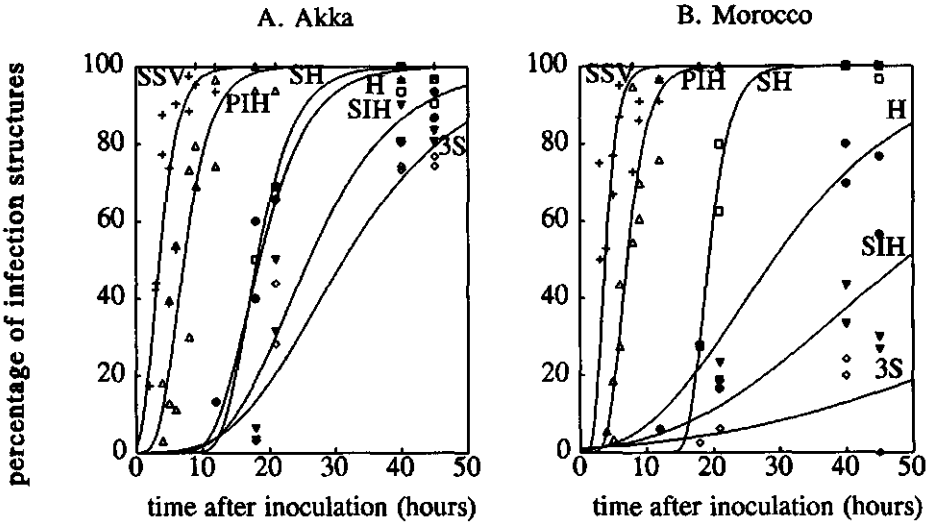


Figure 4. Percentages of infection structures at a specific developmental stage after inoculation with *Puccinia hordei* urediniospores on seedling leaves of **A.** barley cv. **Akka** and **B.** wheat cv. Morocco. Each point in the graph represents the percentage over 30 germlings in one leaf. SSV = substomatal vesicle, PIH = primary infection hypha, SH = secondary hyphae, H = at least one haustorium, SIH = secondary infection hyphae, 3S = three septa in the SSV.

morphology is difficult, since many SIH have been formed. Comparing germling morphology of Barley leaf rust in a host and a non-host plant suggest that observations made in the host at about 20 h after inoculation are similar to those obtained in the non-host at about 40 h after inoculation (Fig. 4).

The results of the experiment suggest that the rate of development and the morphology of the infection structures of grass rust fungi is similar in a host and a non-host plant until the formation of the first haustorium. In any case, the non-host plant should have an epidermis of the gramineous type and the environmental conditions should favour germination and infection.

Results also indicate that when fixed at about 20 h after inoculation on a host plant, the morphology of the urediniospore germlings is similar to those fixed at about 40 hours after inoculation on a non-host plant.

Thus when using the barley line L94 (in Chapter 4) as a standard in which to observe the germling morphology of various grass rust fungi, it is suggested to fix rust fungi collected from barleys at about 20 h after inoculation and rust fungi collected from other grasses at about 40 h after inoculation.

4 GERMLING MORPHOLOGY OF SELECTED RUST FUNGI

Five rust complexes were selected to study the value of the germling morphology of the urediniospores for identification and classification. Each complex comprised at least one economically important rust pathogen.

Puccinia coronata (crown rust, 4.1) and *P. graminis* (stem rust, 4.2) were selected, because between included infraspecific taxa only minor morphological differences in characters of uredinia, telia, and spores exist. Besides, the host range of the infraspecific taxa may show a considerable overlap.

Puccinia brachypodii (4.3) and *P. recondita* (leaf rust, 4.4) were chosen, because taxonomic concepts within each complex differ largely between authors. Besides, included taxa were reported to differ in morphology of sori and spores and in host range.

Finally, *P. hordei* (Barley leaf rust, 4.5) was studied, because the morphology of the sori and spores is very similar to several *Uromyces* species which are also pathogenic on species of barley.

For each rust complex characteristics of uredinia and telia, economic importance, and taxonomic concepts are briefly discussed. Subsequently, the germling morphology is described and its value for identification discussed.

MATERIAL AND METHODS

Rust collections

Rust-infected leaves were collected in the Netherlands, Belgium, Canada, the Czech Republic, France, Germany, Great Britain and Switzerland, or obtained from other researchers from various parts of the world (see Appendix).

Inoculation and incubation

Freshly collected urediniospores were applied onto seedling leaves of barley line L94 with a needle. To facilitate the search for infections, a segment (appr. 2 cm long) of the leaf was marked with a non-toxic paint. One to three leaf segments were inoculated per collection. The inoculated plants were incubated overnight in a nearly water-saturated chamber.

The leaf segments were harvested about 40 h after start of the incubation. When urediniospores collected from barley were used, the leaf segments were harvested about 20 h after start of the incubation (Chapter 3).

Staining

The segments were fixed in acetic acid/ethanol (1:3 v/v) for 30 min. This step could be omitted, or the segments could be kept in the fixative for several days.

For staining, the segments were boiled in 0.03% trypan blue in lactophenol/ethanol (1:2 v/v), for 10 min, and destained in nearly-saturated chloral

hydrate (5:2 w/v), for at least 24 h (after Niks, 1986). Segments were embedded in 100% glycerol. Slides could be kept for 1-2 years.

Observations

Leaf segments were observed with phase contrast microscopy, at 100 ×, (400 ×) and 1000 × magnifications. At least 15 germlings on each leaf were observed and described (Section 5.2). Obviously malformed germlings and germlings under doubly penetrated stomata were ignored.

For each germling characteristics of the appressorium, infection peg, substomatal vesicle (SSV), primary infection hypha (PIH), and haustorial mother cell (HMC) were described and drawn. Characteristics studied include: shape (including the shape of the ends of the SSV), size, presence of septa, number and orientation of PIH, and orientation of the SSV. The orientation of SSV and PIH was recorded as longitudinal or transverse (i.e., parallel or perpendicular to the long axis of the leaf, respectively) and as horizontal (i.e., in the same plane as the epidermis) or vertical (i.e., growing more deeply into the mesophyll). Besides, the presence of secondary (infection) hyphae was recorded. Secondary hyphae are hyphae arising from the SSV side of the HMC septum (SH) or along the PIH (SH-ADD); secondary infection hyphae (SIH) are extra hyphae arising from the SSV which are formed after the PIH (Fig. 2).

4.1 PUCCINIA CORONATA Corda

Introduction

Crown rust fungus (*Puccinia coronata*) is a common heteroecious rust with pycnia and aecia on species of *Rhamnus* and *Frangula* and uredinia and telia on oats (*Avena*) and several wild grasses (i.a. *Agrostis*, *Arrhenatherum*, *Holcus* and *Lolium*). In the telial stage, the crown rust fungus is easily recognized by the predominantly crowned (coronate) apex of the teliospores. In the uredinial stage, crown rust is recognized by bright orange uredinia, mainly formed on the leaves. The urediniospores have 8–11 obscure germ pores with a scattered arrangement. After staining leaf segments to visualize the germling morphology, the pores of the urediniospores were rather clearly visible (Fig. 5A).

In Europe, crown rust presumably overwinters in the uredinial stage on perennials (Straib, 1941; Urban, 1969; Ullrich, 1977). When an aecial host is present, host alternation may be important. Abundant sporulation of uredinia and telia occurs mainly in late summer and autumn; crown rust infection seems to be promoted by high temperatures (Ullrich, 1977).

Crown rust can cause severe crop losses in oats (Simons, 1985) and in ryegrasses (*Lolium* spp., Potter *et al.*, 1990) and, hence, many investigations have dealt with pathogenity (i.a. Michel & Simons, 1977; Wilkins, 1978 a,b; Potter *et al.*, 1990) and host specialization in both the aecial and the uredinial stages (i.a.

Table 5. Characters of urediniospores of four infraspecific taxa of *Puccinia coronata*.

taxon and main host plants	length (µm)	width (µm)	pores (number)
f.sp. *secalis*[c] *Secale cereale*	15–32	13–29	not mentioned
var. *coronata*[a] *Agrostis*, *Holcus*, *Lolium*	(17.5–)20–25(–30)	(14–)18–21(–25)	8–10
var. *avenae* f.sp. *avenae*[a] *Avena* spp.	(22–)25–30(–32)	(20–)22–24(–27)	(8–)9–11(–14)
var. *avenae* f.sp. *graminicola*[b] *Arrhenatherum elatius*	20–27.5(–32)	(15–)19–24(–27)	(7–)8–11(–14)

Data are taken from [a] Urban (1967, 1969), [b] Formanová *et al.* (1989), and [c] Peturson (1954).

Brown, 1937, 1938; Straib, 1941, 1952; Gäumann, 1959; Eshed & Dinoor, 1980, 1981). These investigations have shown that the former subdivision of crown rusts into two species, depending on the ability to produce aecia on either *Rhamnus* or *Frangula* (see i.a. Eriksson, 1894; Klebahn, 1904), is not justified, because no clear morphological and pathogenic differences were found between aecia produced on these aecial host genera (Brown, 1938; Peturson, 1954).

Host range studies conducted in Europe and North America have shown that crown rusts (the uredinial stage) collected from *Agrostis*, *Alopecurus*, *Arrhenatherum*, *Holcus* and *Phalaris* have a narrow host range, while those from *Agropyron repens*, *Avena*, *Festuca*, *Hordeum*, *Lolium* and *Secale* have a considerably wider host range (Brown, 1937; Peturson, 1954; Schwinghamer, 1955; Gäumann, 1959; Cagaš, 1978; Sampson & Watson, 1985; Jin & Steffenson, 1992). In contrast, Eshed & Dinoor (1981) reported that in Israel, the centre of diversity of many grasses and their rusts, all crown rust forms have a wide host range.

Most authors (i.a. Urban, 1967; Cummins, 1971; Boerema & Verhoeven, 1977; Savile, 1984) recognized two varieties within *P. coronata*, viz. *P. coronata* Corda var. *coronata* and *P. coronata* var. *avenae* Fraser & Led. Variety *coronata* is found on wild grasses and has smaller urediniospores and fewer germ pores than var. *avenae* (Table 5), which is mainly found on wild and cultivated oats, but can infect some wild grasses too. Within var. *avenae*, Urban (1967) recognized two *formae speciales*: f.sp. *avenae* Urban which occurs on *Avena* spp. and *Lamarckia aurea* (also host for *P. coronata* var. *coronata*), and f.sp. *graminicola* Urban, being specific for *Arrhenatherum elatius*, but also reported from *Holcus lanatus* and *H. mollis* (also hosts for *P. coronata* var. *coronata*). Both Urban (1967) and Formanová *et al.* (1989) considered the crown rust fungus collected from *A. elatius* as *P. coronata* var. *avenae* f.sp. *graminicola*, because the size of the urediniospores approached the size of *P. coronata* var. *avenae* f.sp. *avenae*. This view is ques-

tionable since the length of the spores from *A. elatius* tends somewhat more to the range of *P. coronata* var. *coronata* (Table 5). Cummins (1971) included the crown rust from *A. elatius* within *P. coronata* var. *coronata*, and reported a range in size of urediniospores similar to that described by Urban (1967) for *P. coronata* var. *coronata*.

Wilson & Henderson (1966) mentioned several varieties of *P. coronata* which were based on host specialization. But Eshed & Dinoor (1981) concluded that a taxonomic subdivision in *P. coronata* can hardly be based on host specialization because of the wide host range of the individual collections which may cover several grass genera.

Crown rust fungi occurring on *Agropyron repens*, *Calamagrostis* spp., *Hordeum vulgare* and *Secale cereale* have been described as separate taxa, viz. f.sp. *agropyri* Eriksson. (Eriksson, 1908), var. *rangiferina* S. Ito (Cummins, 1971), var. *hordei* Jin & Steff. (Jin & Steffenson, 1992) and f.sp. *secalis* Peturson (Peturson, 1954; Schwinghamer, 1955), respectively. These taxa have up to three times longer teliospore appendages than the other *P. coronata* rusts, but the length of the teliospore appendages can vary within a telium and was considered to be of little use for the delimitation of infraspecific taxa within *P. coronata* (Chapter 2). Infection experiments with the rusts from *A. repens*, *S. cereale* and *H. vulgare* showed that urediniospores of one host can also infect the other two host species (Peturson, 1954; Schwinghamer, 1955; Jin & Steffenson, 1992). This suggests that these three rust forms probably are identical, and possibly also with *P. coronata* var. *rangiferina* S. Ito. The host range of the latter rust is, however, uncertain (Savile, 1984).

In summary, the infraspecific delimitation of crown rust fungi is still debatable. Therefore, the germling morphology of urediniospores of *P. coronata* was studied to see whether this criterion can help to elucidate the taxonomic problems within *P. coronata*.

Germling morphology

Description

Puccinia coronata germlings have a square, 15–23 × 15–23 μm, appressorium with short, 1–5 μm long, digitate protuberances at the corners (Fig. 5B). Only occasionally these protuberances are absent. The penetration peg is short. The substomatal vesicle (SSV) is narrowly oblong to narrowly ellipsoid, rarely nodiform, (16–)30–53(–60) × (5–)6–9(–10) μm, with a central or eccentric septum. It is longitudinally orientated (Figs 5 B, D, E). At both ends of the SSV a narrow, (1.5–)2–3(–4) μm wide, often hooked, primary infection hypha (PIH) arises. Most PIH are longitudinally and horizontally orientated, but occasionally the PIH grow towards the adjacent vein and more deeply into the mesophyll. Sometimes a secondary infection hypha arises from the SSV (Fig. 5E). The haustorial mother cells

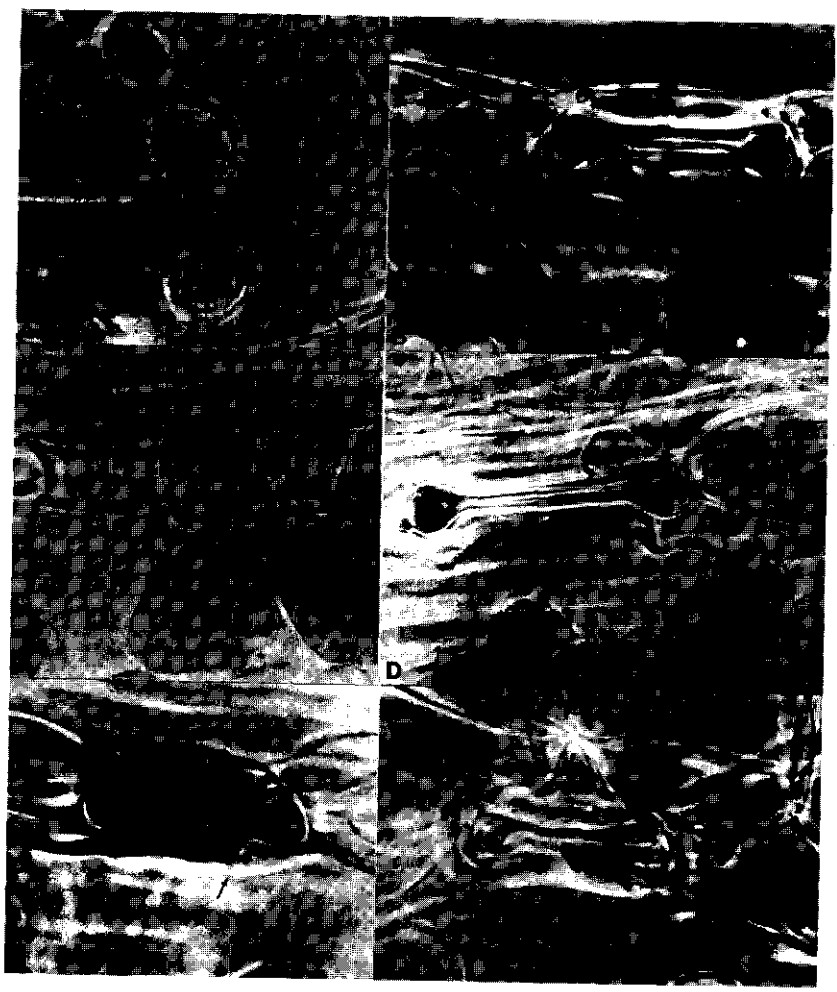


Figure 5. *Puccinia coronata*, phase contrast micrographs, scale bar = 20 μm.
A. Urediniospores collected from *Arrhenatherum elatius* (C.S. 90.091). **B–F.** Germlings of urediniospores collected from: **B.** *Avena sativa* (C.S. 90.122), appressorium and SSV. Note the digitate protuberances of the appressorium (arrows). **C.** *Avena sativa* (C.S. 92.Isr54), HMC. **D.** *Agrostis tenuis* (C.S. 90.093) SSV and two PIH. **E.** *Arrhenatherum elatius* (C.S. 93.050), SSV with a short secondary infection hypha (arrow). **F.** *Agropyron repens* (C.S. 91.048).

Table 6. Length and width (μm) of the substomatal vesicle of *Puccinia coronata* germlings. Collection (country) = the numbers of collections studied and the numbers of countries from which they have been obtained.

host	length	width	collection (country)
Avena	(30–)40–53(–60)	(5–)6–7(–9)	9 (6)
wild grasses[a]	(16–)30–38(–45)	(5–)7–9(–10)	82 (7)

[a] These include collections from *Agrostis* spp, *Alopecurus pratensis*, *Arrhenatherum elatius*, *Festuca* spp., *Glyceria aquatica*, *Holcus* spp., *Lolium* spp., and *Phalaris arundinacea*.

(HMC) are unlobed, long and slender, 10–15(–20) × 2–3(–4) μm. They are frequently hooked (Fig. 5C). Secondary hyphae, 2–3 μm wide, arising at the SSV side of the HMC septum are often formed.

Two morphological groups can be distinguished (Table 6), which differ in length and width of the SSV. The SSV of *P. coronata* collected from *Avena* were considerably larger and smaller than the SSV of *P. coronata* collected from wild grasses.

A total of 9 collections from *Avena*, including one aecial collection from *Rhamnus*, and 83 collections from several wild grasses, were studied from 6 and 7 countries, respectively (Appendix). No overlap in common range measurements were apparent between the two SSV groups (Table 6).

Note: One crown rust collection was observed with an aberrant germling morphology. This collection was found in August 1991 on *Agropyron repens* plants in Ottawa (Ontario, Canada) nearby *Rhamnus catharticus*, on which aecia of crown rust fungus had been observed in spring 1991 (observations of D.B.O. Savile and J. Parmelee; collection C.S. 91.048). As the collection had crowned teliospores it was identified as *P. coronata* s.l. The morphology of the spores and the appressorium was similar to those from the crown rusts observed in the present study, but the shape of the SSV and the number of PIH was clearly different. The SSV was ellipsoid to narrowly ellipsoid, (20–)24–30 × (7–)8–11 μm, with acute ends (Fig. 5F) and the single PIH, 3–4 μm wide, grew vertically. Occasionally a second vertical PIH was present. The HMC were unlobed, 10–14 × 3–4 μm.

Discussion

Crown rust urediniospore germlings are characterized by a square appressorium with digitate protuberances at the corners, a narrowly oblong to narrowly ellipsoid SSV from which two narrow, frequently hooked, PIH arise, and long and slender, also frequently hooked HMC. These features easily distinguish *P. coronata* from the

other grass rust fungi. Identification can be confirmed by observation of the urediniospores and teliospores.

Characters of the urediniospores, teliospores and the shape of the appressorium suggested that the collection from *Agropyron repens* belonged to *Puccinia coronata* s.l. Its germling morphology, however, clearly deviated from the crown rust germlings collected from the other host species. The aberrant morphology may be typical for crown rust from *A. repens*, and possibly also for crown rust fungi collected from *Secale cereale* and *Hordeum vulgare* in North America and Europe, as urediniospores from these three hosts can also infect the other host (Peturson, 1954; Schwinghamer, 1955; Jin & Steffenson, 1992). This type of germling morphology may also be found in crown rusts collected from *Calamagrostis*. Additional studies of the germling morphology of crown rust collections from *A. repens*, *S. cereale*, *H. vulgare* and *Calamagrostis* are needed to investigate whether rusts from these hosts are conspecific.

The similar germling morphology of the *P. coronata* collections studied, except the collection from *A. repens*, suggests that the various crown rust fungi are closely related. The observation of two quantitatively differing morphological groups within *P. coronata* among the taxa studied also indicates that the delimitation of varieties within *P. coronata* is justified.

Germling morphology also suggests that the recognition of *formae speciales* within var. *avenae* (Urban, 1967) can be doubted as the SSV of *P. coronata* collections from *A. elatius*, which was also observed in the Czech collections studied, were clearly smaller than those from *Avena* and similar to the SSV from the other *P. coronata* collections from wild grasses. Besides, in the present study the urediniospores of *A. elatius* were smaller than reported by Formanová *et al.* (1989) for *P. coronata* var. *avenae* f.sp. *graminicola* (Table 5), also in the Czech collections. Urediniospores measured 22–25(–27) × 18–24 μm, which was similar to the measurements of *P. coronata* urediniospores from i.a. *Lolium perenne* and *Agrostis* spp. (data not shown). The observed overlap in size of urediniospores from *P. coronata* var. *coronata*, *P. coronata* var. *avenae* f.sp. *avenae* and *P. coronata* var. *avenae* f.sp. *graminicola* (Table 5) confirms that the size of urediniospores is a rather variable feature and therefore of little use for identification (Chapter 2).

Since the typical range in size of the SSV did not overlap between collections from *Avena* and the wild grasses, this character seems to be a more reliable criterion for distinction between these host groups (see also Section 5.1). Based on the results reported here, it may be suggested to exclude the rust of *A. elatius* from *P. coronata* var. *avenae* and to follow the species concept of Cummins (1971) and subsume this rust under *P. coronata* var. *coronata*.

In summary, the quantitative differences in germling morphology and characters of urediniospores and the differences in host range suggest to recognize two varieties within *P. coronata*, viz. var. *avenae* and var. *coronata*.

4.2 PUCCINIA GRAMINIS Pers. : Pers.

Introduction

Stem rust fungus, *Puccinia graminis*, is a common heteroecious rust with pycnia and aecia on species of *Berberis*, rarely on *Mahonia*, and uredinia and telia on cereals and wild grasses (Wilson & Henderson, 1966; Urban, 1969; Savile, 1984).

Stem rust is easily recognized by the long brown uredinia formed mainly on the stems and sheaths and, to a lesser extent, on the upper side of the leaves of the infected host plant. Urediniospores are ellipsoid, with generally 3–5 distinct germ pores in an equatorial arrangement (Fig. 6A). In the telial stage the pustules are black and early-ruptured. Within a pustule the teliospores vary greatly in shape and in length of the persistent pedicels. The teliospores can be either clavate and long-pedicellate, or fusoid and short-pedicellate. In addition, one-celled teliospores are occasionally to be found, especially at the edges of young sori (Urban, 1967; Savile, 1984).

Urediniospores of *Puccinia graminis* require high temperatures (18–30 °C) and high light intensities (≥ 10 000 lux) for optimal infection (Sharp *et al.*, 1958; Martens, 1985; Roelfs, 1985). Therefore, in temperate Europe attack of stem rust is most severe in late summer and early autumn (Wilson & Henderson, 1966; own observations).

Especially in wheat, stem rust fungus has caused severe crop losses until the mid-1950s, when Wheat stem rust-resistant cultivars were grown widely and campaigns started to eradicate the aecial host (Urban, 1969; Roelfs, 1985). Nowadays crop losses due to Wheat stem rust are less severe. Stem rust of oats only periodically causes crop losses (Martens, 1985), whilst stem rusts of rye and barley are of minor importance (Roelfs, 1985). Recently, severe Barley stem rust epidemics have been reported in barley crops due to break-down of the T-gene resistance (Steffenson, 1992).

Most authors (i.a. Cummins, 1971; Boerema & Verhoeven, 1977; Ullrich, 1977; Savile, 1984) follow Guyot *et al.* (1946) and Urban (1967), who recognized two subspecies within *P. graminis*. *Puccinia graminis* subsp. *graminicola* Urban occurs on wild grasses (i.a. *Agropyron*, *Agrostis*, *Arrhenatherum*, *Dactylis glomerata*, *Festuca*, *Lolium*, *Phleum pratense*) and has smaller, rather ovoid urediniospores and fewer germ pores than *Puccinia graminis* subsp. *graminis*. The latter occurs on cereals and closely related genera (*Aegilops*, *Agropyron* and *Elymus*), but occasionally other wild grasses may be infected. This subspecies has longer, ellipsoid urediniospores and more germ pores (Table 7). In addition, Urban (1967) distinguished two varieties within subsp. *graminis*. Variety *graminis* is mainly found on *Triticum*, *Aegilops* and *Elymus*, but can also infect *Hordeum*, *Secale* and *Agropyron*, and has somewhat larger urediniospores (Table 7) than var. *stakmanii* Guyot, Massenot & Saccas ex Urban. The latter variety occurs mainly on *Avena*, *Secale* and *Hordeum*, but has also been reported from *Agropyron*, *Elymus* and *Dactylis glomerata* (Urban, 1967, 1969). Spore size, however, is very variable

among taxa, making diagnostic morphological differentiation often very difficult (Table 7).

Due to the variation in spore size, other scientists (mainly plant pathologists) have given more value to host specialization and recognized several *formae speciales* either within *P. graminis* (Wilson & Henderson, 1966; Martens, 1985; Roelfs, 1985) or within *P. graminis* subsp. *graminis* (Boerema & Verhoeven, 1977). On cereals these *formae speciales* include f.sp. *tritici* Erikss. & E. Henn. occurring mainly on *Triticum* and *Hordeum*; f.sp. *secalis* Erikss. & E. Henn. mainly on *Secale* and *Hordeum*, and f.sp. *avenae* Erikss. & E. Henn. on *Avena*.

Host specialization, however, is not always strict, as can be seen from the host ranges mentioned for *P. graminis* subsp. *graminis* (Table 7). In addition, Savile (1984) reported that subsp. *graminicola* could also infect cereals (e.g. *Hordeum*). As a consequence, several host genera (e.g. *Agropyron*, *Dactylis* and *Hordeum*) can be infected with both *P. graminis* subsp. *graminicola* and subsp. *graminis*, and also with both varieties of subsp. *graminis* (Savile & Urban, 1982; Urban & Marková, 1983, 1984a,b; Savile, 1984). This makes host identity unsuitable for rust identification, unless extensive host range experiments are carried out.

The overlap between the taxa included in *P. graminis* in host range, spore size and numbers of germ pores (Table 7) indicate that the taxonomic concept in *P. graminis* is not satisfactory. Therefore, the germling morphology of the urediniospores was studied to see whether this criterion is a useful additional tool for the identification and classification of the *P. graminis* complex.

Germling morphology

Description

Puccinia graminis germlings have a narrowly oblong appressorium, 30–45 × 7–12 μm (Fig. 6B). The penetration peg is short. The SSV is fusiform, 20–35(–40) × (7–)8–9(–12) μm, aseptate and longitudinally and horizontally orientated (Figs 6 C, D, E, F). At one of the ends of the SSV a longitudinally and horizontally orientated primary infection hypha, (2.5–)3–4(–6) μm wide, arises at the end of which a haustorial mother cell is formed (Fig. 6C). The PIH may be very short (Figs 6 D, E) and occasionally an HMC is formed without differentiation of a PIH (Fig. 6F, left side of the SSV). Total length of SSV + PIH ranges from 20–95(–140) μm. At the other end of the SSV a short appendix or SIH may arise which may also delimit an HMC (Fig. 6F). Occasionally additional septa are formed in the PIH and secondary hyphae may be formed along the PIH (SH-ADD, Fig. 6C). The HMC are unlobed with a rounded tip and long and slender, (10–)15–22 × 3–5(–6) μm (Figs 6 C, D). The HMC rarely has an elongated tip (Fig. 6E). Secondary hyphae arising at the SSV side of the HMC septum are frequently formed.

A total of 69 collections of *P. graminis* obtained from cereals, several wild grasses and *Berberis* were studied (Appendix). Only the stem rust fungi from *Avena*

Table 7. Hosts and morphological characters of urediniospores and teliospores of the infraspecific taxa of *Puccinia graminis*, after Urban (1967, 1969, hosts and size) and Savile (1984, numbers of germ pores).

taxon	host[a]	urediniospores		teliospores
		size (l × w, in μm)	number of pores[b]	size (l × w, in μm)
subsp. *graminis* var. *graminis*	*Triticum, Aegilops, Elymus* (*Hordeum, Secale, Agropyron*)	(20–)26–36(–45) × (13–)16–21(–22)	(3–)4–5(–6)((–7))	(33–)38–61(–72) × (12–)15–23(–26)
subsp. *graminis* var. *stakmanii*	*Avena, Secale, Hordeum* (*Agropyron, Elymus, Dactylis glomerata*)	(20–)23–36(–39) × (13–)14–21(–23)[c]	(3–)4–5((–6))	(25–)33–59(–74) × (13–)16–25(–28)
subsp. *graminicola*	wild grasses	(17–)19–28(–34) × (11–)13–20(–22)	(2–)3–4((–5))	(21–)26–60 × (12–)13–24(–30)

[a] Hosts within parentheses are occasional hosts.
[b] Values within double parentheses are rare extremes.
[c] Urban (1967, 1969) reported (20–)33–36(–39) × (13–)14–21(–23) μm, which did not fit his statement of var. *stakmanii* having smaller urediniospores. By comparing the original work of Guyot *et al.* (1946), which Urban (1967) had used for his study, it was found that Urban had exchanged some digits in his manuscript.

spp., *Triticum*, *Secale*, *Hordeum* spp. and some of the *Berberis* collections obtained from and studied at Agriculture Canada Research Station, Winnipeg (ACRS isolates in the Appendix), are included in the discussion, as for the other stem rust fungi too few germlings could be observed due to low germination and poor stomatal penetration. However, the germling morphology of these collections was similar to those observed in the ACRS isolates. Besides, aecial and uredinial collections had identical germling morphology.

Within the ACRS isolates studied, three groups of germling morphology were recognized based on quantitative differences (Table 8). These groups coincided with the *forma specialis* designation obtained from the ACRS (K. Dunsmore, pers. comm., 1991). For convenience, the groups are referred to here by their respective *forma specialis* name. Germlings from f.sp. *avenae* have the highest values for mean SSV + PIH length and mean percentages of germlings with SH-ADD and with an appendix or SIH. Germlings from f.sp. *secalis* have the lowest values for these parameters, and germlings from f.sp. *tritici* are intermediate. However, the range of SSV + PIH length and percentage of germlings with an appendix each showed a large overlap (Table 8).

Within a *forma specialis* the urediniospore collections from different host species showed considerable variation in quantitative features of germling morphology (Table 9). Mean length of SSV + PIH was larger in f.sp. *tritici* collections from *Hordeum vulgare* than in the other collections, whereas in f.sp. *secalis* the collections from *Secale* were larger than the collections from the other hosts. The percentage of germlings with SH-ADD was also larger in collections from *Secale*, which, however, was not observed in f.sp *tritici* from *Hordeum vulgare*. In all three *formae speciales* the percentage appendix or SIH formation was very variable (Table 9).

Discussion

Puccinia graminis germlings are recognized by a narrowly oblong appressorium, a fusiform substomatal vesicle with mostly one, sometimes two, longitudinally and horizontally orientated primary infection hyphae, and long and slender haustorial mother cells. Only a *P. recondita* rust fungus collected from *Alopecurus pratensis* had a similar SSV + PIH germling morphology. This collection, however, had an oblong appressorium and the germ pores of the urediniospores had a scattered arrangement (Section 4.4).

The mean length of SSV + PIH and the percentage of germlings with SH-ADD differed between f.sp. *avenae*, f.sp. *secalis* and f.sp. *tritici* (Table 8). The differences between the three *formae speciales* partly agree with values found by Niks (1986). He rarely observed germlings with a PIH (SSV + PIH length $\leq$ 35 (–40) μm) in f.sp *tritici*, whereas in the present study several germlings with a PIH were observed (mean SSV + PIH length 41.2 $\pm$ 1.4 μm, Table 9). Lennox & Rijkenberg (1989) also reported the presence of PIH in wheat stem rust after

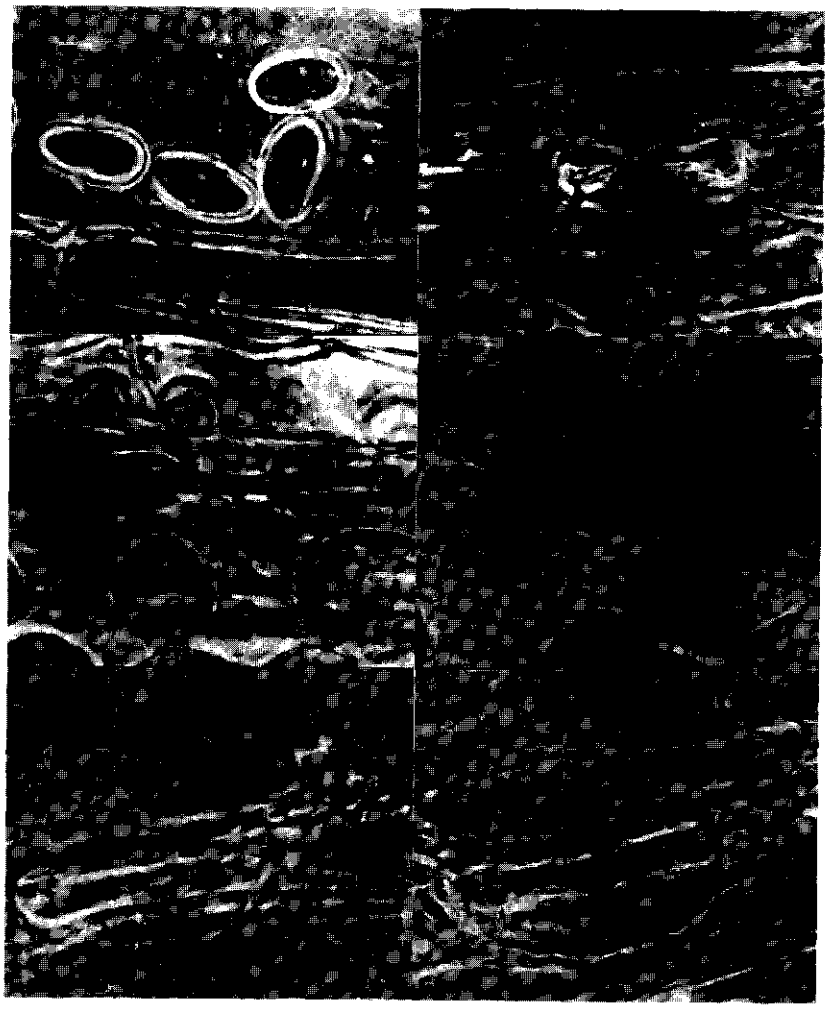


Figure 6. *Puccinia graminis*, phase contrast micrographs, scale bar = 20 μm.
A. Urediniospores collected from *Secale cereale* (Canada ACRS-F1221). **B–F.** Germlings of urediniospores collected from: **B.** *Avena fatua* (Canada ACRS-661), appressorium. **C.** *Avena sativa* (f.sp. *avenae*, Canada ACRS-697), SSV with long PIH and SH-ADD (right arrow). Note short appendix (left arrow). **D.** *Lolium perenne* (C.S. 92.100), SSV with short PIH and HMC. **E-F.** *Hordeum jubatum* (f.sp. *tritici*, Canada ACRS-F1319): **E.** SSV with short PIH and HMC (arrow). **F.** Two-sided SSV (arrows). Note HMC at left side of the SSV (left arrow).

inoculation on barley. The observations of Niks (1986) may be extreme values of the data set presented here, as he studied only one isolate of each taxon, which does not represent the range of variation within the taxon.

Niks (1986) also recorded the percentage of germlings with an appendix. His values were somewhat lower than those reported here, though the ranking of taxa was similar. However, the percentage of germlings with an appendix or an SIH was very variable. Therefore, this criterion does not seem to be useful as an aid in identifying taxa.

The observed differences between germlings from the same *forma specialis* but from different host species (Table 9) are not easily to be explained. Firstly, environmental conditions are not likely to account for these differences as all experiments were done under the same conditions. Secondly, the susceptibility of the barley line used for the inoculation of urediniospores to a specific *forma specialis* does not seem to influence germling morphology. Germlings of f.sp. *tritici* collected from *Hordeum vulgare* had longer SSV + PIH than collections from i.a. *Triticum*, but germlings of f.sp. *secalis* from *Hordeum vulgare* were shorter than those from *Secale*, but only one collection was studied. Results indicate that the germling morphology enables a distinction between f.sp. *secalis* and f.sp. *tritici* when occurring on *Hordeum vulgare* and *Hordeum jubatum* (Table 9), but additional collections need to be studied to corroborate this trend.

The observed similarities in germling morphology of stem rust fungus collections from cereals and wild grasses show that stem rusts are closely related and that it is justified to consider them as one species. Whether it is also justified to recognize subspecies from cereals and wild grasses cannot be assessed on the basis of this study, as only a few germlings of stem rusts of the wild grasses have been studied. The few germlings observed of wild grasses had PIH which were similar in width (3–4 μm) to those observed in stem rusts of cereals. In addition, length of SSV + PIH was similar to that in f.sp. *tritici* and f.sp. *secalis* (20–70(–105) μm). These findings contrast with the study of Pole Evans (1907), who reported smaller SSV and thinner PIH for *P. phlei-pratensis*, a synonym for *P. graminis* subsp. *graminicola* (Urban, 1967; Cummins, 1971). Additional experiments with stem rust urediniospores collected from wild grasses and cereals are required to get more insight in the significance of the delimitation of subspecies within *P. graminis*.

The quantitative variation observed in germlings of the three forms of *P. graminis* on cereals suggest to recognize them as three separate taxa and not to subsume them under two as suggested by Urban (1967). Forms from *Avena* and *Secale*, both included in variety *graminis* (Urban, 1967), differ largely in germling features, whereas the *formae speciales* from *Secale* and *Triticum*, varieties sensu Urban (1967), are more similar in germling morphology (Table 8).

Somatic and sexual hybridization and nuclear exchanges may introduce genes from one taxon into another (Savile, 1984). Somatic hybridization between forms of *P. graminis* from cereals and wild grasses has been reported to occur in greenhouse experiments (Johnson *et al.* 1932; Johnson & Newton, 1933; Green, 1971) and in

Table 8. Main hosts, range and mean length ± standard error of the substomatal vesicle + primary infection hypha (SSV + PIH), percentages of germlings with secondary hyphae along the primary infection hypha (% SH-ADD), and of germlings with an appendix or SIH (% appendix) of three *formae speciales* of *Puccinia graminis*. N = number of collections studied.

f.sp.	main hosts[a]	SSV + PIH (μm)		% SH-ADD		% appendix		N
		range	mean ± s.e.	range	mean ± s.e.	range	mean ± s.e.	
avenae	*Avena sativa, Avena fatua*	20–95(–140)	55.5 ± 2.5	(10–)27–50(–67)	35.2 ± 4.4	13–53	34.0 ± 4.0	12
tritici	*Triticum* spp., *Hordeum vulgare, Hordeum jubatum*	20–75(–90)	47.3 ± 1.6	3–16(–33)	13.7 ± 3.2	10–27(–43)	21.7 ± 3.4	10
secalis	*Secale cereale, Hordeum vulgare, Hordeum jubatum*	20–70(–100)	39.1 ± 3.2	0–16	7.1 ± 2.2	(0–)23–37	19.2 ± 5.1	7

[a] *Formae speciales* were provided and identified by K. Dunsmore of the Agriculture Canada Research Station, Winnipeg.

Table 9. Mean length ± standard error of the substomatal vesicle and primary infection hypha (SSV + PIH), percentages of germlings with secondary hyphae along the primary infection hypha (% SH-ADD), and of germlings with an appendix or SIH (% appendix) of *Puccinia graminis formae speciales avenae*, *tritici* and *secalis* collected from various hosts[a]. N = number of collections studied.

f.sp.	host	SSV + PIH (μm)	% SH-ADD	% appendix	N
avenae	*Avena sativa*	56.4 ± 2.8	38.0 ± 4.8	37.7 ± 3.8	10
	Avena fatua	50.9 ± 7.1	20.9 ± 2.4	15.9 ± 2.6	2
tritici	*Triticum* spp.	41.2 ± 1.4	11.1 ± 1.1	16.7 ± 6.0	3
	Hordeum vulgare	53.7 ± 1.7	14.2 ± 6.6	21.7 ± 4.0	4
	Hordeum jubatum	45.0 ± 0.6	15.6 ± 7.8	26.7 ± 8.4	3
secalis	*Secale cereale*	47.9 ± 0.5	12.5 ± 3.0	22.9 ± 8.2	3
	Hordeum vulgare	33.8	0.0	23.3	1
	Hordeum jubatum	32.0 ± 2.0	3.8 ± 2.3	14.0 ± 9.5	3

[a] Collections were provided and identified by K. Dunsmore of the Agriculture Canada Research Station, Winnipeg.

nature (Burdon *et al.*, 1981). Besides, Savile (1984) found a collection on *Elymus triticoides* with spore features that suggested this rust to be a hybrid between subsp. *graminis* var. *graminis* and subsp. *graminicola*. Since f.sp. *tritici*, f.sp. *secalis*, and subsp. *graminicola* can grow on the same host, nuclear exchange is possible. The wide range and overlap in size of urediniospores, teliospores (Urban, 1969; Savile, 1984) and urediniospore germling morphology (Pole Evans, 1907; Niks, 1986; present study) between forms collected from *Secale*, *Triticum*, *Hordeum* spp., and wild grasses could also indicate that the taxa are not (completely) isolated genetically.

In conclusion, the germling morphology easily distinguishes *P. graminis* from the other grass rust fungi. Since the long pustules, the arrangement of the germ pores on the urediniospores, and the shape of the teliospores also reliably identify a collection as *P. graminis*, germling morphology is not needed as additional criterion. However, germling morphology enables the recognition of three groups in *P. graminis*, which differ in host range and spore size.

4.3 PUCCINIA BRACHYPODII Otth

Introduction

Puccinia brachypodii sensu lato covers a complex of grass rust fungi characterized by numerous clavate-capitate, thick-walled, intra-uredinial paraphyses (Fig. 1B), urediniospores with 6–11 rather distinct germ pores with a scattered arrangement (Fig. 8A), and covered telia. In Europe, grasses which can be infected by *P. brachypodii* are amongst others *Anthoxanthum odoratum*, *Arrhenatherum elatius*, *Brachypodium* spp., *Deschampsia cespitosa*, and *Poa* spp. Cereals are not infected (Cummins & Greene, 1966; Wilson & Henderson, 1966; Urban, 1969).

For the rust fungi collected from *Brachypodium* spp. and *A. elatius* aecia have been recorded on species of *Berberis* (and in North America *Mahonia*), but generally host alternation does not occur. The fungus overwinters as mycelium in, or as urediniospores on the leaves of grasses (Viennot-Bourgin, 1949; Straib, 1952; Cummins & Greene, 1966; Wilson & Henderson, 1966; Ullrich, 1977; Cagaš & Marková, 1985) and therefore rust infections can be found from early spring onwards.

Economically, *P. brachypodii* rusts are of minor importance. Only on *Poa* spp., especially *P. pratensis* which is commonly grown in sports fields, along roads and in meadows (Bakker & Vos, 1975), *P. brachypodii* s.l. can decrease fodder quality and, as with powdery mildews and leaf spots, can lead to a decrease in seed yield (Cagaš, 1981, cited in Cagaš, 1989).

Host range experiments to recognize resistant *Poa* cultivars have led to conflicting reports. In 1985, Cagaš & Marková reported that *P. brachypodii* rusts from *P. pratensis* and *P. palustris* were strictly confined to the host species they

were collected from, though some of the host cultivars tested could be resistant. In further experiments, however, the same authors (Cagaš & Marková, 1988) obtained infections of the rust from *P. pratensis* on several *Poa* spp., including *P. palustris*. In addition, infection was observed on *Alopecurus myosuroides* and *Lolium temulentum* in 24 and 43%, respectively, of the inoculated plants. They did not mention the infection type. They concluded that the host range of the *P. brachypodii* rust from *P. pratensis* was limited to species of *Poa*. The other grass species showing signs of infection were not thought to be important in the life cycle of the rust as no spontaneous field infections were found (Cagaš & Marková, 1988). As far as known, no host range studies have been carried out with other taxa belonging to *P. brachypodii* s.l.

Taxonomic concepts of *P. brachypodii* s.l. vary considerably among authors. Authors not only use various taxonomic ranks, viz. species or varieties, but they also recognize different numbers of taxa (Table 10). Wilson & Henderson (1966) and Cummins & Greene (1966) recognized three species and three varieties, respectively, and Urban (1969) recognized four species. Besides, classification of the *P. brachypodii* rusts from *A. elatius* and *D. cespitosa* is very confusing. Urban (1969) recognized the rust from *A. elatius* as a separate species (*P. magelhaenica*

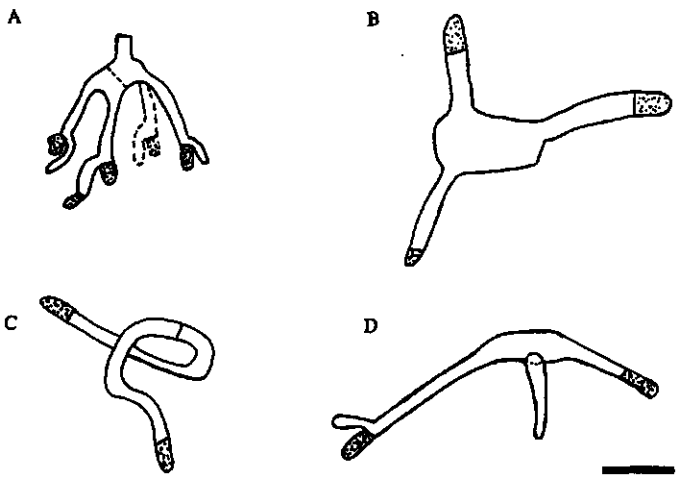


Figure 7. Drawings of types of germling morphology of *Puccinia brachypodii* s.l. Scale bar = 20 μm.
A. *Arrhenatherum* type from *A. elatius* (C.S. 90.007). **B.** *Brachypodium* type from *B. sylvaticum* (C.S. 90.071). **C.** *Deschampsia* type from *D. cespitosa* (C.S. 90.213). **D.** *Poa* type from *P. palustris* (C.S. 92.029).

Table 10. Comparison of the species concepts within *Puccinia brachypodii* s.l. by several authors.

host	Cummins & Greene (1966)	Wilson & Henderson (1966)	Urban (1969); this study
Arrhenatherum elatius	*P. brachypodii* var. *arrhenatheri* (Kleb.) Cumm. & H.C. Greene[b]	*P. poae-nemoralis* Otth[a]	*P. magelhaenica* Peyritsch in Magn.
Brachypodium spp.	*P. brachypodii* var. *brachypodii* Otth	*P. brachypodii* Otth	*P. brachypodii* Otth
Deschampsia cespitosa	*P. brachypodii* var. *arrhenatheri* (Kleb.) Cumm. & H.C. Greene[b]	*P. deschampsiae* Arth.	*P. deschampsiae* Arth.
Poa spp. + *Anthoxanthum odoratum*	*P. brachypodii* var. *poae-nemoralis* (Otth) Cumm. & H.C. Greene	*P. poae-nemoralis* Otth[a]	*P. poae-nemoralis* Otth

[a] Also mentioned by Jørstad (1950). [b] Also mentioned by Braun (1982) as *P. magelhaenica*.

Table 11. Arrangement of uredinia, total length (in μm) of the uredinial paraphyses, and size (length × width, in μm) of the urediniospores and teliospores of *Puccinia brachypodii* s.l.

host	arrangement	length of paraphyses	size of urediniospores	size of teliospores
Arrhenatherum elatius	scattered	50–90(–100)	(23–)25–29(–32) × (20–)21–26	(30–)34–50(–60) × 14–21
Brachypodium spp.	linear	30–60(–70)	(18.5–)21–25(–27) × 16–20(–25)	(20–)30–40(–44) × (13–)16–23
Deschampsia cespitosa	scattered	70–90(–100)	(26–)28–32 × 21–25(–26)	not present
Poa spp. + *Anthoxanthum odoratum*	scattered	50–85(–100)	(19–)22–26(–31) × (15–)17–21(–25.5)	(28–)32–45(–50) × 14–23[a]

Data are from own measurements, in heated lactophenol.
[a] Not formed on *A. odoratum*

Figure 8. *Puccinia brachypodii* s.l., phase contrast micrographs, scale bar = 20 μm. **A.** Urediniospores of *P. poae-nemoralis* from *Anthoxanthum odoratum* (C.S. 90.078). **B–F.** Germlings of urediniospores: **B.** *P. poae-nemoralis* from *Anthoxanthum odoratum* (C.S. 90.077), oblong appressorium. **C.** *P. magelhaenica* from *Arrhenatherum elatius* (C.S. 90.070), SSV with two vertical PIH. Note elongated infection peg and septum. **D–F.** *P. brachypodii*: **D.** from *Brachypodium sylvaticum* (C.S. 90.071), SSV with two horizontal and one vertical PIH (arrows) and a HMC. **E.** from *Brachypodium pinnatum* (C.S. 90.073), SSV with two horizontal PIH. **F.** from *Brachypodium sylvaticum* (C.S. 90.140), secondary infection hypha.

Peyritsch in Magn.), whereas Cummins & Greene (1966) included this together with the rust from *D. cespitosa* in one variety (*P. brachypodii* var. *arrhenatheri* Kleb.). Wilson & Henderson (1966) and Jørstad (1950) classified the rust from *A. elatius* together with the rusts from *Poa* and *Anthoxanthum odoratum* as *P. poae-nemoralis* Otth. Both Urban (1969) and Wilson & Henderson (1966) recognized the rust from *D. cespitosa* as a separate species, *P. deschampsiae* Arth. (Table 10).

One of the causes of the complicated taxonomy is the almost continuous variation in characters of uredinia and telia. Only the rust on *Brachypodium* is easily recognized by its linear arrangement of sori on blackish rectangular flecks in the leaf, whereas the rusts from *A. odoratum* and *Poa* spp., *A. elatius*, and *D. cespitosa* have sori with a scattered arrangement (Table 11). It is difficult to identify rusts of the latter four hosts because the quantitative characters of uredinia and telia show a considerable overlap in size. The rust from *A. elatius* and *D. cespitosa* tend to have somewhat larger urediniospores than those from *Poa* spp. and *A. odoratum*, but smaller urediniospores are also frequently found (Table 11). Moreover, the other criterion used for identification of *P. brachypodii* rusts, viz. the shape of the paraphyses, appeared to be very variable (Chapter 2).

As the host range is probably limited (Cagaš & Marková, 1988), the identity of the host seems to be more valuable for identification of *P. brachypodii* rusts than the morphological characters of the spores. Host identity, however, should be used with caution (Savile, 1984) and therefore germling morphology of the urediniospores was studied to see whether this criterion can help to elucidate the identification of taxa.

Germling morphology

Four morphological types of germling morphology were recognized in *Puccinia brachypodii* s.l., viz. the *Arrhenatherum*, *Brachypodium*, *Deschampsia*, and *Poa* types (Fig. 7).

Description

Arrhenatherum type (Figs 7A, 8C)

The appressorium is oblong, 25–37 × 10–18 μm. The penetration peg is vertically elongated and 5–7(–15) μm long. The SSV is ellipsoid-oblong to narrowly ellipsoid, (11–)15–25 × 7–10 μm. In many SSV a central or eccentric septum is formed. The SSV is longitudinally orientated and the ends are curved deeply into the mesophyll. At the ends of the SSV 2–3(–4) primary infection hyphae, (4–)5–7(–9) μm wide, arise. The PIH are vertically orientated. Occasionally a septum is present in one of the PIH. The HMC are unlobed and short, (8–)10–14 × (3–)4–5 μm. Frequently secondary hyphae, 4–6 μm wide, are formed at the SSV side of the HMC septum.

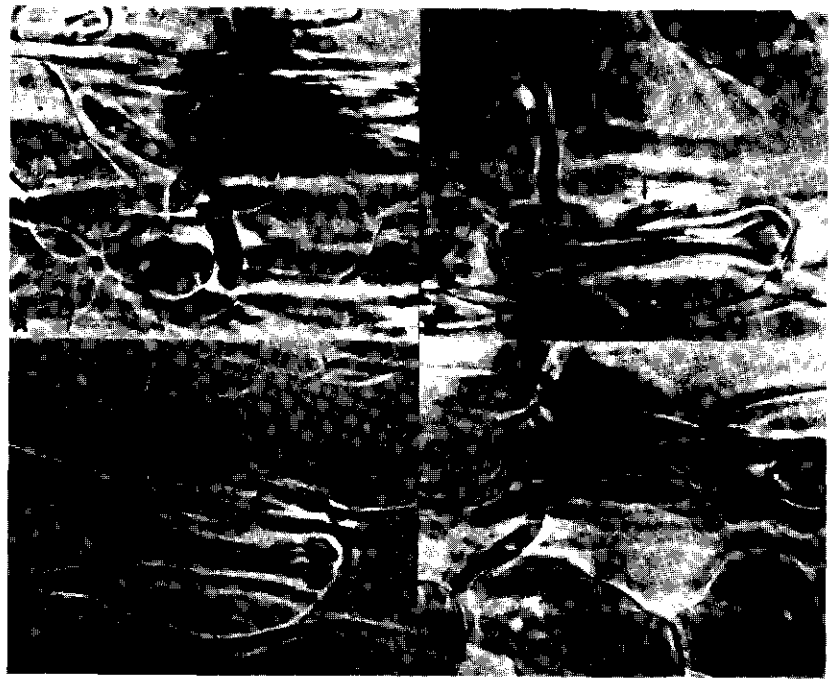


Figure 9. *Puccinia brachypodii* s.l., phase contrast micrographs, scale bar = 20 μm. Germlings of urediniospores: **A.** *P. deschampsiae* from *Deschampsia cespitosa* (C.S. 90.213), SSV with two vertical PIH and a central septum. Note absence of elongated infection peg. **B–D.** *P. poae-nemoralis*: **B.** from *Poa nemoralis* (C.S. 90.066), SSV with three horizontal PIH (arrows), and a septum. **C.** from *Poa pratensis* (C.S. 91.014), SSV with four horizontal PIH and a central septum. **D.** from *Anthoxanthum odoratum* (C.S. 90.056), two longitudinal PIH and an eccentric septum.

The *Arrhenatherum* type of germling morphology is characterized by an elongated infection peg and a vertical orientation of the PIH. This type was observed in 16 collections from *Arrhenatherum elatius*, obtained from five European countries (Appendix).

Brachypodium type (Figs 7B, 8 D, E, F)

The appressorium is oblong, 20–30 × 10–15 μm. The penetration peg is short. The SSV is ellipsoid-oblong to deltoid, (11–)15–22(–25) × (8–)10–12(–15) μm, without a septum. From the SSV 2–4(–5) primary infection hyphae, (3–)4–5(–7) μm wide, arise. Orientation of the PIH is variable. Mostly two PIH grow horizontally and transversely and the other(s) grow vertically. Occasionally one hypha is longitudinally, or all hyphae are vertically orientated. The HMC are unlobed and short, (6–)8–10(–12) × (3–)4–5(–6) μm. Very rarely secondary hyphae arise at the SSV side of the HMC septum. SH are always associated with a haustorium formed in the invaded barley mesophyll cell. The SH are 9–10 μm wide and nodiform.

The *Brachypodium* type of germling morphology is characterized by an ellipsoid-oblong to deltoid SSV with 2–4(–5) horizontally and vertically orientated PIH. This type was observed in 14 collections from *Brachypodium* spp., obtained from four European countries (Appendix). Collections from *B. sylvaticum* more often formed three and four PIH while in collections from *B. pinnatum* two PIH were more often present (Table 12).

Deschampsia type (Figs 7C, 9A)

The appressorium is oblong, 30–40 × 10–20 μm. The penetration peg is short. The SSV is ellipsoid-oblong to narrowly ellipsoid, 20–22 × 7–10 μm, often with a septum. It is longitudinally orientated and the ends are curved down into the mesophyll. At the ends of the SSV two or three primary infection hyphae, 6–7 μm wide, arise. The PIH grow vertically and are deflected towards the nearest vein. The PIH often cross each other when growing downwards. The HMC are unlobed and relatively long, 15–21 × 5–6 μm. Secondary hyphae arising from the SSV side of the HMC septum are sometimes formed.

The *Deschampsia* type of germling morphology is similar to the *Arrhenatherum* type, but can be distinguished from the latter by the absence of an elongated infection peg and the longer HMC. The *Deschampsia* type was observed in one collection from *Deschampsia cespitosa*, collected in Edinburgh, Great Britain (Appendix). In Limburg, the Netherlands, a rust fungus with thin-walled paraphyses was found on *D. cespitosa*. It had a germling morphology similar to *P. pygmaea* collected from *Calamagrostis* spp. (unpublished data), and was similar to the description of *P. pygmaea* by Niks (1986). *P. pygmaea* has thin-walled paraphyses (Urban, 1969), but has not yet been reported from *D. cespitosa*. The collection from

Limburg possibly represents a jump of *P. pygmaea* from *Calamagrostis* to *D. cespitosa*.

Poa type (Figs 7D, 8B, 9 B, C, D)

The appressorium is oblong, 24–32(–35) × 10–20 μm. The penetration peg is short. The shape of the SSV depends on the number of primary infection hyphae. When two or three PIH are present, the SSV is fusiform, with a widest diameter of (6–)7–9(–11) μm. The SSV slightly tapers to form a PIH, (3–)4–6(–7) μm wide, at both ends of the SSV. PIH are often longitudinally and horizontally orientated, but PIH can also be deflected toward the nearest vein and grow more deeply into the mesophyll. A third PIH, either vertically or transversely orientated, may be present. When four or five PIH are present, the SSV is rectangular, 20–26 × (7–)8–9(–10) μm. Orientation of the PIH is either transverse and horizontal, or some PIH are transversely and horizontally, and the others vertically orientated. Occasionally a septum is formed, either in the SSV (central or eccentric) or in one of the PIH. The HMC are unlobed and short, (7–)8–15(–25) × 3–5(–6) μm. Frequently secondary hyphae, 4–6 μm wide, are formed at the SSV side of the HMC septum and occasionally they arise along the PIH.

The *Poa* type is characterized by a fusiform SSV with a variable number of PIH, but each collection always had some germlings with two PIH. This type was observed in 51 collections from *Poa* and *Anthoxanthum odoratum*, obtained from seven European countries (Appendix). The distribution of the number of PIH appeared to depend on the species from which the rust fungus had been collected, but the differences could be very small, e.g. between the rust fungi collected from *P. nemoralis* and *P. palustris* (Table 12).

Discussion

The germlings of *P. brachypodii* s.l. can be divided into four clearly different morphological groups, viz. rust fungi collected from *Arrhenatherum elatius*, *Brachypodium* spp., *Deschampsia cespitosa*, and *Poa* spp. + *Anthoxanthum odoratum* (Figs 7, 8, 9). These observations suggest that within *P. brachypodii* s.l. germling morphology can be very valuable for both identification and classification of rust taxa. Results of this study agree with the findings of Niks (1986) who studied germlings of urediniospores collected from *A. elatius*, *P. nemoralis*, and *P. pratensis*.

Results also suggest that host specificity is associated with germling morphology and that it might be possible to identify the host species from which the urediniospores were collected by overall germling morphology (Figs 8, 9) and by number of PIH (Table 12). This possibility was investigated by observing several collections of *P. brachypodii* s.l. in a blind experiment in which the name of the host species from which the rust was collected was put under code.

The four types of germling morphology and thus the four host groups were easily recognized according to the differences in germling morphology. Recognition of the host species of *P. brachypodii* rust fungi which had similar overall germling morphology but differed slightly in number of PIH, viz. collections from *Brachypodium* spp., *Poa* spp. and *A. odoratum* was more difficult. *P. annua*, *P. pratensis* and *A. odoratum* could be distinguished, but the rusts collected from *B. pinnatum* and *B. sylvaticum* were too similar to tell them apart as was the case for the collections from *P. palustris* and *P. nemoralis*. The recognition of rusts collected from *Poa* spp. may even become more difficult when germlings of rusts collected from other hosts, like *Poa trivialis*, are included in the study. Thus, the germling morphology may be helpful in some, but not all, cases to identify the host species of *P. brachypodii*.

The similar germling morphology of the rusts from *Poa* spp. and *A. odoratum* suggests that these rusts are closely related and that it is justified to subsume

Table 12. Numbers of primary infection hyphae (PIH) of *Puccinia brachypodii* s.l. collected from different hosts. Collection (country) = the numbers of collections studied and the numbers of countries from which they had been obtained.

host	number of PIH[a]	collection (country)
Arrhenatherum type		
Arrhenatherum elatius	**2**, **3**, **4**	16 (5)
Brachypodium type		
Brachypodium pinnatum	**2**, **3**, 4	5 (3)
Brachypodium sylvaticum	**2**, **3**, 4, (5)	9 (4)
Deschampsia type		
Deschampsia cespitosa	**2**, **3**	1 (1)
Poa type		
Anthoxanthum odoratum	**2**, (3)	6 (1)
Poa annua	**2**, **3**, (4, 5)	13 (4)
Poa nemoralis	**2**, **3**, 4, (5)	13 (6)
Poa palustris	**2**, **3**, (4)	7 (3)
Poa pratensis	**2**, **3**, **4**, 5	12 (6)

[a] Prevailing numbers of PIH are printed in bold face, numbers in parentheses were rarely observed.

Table 13. Aecial hosts and notes on host range of uredinial hosts included in *Puccinia recondita sensu lato.*

main host	aecial host	notes on additional host species (reference)
Agropyron junceiforme[a]	probably *Thalictrum*	no data available
Agropyron repens[b]	probably hibernating	*Bromus arvensis* (Eriksson, 1899), *Triticum*, *Secale* (Urban & Marková, 1978, 1985), *Hordeum jubatum* (Niks, unpub.)
Alopecurus pratensis	*Ranunculus acris*	*Avena elatior* (Plowright, 1885, cited in Mains, 1933)
Arrhenatherum elatius	unknown	*Secale cereale* (Niks, 1987)
Bromus hordeaceus	probably hibernating	not on *B. erectus*, *B. inermis*, *B. sterilis* (Marshall Ward, 1903; Mains, 1933)
Bromus inermis, *B. erectus*	*Symphytum*, *Pulmonaria*	not on *B. sterilis*, *B. hordeaceus* (Mains, 1933)
Bromus sterilis	probably hibernating	not on *B. inermis*, *B. erectus* (Mains, 1933), not on *B. hordeaceus*, nor on *Agropyron repens*, *Avena sativa*, *Hordeum vulgare*, and *Triticum aestivum* (Urban & Marková-Ondráčková, 1975)
Holcus lanatus, *H. mollis*	unknown	no additional host species found (Mains, 1933)
Lolium perenne, *L. multiflorum*	unknown	*Festuca pratensis*; not on *Hordeum vulgare*, *H. murinum*, *Holcus lanatus*, *Koeleria gracilis*, *Secale cereale*, *Bromus mollis*[c] (Wilkins, 1973)
Secale cereale, *S. montanum*	*Anchusa*, *Lycopsis*, *Echium*, *Clematis*	*Avena sativa* (Urban, 1969), *Bromus*, *Hordeum*, *Elymus* (Anikster & Wahl, 1979), *Aegilops ovata*, (*Agropyron repens*) (Anikster, unpubl.)
Trisetum flavescens	*Sedum nicaeense*	*Koeleria phleoides* (Dupias, 1958)
Triticum spp.	*Thalictrum*, *Anchusa*, *Isopyrum*	*Hordeum* (Eriksson, 1899; Urban, 1969), *Aegilops crassa*, trace infection on several grasses and cereals (Mains, 1933), *Aegilops*, *Agropyron*, *Bromus* (in Anikster & Wahl, 1979), *Secale* (Savile, 1984), *Aegilops squarrosa* (a.o. Valkoun *et al.*, 1985)

[a] Synonymous with *Elymus farctus*, [b] synonymous with *Elymus repens*, [c] synonymous with *Bromus hordeaceus*.

them under one taxon. Similarly, the rusts collected from *Brachypodium* spp. can be subsumed under a second taxon. The large differences in germling morphology between the rusts from *A. elatius* and the rust from *Poa* spp. + *A. odoratum* show that they represent separate taxa. As the germlings from *D. cespitosa* (although only one collection was studied) were clearly different from those from *A. elatius*, this indicates the existence of a fourth taxon.

This study showed that the germling morphology greatly contributes to the recognition of taxa in the large species complex *P. brachypodii*, whereas recognition on the basis of characters of sori and spores is often difficult. The large qualitative differences in germling morphology between rusts collected from various host genera and the merely quantitative differences between germlings collected from species of one genus, suggest to recognize four species within *P. brachypodii* s.l., viz. *P. brachypodii* Otth, *P. poae-nemoralis* Otth, *P. magelhaenica* Peyritsch in Magn. and *P. deschampsiae* Arth. as suggested by Urban (1969).

4.4 PUCCINIA RECONDITA Rob. ex Desm.

Introduction

Puccinia recondita sensu lato covers a complex of grass rust fungi characterized by brown, scattered, aparaphysate uredinia, urediniospores with (4–)7–12(–14) rather distinct germ pores with a scattered arrangement (Fig. 11A), black, loculate, long-covered telia and two-celled teliospores. Occasionally one- and three-celled teliospores are formed (Wilson & Henderson, 1966; Urban, 1969; Savile, 1984; own observations).

Uredinia and telia are formed on cereals and grasses, i.a. species of *Aegilops*, *Agropyron*, *Agrostis*, *Alopecurus*, *Arrhenatherum*, *Bromus*, *Holcus*, *Lolium*, *Secale*, *Trisetum* and *Triticum*. Most taxa (species, varieties, '*formae speciales*') subsumed under *P. recondita* are heteroecious. Pycnia and aecia are formed on species of Ranunculaceae, Boraginaceae or Crassulaceae. For some taxa the aecial host is unknown (Table 13).

The optimum temperature for germination and stoma penetration of urediniospores of the Wheat leaf rust fungus, *Puccinia recondita/Triticum*, is 10–22 °C (Urban & Marková, 1978, 1986; Kramer & Eversmeyer, 1992; Roelfs *et al.*, 1992). For the other *P. recondita* rusts the optimum temperatures are not known. Most *P. recondita* rusts occur worldwide.

Wheat leaf rust can cause severe crop losses when susceptible cultivars are grown (Roelfs, 1985; Roelfs *et al.*, 1992). In rye and wild grasses *P. recondita* s.l. is regularly observed, but, in general, does not cause severe economic losses (Wilson & Henderson, 1966; Urban, 1969; own observations). Leaf rust infections in *Agropyron repens* may be dangerous to nearby-situated wheat fields as infection

Table 14. Overview of the taxa recognized within *Puccinia recondita sensu lato* by several authors.

Uredinial host	Gäumann, 1959	Wilson & Henderson, 1966	Urban, 1969	Cummins, 1971	Swertz, this study
Agropryon junceiforme	*P. agropyri* Ellis & Everh.[a]	*P. recondita* f.sp. *persistens* Plowr.	not mentioned	*P. recondita* Rob. ex Desm.	*P. agropyri-juncei* Kleb.
Agropyron repens	*P. persistens* Plowr.	*P. recondita* f.sp *agropyrina* Erikss.[b]	*P. perplexans* var. *triticina* f.sp. *persistens* (Plowr.) Urban	*P. recondita* Rob. ex Desm.	*P. agropyrina* Erikss.
Alopecurus pratensis	*P. perplexans* Plowr.	*P. recondita* f.sp. *perplexans* Plowr.	*P. perplexans* Plowr. var. *perplexans*	*P. recondita* Rob. ex Desm.	*P. perplexans* Plowr.
Arrhenatherum elatius	*P. arrhenathericola* Fischer	not mentioned	*P. arrhenathericola* Fischer	*P. recondita* Rob. ex Desm./*P. hordei* Otth[c]	*P. arrhenathericola* Fischer
Bromus hordeaceus	*P. symphyti-bromorum* F. Müller	*P. recondita* f.sp. *bromina* Erikss.	*P. bromina* Erikss. var. *bromina*	*P. recondita* Rob. ex Desm./*P. hordei* Otth[c]	*P. bromina* Erikss. var. *bromina*
Bromus inermis, *B. erectus*	*P. symphyti-bromorum* F. Müller	*P. recondita* f.sp. *symphyti-bromorum* Müll.	*P. bromina* var. *paucipora* Urban	*P. recondita* Rob. ex Desm./*P. hordei* Otth[c]	*P. bromina* var. *paucipora* Urban
Bromus sterilis	*P. symphyti-bromorum* F. Müller	*P. recondita* f.sp. *bromina* Erikss.	*P. bromina* Erikss. var. *bromina*	*P. recondita* Rob. ex Desm./*P. hordei* Otth[c]	*P. bromina* Erikss. var. *bromina*
Holcus lanatus, *H. mollis*	*P. holcina* Erikss.	*P. recondita* f.sp. *holcina* Erikss.	*P. holcina* Erikss.	*P. hordei* Otth	*P. holcina* Erikss.
Lolium perenne, *L. multiflorum*	*P. loliina* Sydow	*P. schismi* Bubák[d]	not mentioned	*P. recondita* Rob. ex Desm./*P. hordei* Otth[c]	*P. loliina* Sydow
Secale cereale, *S. montanum*	*P. dispersa* Erikss.	*P. recondita* Rob. ex Desm. f.sp. *recondita*	*P. recondita* Rob. ex Desm.	*P. recondita* Rob. ex Desm.	*P. recondita* Rob. ex Desm.
Trisetum flavescens	*P. triseti* Erikss.	*P. recondita* f.sp. *triseti* Erikss.	*P. triseti* Erikss.	*P. recondita* Rob. ex Desm./*P. hordei* Otth[c]	*P. triseti* Erikss.
Triticum spp.	*P. triticina* Erikss.	*P. recondita* f.sp. *tritici* Erikss. & E. Henn.	*P. perplexans* var. *triticina* (Erikss.) Urban f.sp. *triticina*	*P. recondita* Rob. ex Desm.	*P. triticina* Erikss.

[a] Synonymous with *Puccinia agropyri-juncei* Klebahn (Gäumann, 1959).
[b] Aecial host unknown; when the aecial host is *Thalictrum*, it is called f.sp. *persistens* Plowr.. Taxa are morphologically similar.
[c] Distinction of *P. recondita* and *P. hordei* is extremely difficult; the colour of the urediniospore wall is brownish or pale yellowish, respectively.
[d] Also named *P. recondita* s.l. (Wilkins, 1973).

in *A. repens* is easily transferred to wheat (Azbukina, 1980; Urban & Marková, 1985, 1986).

Taxa belonging to *Puccinia recondita* s.l. exhibit parasitic specialization on both the telial (uredinial) and the aecial hosts. Inoculation experiments showed that in the uredinial stage most rust fungi belonging to *P. recondita* s.l. are strictly limited to the original host species from which they were collected. Occasionally, additional grass species can be infected (Table 13). It is uncertain to what extent additional species are important hosts in nature, as artificially proven infections may not occur in nature (Roelfs *et al.*, 1992). *Aegilops* spp., *Agropyron repens*, *Hordeum* spp. and *Secale* spp. probably serve as additional hosts for Wheat leaf rust as these grasses were occasionally found infected with the Wheat leaf rust fungus in nature (Urban, 1969; Savile, 1984; Urban & Marková, 1985, 1986).

In the aecial stage, many taxa are strictly limited to one or two aecial host species belonging to the same angiosperm family (Table 13). Exceptions are *P. recondita*/*Triticum* and *P. recondita*/*Secale* which both have been reported to alternate with species from the Boraginaceae and the Ranunculaceae (Oliveira, 1960, cited in Anikster & Wahl, 1979; Oliveira & Samborski, 1966).

Many authors have applied a broad species concept and regard all leaf rusts as one species, *P. recondita* Rob. ex Desm., in which many *formae speciales* are distinguished (i.a. Wilson & Henderson, 1966; Cummins, 1971; Gjaerum, 1974; Boerema & Verhoeven, 1977; Samborski, 1985; Table 14). The *formae speciales* are defined mainly by their pathogenity to a specific uredinial plant species or group of species, since the differences in morphological characters of uredinia and telia are very small (Wilson & Henderson, 1966; Cummins, 1971; Anikster, 1984; Table 15).

Other authors have followed a narrow species concept and regard most leaf rust fungi collected from different plant genera as true species (i.a. Fischer, 1904; Gäumann, 1959; Urban, 1969; Savile, 1984; Table 14). They attach a high value to the small but consistent differences in morphological characters of the uredinia, telia (Table 15) and aecia (Savile, 1973, 1984) between rust fungi collected from various plant species. Whilst Gäumann (1959) recognized ten species in the complex studied here, Urban (1969) accepted six species, and additionally introduced some varieties and *formae speciales*. The varieties subsumed under (i) *P. bromina* Erikss., viz. var. *bromina* and var. *paucipora* Urban, and (ii) *P. perplexans* Plowr., viz. var. *triticina* (Erikss.) Urban and var. *perplexans* (Erikss.) Urban, differ in the numbers of germ pores. In *P. persistens* Plowr. subsp. *persistens* (a new combination for *P. perplexans* var. *triticina* introduced in 1977 by Marková & Urban, cited in Urban & Marková, 1986) the varieties *triticina* (Erikss.) Urban & Marková and *persistens* differ in colour and thickness of the urediniospore wall (Urban & Marková, 1986). Recently, Urban & Marková (1992) changed their taxonomic concepts in *P. recondita* s.l. This did not contribute to facilitate the identification of *P. recondita* s.l. rusts since many

Table 15. Morphological characters of urediniospores and teliospores of taxa subsumed under *Puccinia recondita sensu lato.*

uredinial host	urediniospores			teliospores
	number of germ pores	echinulation distance (μm)	dimensions (μm)	length (μm)
Agropyron junceiforme	7–9[6]	1.5–2.5[6]	(24–)26–32(–36) × 20–24[6]	43–58 × 12–17[6]
Agropyron repens, *Hordeum jubatum*[6]	(7–)8–9(–11)[3]	(1.7–)2–2.5(–3)[3]	(20–)25–30(–32.5) × (17–)20–25[3]	34–55(–72) × 14–20(–25)[3]
Alopecurus pratensis	(5–)6–8(–10)[2]	2–3[2]	(21–)24–30 × 21–24(–25)[2]	35–60 × 14–23[1]
Arrhenatherum elatius	(8–)9–10(–13)[3]	(1.5–)2–2.5(–2.7)[3]	25–28 × 23–26[1]	35–72.5 × 19–30[3]
Bromus erectus	(5–)7–8(–9)[6]	(1–)2–3[6]	23–27 × 20–24[6]	not present
Bromus hordeaceus, *Br. sterilis*	(8–)10–12(–14)[2]	(1.5–)2–2.5(–2.7)[2]	(23–)25–33(–37) × (18–)20–28(–30)[2]	30–95(–65) × 15–25(–27)[5]
Bromus inermis	(4–)5–6(–8)[2]	2–3(–3.5)[2]	(25–)30–37(–41) × (20–)26–32(–43)[2]	40–70 × 10–20[4]
Holcus lanatus, *H. mollis*	(9–)10–12(–13)[3]	1.5–2(–2.5)[3]	28–33 × 24–28[3]	38–65 × 19–26[3]
Lolium perenne	8–11[6]	1–1.5(–2)[6]	(20–)23–28(–30) × (19–)20–24[6]	42–56 × 18–23(–27)[6]
Secale cereale, *S. montanum*, *Aegilops ovata*[6]	(6–)7–9(–10)[2]	2.5–3[2]	22–31 × 22–27[2]	37–60(–80) × 14–22(–28)[2]
Trisetum flavescens	(6–)8–11(–14)[3]	(1.5–)1.7–2.5(–3)[3]	18–26 × 15–23[1]	35–55 × 18–28[3]
Triticum aestivum, *Aegilops squarrosa*[6]	(7–)8–9(–11)[3]	(1.7–)2–2.5(–3)[3]	(20–)25–30(–32.5) × (17–)20–25[3]	34–55(–72) × 14–20(–25)[3]

References: [1] Gäumann, 1959; [2] Urban, 1967; [3] Urban, 1969; [4] Ullrich, 1977; [5] Urban & Marková, 1977.

[6] Own measurements, spores mounted in glycerol after staining in lactophenol/ethanol (1:2 v/v) with 0.03% trypan blue. Experiments showed that size of the spores remained fairly constant for at least half a year after embedding in glycerol after this staining (Swertz, unpublished data).

new infraspecific taxa were recognized. Recognition of these taxa is mainly based on the identity and/or presence of the aecial host and characters of the teliospores, data which are often not easily obtained.

Studies on germling morphology of some rust fungi belonging to *P. recondita* have already shown that large qualitative differences exist in germling morphology of urediniospores collected from various host species (Pole Evans, 1907; Niks, 1986, 1987; Helfer, 1987). The present study aimed to further evaluate the value of germling morphology for the identification and classification of *P. recondita* s.l.

Germling morphology

All rusts belonging to *Puccinia recondita* s.l. have an oblong appressorium, 20–33(–40) × 9–12(–16) μm (Fig. 11B). Only the leaf rust from *Agropyron junceiforme* had a larger appressorium, viz. (20–)30–45(–48) × 10–15(–25) μm. The penetration peg of all *P. recondita* rusts studied was short.

Based on the shape and septation of the substomatal vesicle and the numbers and orientation of the primary infection hyphae, four morphological types of germling morphology were recognized, viz. the *Alopecurus*, *Holcus*, *Secale*, and *Triticum* types (Fig. 10). In the latter two several subtypes were recognized.

Description

Alopecurus type (Figs 10A, 11D)

The SSV is fusiform, (22–)26–35(–38) × 9–11(–13) μm, without a septum. Generally, the orientation of the SSV is longitudinal and horizontal, rarely somewhat vertical. At one of the ends of the SSV a longitudinally and horizontally orientated PIH, 4–5 μm wide, arises. Rarely a second infection hypha is formed at the other end of the SSV. The HMC are usually lobed, rarely unlobed, 10–17 × 4–6 μm. Frequently secondary hyphae, 4–6 μm wide, arise at the SSV side of the HMC septum and occasionally along the PIH.

The *Alopecurus* type of germling morphology is very similar to the *P. graminis* germlings observed (Section 4.2). It can be distinguished from germlings of the latter by the spheroid urediniospores with 6–8 germ pores in a scattered arrangement, the oblong shape of the appressorium, the wider SSV and PIH, and the lobed HMC.

Despite intensive search, only two urediniospore collections of *P. recondita* s.l. on *Alopecurus pratensis* were found, one in Bohemia, Czech Republic, and one in Gelderland, the Netherlands (1992; Appendix). This rust has been reported to alternate with *Ranunculus acris* (i.a. Wilson & Henderson, 1966; Table 13). In

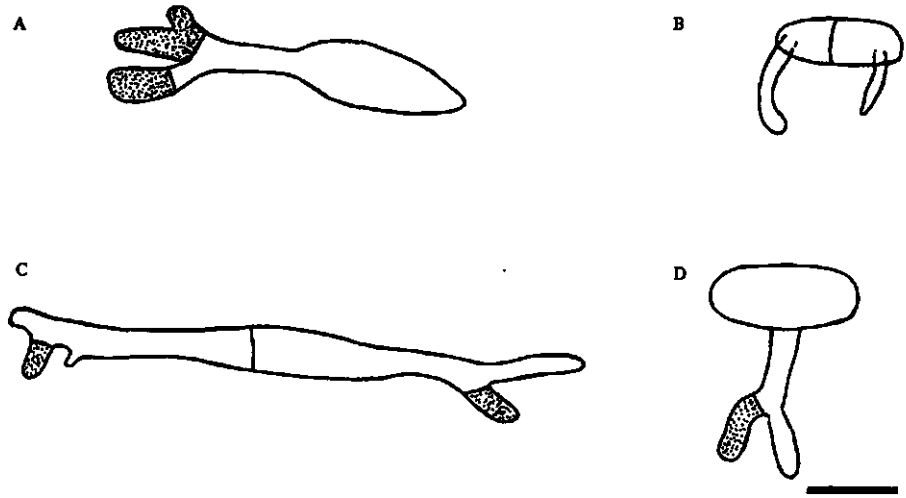


Figure 10. Drawings of types of germling morphology of *Puccinia recondita* s.l. Scale bar = 20 μm.
A. *Alopecurus* type from *A. pratensis* (C.S. 92.050). **B.** *Holcus* type from *H. lanatus* (C.S. 93.012). **C.** *Secale* type from *Aegilops ovata* (C.S. 92.Isr.23). **D.** *Triticum* type from *Aegilops squarrosa* (Flamingo).

1993, the same locality in Gelderland was visited again and aecia on *R. acris* were found. The aeciospores gave the same germling morphology as the urediniospore collection from 1992 and induced infection on *A. pratensis* in the greenhouse. The germling morphology of the urediniospores produced in the greenhouse was also similar to that of the other collections studied. Probably *P. recondita/Alopecurus* is strictly host-alternating with *Ranunculus acris*, which may explain the few observations of this rust fungus.

Holcus type (Figs 10B, 11C)

The SSV is oblong to narrowly oblong, 21–30 × 9–13(–15) μm, with a central septum. The orientation of the SSV is longitudinal and horizontal. The ends of the SSV are rounded. At the under side of both ends of the SSV a PIH, 3–4(–5) μm wide, arises. PIH usually grow vertically, rarely they are deflected towards the nearest vein. The HMC are unlobed, (10–)15–20 × 4–5(–6) μm. Secondary hyphae are rarely formed.

The *Holcus* type is characterized by an oblong SSV with a central septum, rounded ends, and two vertically orientated PIH. It resembles the germling morphology of

isolate 28 of *P. hordei* (Section 4.5) of which it is distinguished by the narrow PIH, the unlobed SSV, and the rare formation of secondary hyphae. It was observed in 15 and 2 collections from *Holcus lanatus* and *H. mollis*, respectively, obtained from the Netherlands, Austria, Belgium, France, Great Britain, and Madeira (Appendix). No differences were found between collections.

Secale type

The *Secale* type is characterized by an occasionally septate, longitudinally and horizontally orientated fusiform SSV. At both ends the SSV tapers to form a, generally, horizontally and longitudinally orientated PIH.

Five subtypes were recognized: the *Secale*, *Arrhenatherum*, *Bromus inermis*, *Lolium*, and *Trisetum* subtypes.

Secale subtype (Figs 10C, 11E)

The SSV is (20–)25–35(–40) × (9–)10–13 μm and mostly septate. The septum is either central, eccentrical or absent. The PIH are (4–)5–6(–7) μm wide and straight. The HMC are usually lobed, (7–)10–15(–17) × (4–)5–6 μm. Some HMC can be unlobed. Occasionally secondary hyphae are formed at the SSV side of the HMC septum or along the PIH.

The *Secale* subtype is characterized by a fusiform, mostly septate SSV, straight PIH and usually lobed HMC. It is distinguished from the *Arrhenatherum* subtype by its thicker PIH. It was observed in 19 collections from *Aegilops ovata* (2), *A. longissima* (1), *Secale cereale* (14), *S. montanum* (1) and *Triticale* (1), and in three aeciospore collections from *Anchusa* and *Echium* (Appendix). Most collections originated from Israel. No differences were found between germlings obtained from aecial or uredinial collections, irrespective of the host species.

Arrhenatherum subtype (Figs 11 F, G)

The SSV is 20–30 × (7–)8–10(–12) μm and mostly septate. The septum is either central, eccentrical or absent. The PIH are (2–)3–4(–5) μm wide and straight. The HMC are usually lobed, 10–15 × 4–5 μm. Some HMC are unlobed. Secondary hyphae arising at the SSV side of the HMC septum are occasionally formed.

Note: In many collections one or more germlings were observed which had formed additional septa and secondary infection hyphae. The PIH of these germlings were clearly thicker (5–7 μm) than of germlings without additional septa and they often had formed a haustorium in a barley mesophyll cell (Fig. 11G). As these germlings were clearly further developed than regular primary infection structures of the rust from *A. elatius* they are regarded as aberrant structures and not discussed.

The *Arrhenatherum* subtype is very similar to the *Secale* subtype, but can be distinguished from the latter by the narrower PIH. It was observed in 25 collections from *Arrhenatherum elatius* obtained from the Netherlands, Belgium, the Czech Republic, France, and Great Britain (Appendix).

Bromus inermis subtype (Fig. 11H)

The SSV is (35–)40–60 × (8–)9–11(–16) μm with a more or less central septum. The PIH are (2–)3–4(–5) μm wide and clearly smaller than the SSV. The HMC are usually unlobed, 10–12 × 4–5 μm. Occasionally the HMC are lobed. Secondary hyphae arising at the SSV side of the HMC septum are occasionally formed.

The *Bromus inermis* subtype is distinguished from the other subtypes of the *Secale* type by the long SSV with a more or less central septum. It was observed in three collections of *Bromus inermis* collected in the Czech Republic (Appendix).

Lolium subtype (Figs 11I, 12B)

The SSV is (25–)30–40 × 7–9(–10) μm, with a central septum. Occasionally an additional septum is present in the SSV. The SSV gradually tapers at both ends to form a PIH. They are (3–)4–5 μm wide. Mostly the PIH are vertically orientated, sometimes they are horizontal and slightly deflected towards the nearest vein. Occasionally secondary infection hyphae arise near the septa in the SSV. The HMC are unlobed, 10–16 × 3–4 μm. Secondary hyphae arising at the SSV side of the HMC septum are frequently formed.

The *Lolium* subtype is distinguished from the other subtypes by its central septum and, generally, vertically orientated PIH. It can be distinguished from the *Bromus inermis* subtype by shorter SSV, and from the *Trisetum* subtype by the presence of SIH and SH and the occasional formation of additional septa. It closely resembles that of *P. hordei* (Section 4.5), especially when secondary infection hyphae are formed. However, the *Lolium* subtype has smaller SSV and the PIH mostly grow vertically, whereas PIH of *P. hordei* generally grow horizontally. It was observed in 14 collections from *Lolium italicum* (1), *L. multiflorum* (2) and *L. perenne* (11) obtained from the Netherlands, Belgium, France, Germany, Great Britain, and Switzerland (Appendix).

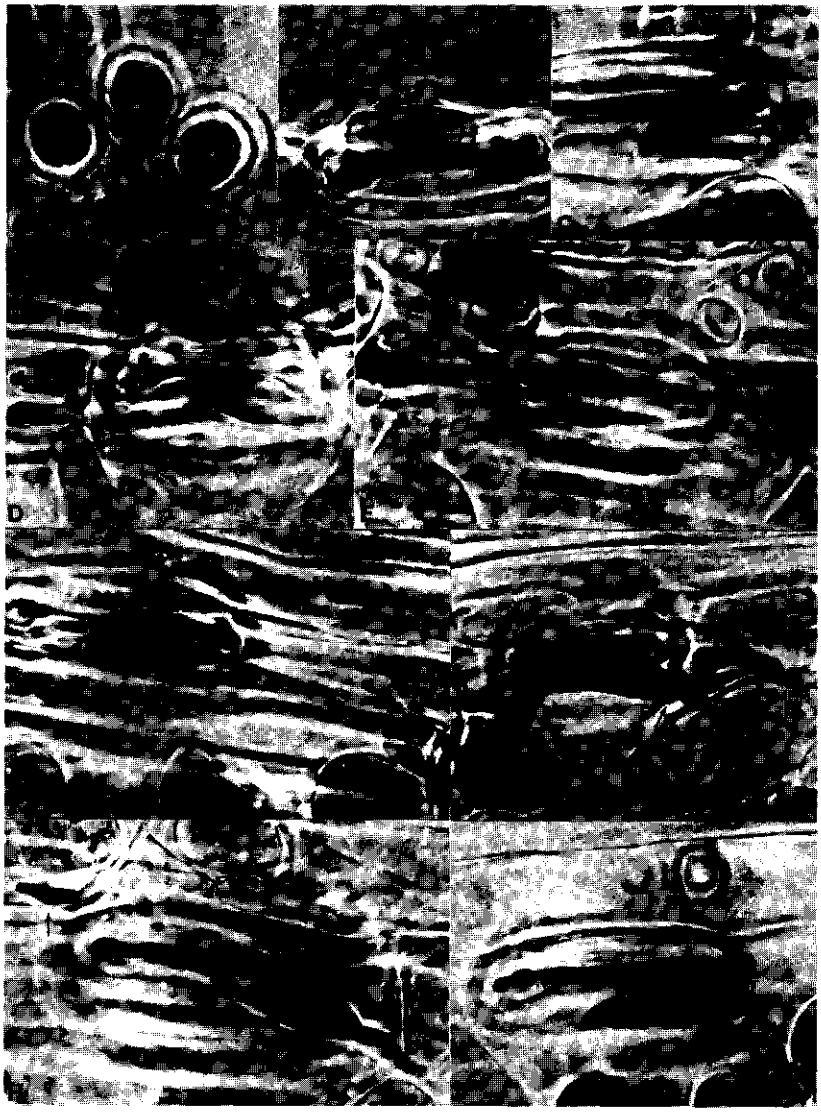


Figure 11. *Puccinia recondita*, phase contrast micrographs, scale bar = 20 μm.
A. Urediniospores of *P. triticina* from *Triticum aestivum* (Flamingo), **B–H.** Germlings of urediniospores: **B.** *P. triticina* from *Triticum aestivum* (Flamingo), appressorium. **C.** *P. holcina* from *Holcus lanatus* (J.M. IC), SSV with rounded ends and central septum. **D.** *P. perplexans* from *Alopecurus pratensis* (C.S. 92.050). **E.** *P. recondita* s. str. from *Secale montanum* (C.S. 92.Isr10). **F–G.** *P. arrhenathericola* from *Arrhenatherum elatius*: **F.** C.S. 90.060. **G.** C.S. 93.030, SSV with additional septa (arrows) and infection hypha. Note haustorium (H). **H.** *P. bromina* var. *paucipora* from *Bromus inermis* (J.M. IID). Note HMC (arrow). **I.** *P. loliina* from *Lolium multiflorum* (C.S. 91.001).

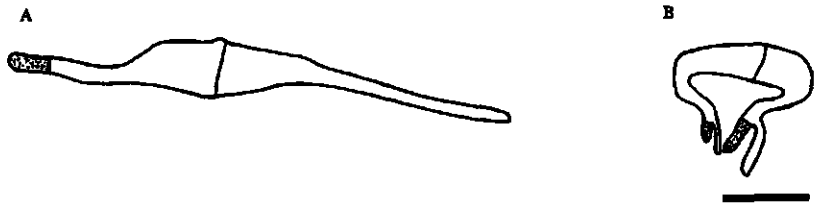


Figure 12. *Puccinia recondita*, drawings, scale bar = 20 μm.
Germlings of urediniospores: **A.** *P. triseti* from *Trisetum flavescens* (C.S. 90.168). **E.** *P. loliina* from *Lolium multiflorum* (C.S. 91.001).

Trisetum subtype (Fig. 12A)

The SSV is (20–)25–28(–30) × 8–10 μm, with a central septum. The PIH are 3–4.5 μm wide, clearly thinner than the SSV. Rarely the PIH are slightly deflected towards the nearest vein, but grow horizontally. The HMC are unlobed, 10–12(–15) × 3–4 μm. Secondary hyphae are rarely formed.

The *Trisetum* subtype is characterized by a central septum, the rare formation of secondary hyphae and the pronounced delimitation between SSV and PIH. It can be distinguished from the *Bromus inermis* subtype by its shorter SSV. It was observed in 13 collections from *Trisetum flavescens* obtained from the Netherlands, the Czech Republic, France, and Switzerland (Appendix).

Triticum type

The *Triticum* type is characterized by a (generally) aseptate, longitudinally and horizontally orientated ellipsoid SSV with mostly one, rarely two, primary infection hyphae with a variable orientation.

Six subtypes were recognized: the *Triticum*, *Agropyron junceiforme*, *Agropyron repens*, *Bromus erectus*, *Bromus hordeaceus*, and *Bromus sterilis* subtypes.

Triticum subtype (Figs 10D, 13A)

The SSV is ellipsoid (to narrowly ellipsoid), (21–)24–34(–38) × 11–15(–17) μm, with acute-rounded ends. One PIH, (4–)5–6 μm wide, arises at the under side of the SSV and is vertically orientated. Rarely the PIH arises at one end of the SSV and grows horizontally and longitudinally. The HMC are unlobed, 9–15 × 5–6 μm. Secondary hyphae are occasionally formed.

Figure 13. *Puccinia recondita*, phase contrast micrographs, scale bar = 20 μm.
Germlings of urediniospores: **A.** *P. triticina* from *Triticum aestivum* (Felix). **B.** *P. agropyri-juncei* from *Agropyron junceiforme* (C.S. 90.200). **C–D.** *P. agropyrina* from *Agropyron repens* (C.S. 93.009): **C.** SSV. **D.** SSV with septum and second infection hypha. **E.** *P. bromina* s.l. from *Bromus erectus* (C.S. 92.025). **F–G.** *P. bromina var. bromina*: **F.** from *Bromus hordeaceus* (C.S. 91.019). **G.** from *Bromus sterilis* (C.S. 91.015).

The *Triticum* subtype is characterized by an ellipsoid SSV with acute-rounded ends and a vertically orientated PIH. It was observed in 43 collections from *Triticum* spp. obtained from numerous regions of the world, in one collection from *Aegilops squarrosa* and in 4 aeciospore collections from *Thalictrum* spp. in Israel (Appendix).

Agropyron junceiforme subtype (Fig. 13B)

The SSV is narrowly ellipsoid, (30–)45–57(–65) × (7–)10–15 μm, with acute-rounded ends. Rarely a central septum is formed. Generally one, rarely two, PIH, (4–)5–6(–7) μm wide, arise at the under side of the SSV. Orientation of the PIH is mostly vertical, but occasionally the PIH arises at one of the ends of the SSV and grows horizontally and longitudinally. The HMC are unlobed, (8–)10–13(–15) × (4–)5–6(–7) μm. Secondary hyphae arising at the SSV side of the HMC septum are frequently formed.

The *Agropyron junceiforme* subtype is easily distinguished from the other subtypes by its long SSV. The shape of the SSV is similar to the *A. repens* subtype. It was observed in 3 collections from *Agropyron junceiforme* obtained from the Netherlands, Germany, and Great Britain (Appendix).

Agropyron repens subtype (Figs 13 C, D)

The SSV is (ellipsoid to) narrowly ellipsoid, (25–)29–42 × 9–12(–14) μm, with short-acuminate, occasionally rounded ends. An eccentric septum may be present (Fig. 13D). Generally one PIH, (2–)4–5(–6) μm wide, arises at the under side of the SSV. The PIH mostly grows vertically, sometimes it arises at one of the ends of the SSV and grows longitudinally and horizontally. Occasionally a second PIH arises which grows either horizontally or vertically. The HMC are usually unlobed, 12–15 × 4–5 μm. Rarely the HMC are lobed. Secondary hyphae are occasionally formed.

The *Agropyron repens* subtype resembles the *Triticum* and *Bromus erectus* subtypes. It can be distinguished from the *Triticum* subtype by its generally narrowly ellipsoid SSV with short-acuminate ends. The *Bromus erectus* subtype has long projections at the SSV-ends (Fig. 13E). It was observed in 22 collections from *Agropyron repens*, obtained from the Netherlands, the Czech Republic, France, Germany, and Great Britain, and in one collection from *Hordeum jubatum* found in Wageningen, the Netherlands (Appendix). No differences were found between these collections.

Bromus erectus subtype (Fig. 13E)

The SSV is (ellipsoid to) narrowly ellipsoid, 32–40(–43) × 10–13 μm, with long papillate ends; the projections are 5–8 μm long. One vertically orientated PIH, 3–4(–5) μm wide, arises at the under side of the SSV. Occasionally a second PIH is formed. HMC are unlobed and appr. 10 × 4 μm, but rarely observed. Secondary hyphae are rarely formed.

Though only two collections could be studied from *Bromus erectus* collected in Switzerland (Appendix), the *Bromus erectus* subtype can be distinguished from the other subtypes by its long projections at the SSV-ends and the vertically orientated PIH.

Bromus hordeaceus subtype (Fig. 13F)

The SSV is narrowly ellipsoid (to ellipsoid), (22–)28–33(–37) × (10–)12–14(–16) μm, with acute–rounded ends. One PIH, 3–4(–5) μm wide, arises along or at the under side of the SSV and generally grows horizontally and transversely, either towards the nearest vein or away from it. Sometimes the PIH grow more deeply into the leaf. The PIH tend to be somewhat constricted at the base. Rarely a short secondary infection hyphae is formed. The HMC are unlobed, 12–20(–23) × 4–5(–6) μm. Secondary hyphae are rarely formed.

The *Bromus hordeaceus* subtype is characterized by a narrowly ellipsoid SSV with acute–rounded ends and a horizontally and transversely orientated PIH. It closely resembles the *Bromus sterilis* subtype which has a more oblong SSV with rounded ends and a vertically orientated PIH. It was observed in 10 collections from *Bromus hordeaceus* obtained from the Netherlands and in one collection from France (Appendix).

Bromus sterilis subtype (Fig. 13G)

The SSV is ellipsoid–oblong to oblong, (20–)25–29(–32) × (9–)11–13(–14) μm, with rounded ends. One PIH, (3–)4–5 μm wide, arises at the under side of the SSV and generally grows vertically or is deflected towards the nearest vein. Rarely the PIH grows horizontally and longitudinally. The HMC are unlobed, 12–20 × 4–5 μm. Secondary hyphae are rarely formed.

The *Bromus sterilis* subtype is characterized by an oblong SSV with rounded ends and a vertically orientated PIH. It was observed in 13 collections from *Bromus sterilis*, obtained from the Netherlands, the Czech Republic, Great Britain, and Switzerland (Appendix).

Discussion

The germling morphology of the urediniospores of rust fungi belonging to *Puccinia recondita sensu lato* was very variable. Four different types and 11 subtypes could be distinguished. The descriptions reported here for rusts collected from *Agropyron repens*, *Arrhenatherum elatius*, *Secale cereale* and *Triticum aestivum* wholly agree with previous descriptions (Pole Evans, 1907; Allen, 1926; Niks, 1986, 1987; Helfer, 1987).

Generally, rust fungi collected from different plant genera (e.g. collections from *Alopecurus*, *Holcus*, *Lolium*, *Trisetum*, *Triticum*) differed in germling morphology, though occasionally they were similar. Collections of *Triticum* spp. and *Aegilops squarrosa*, reported to have a Ranunculaceous aecial host plant (Oliveira & Samborski, 1966), had a *Triticum* subtype germling morphology (Figs 10D, 13A) and collections of *Secale* spp. and *Ae. ovata*, reported to have a Boraginaceous aecial host plant (Oliveira & Samborski, 1966), had a *Secale* subtype germling morphology (Figs 10C, 11E). The identical germling morphology of collections with aecia produced on the same host family seems to agree with the suggestion of Anikster & Wahl (1979) that leaf rust has apparently followed two lines of evolution, one involving alternate hosts of the Ranunculaceae and the other including hosts of the Boraginaceae.

Identical germling morphology between collections from different uredinial host species was also observed for urediniospores collected from *Agropyron repens* and *Hordeum jubatum*. Urediniospores collected from *Agropyron repens*, isolates C.S. 92.009, C.S. 92.099, C.S. 93.009, C.S. 93.021, were infectious to *Hordeum jubatum* and urediniospores collected from *H. jubatum* could infect several *A. repens* genotypes (Niks, unpublished data).

It has also been reported that leaf rust collections from *Triticum aestivum* could infect *Aegilops squarrosa* (Valkoun *et al.*, 1985; own observations with isolate Flamingo). The fact that an isolate could infect members of different plant species and different genera (*P. recondita/Triticum* on *T. aestivum* and *Ae. squarrosa*; *P. recondita/A. repens* on *A. repens* and *H. jubatum*) and that the urediniospores produced on both hosts had identical germling morphology suggests that the germling morphology is specific of a rust taxon and not influenced by the host plant on which the urediniospores were produced.

Moreover, rust fungi collected from species included in different sections of a genus (e.g. *Bromus hordeaceus*, section *Bromus* and *B. sterilis*, section *Genea* (Smith, 1970)) differed in germling morphology, while rusts collected from plant species belonging to the same section could have a similar germling morphology. A leaf rust collected from *B. secalinus* (section *Bromus*) observed by Marshall Ward (1903) had a germling morphology identical to the *B. hordeaceus* subtype described here.

However, collections from the same section could also differ in germling morphology. Collections from *Bromus inermis* and *B. erectus* (Figs 11H, 13E), both included in *Bromus* section *Zerna* (Smith, 1970) and from *Agropyron junceiforme* and *A. repens*, included in *Agropyron* section *Elytrigia* (Hubbard, 1985) differed in shape and size of the SSV, respectively (Figs 13 B, C, D). The rusts from the two species of *Bromus* also differed somewhat in morphological characters of the urediniospores (Table 15), whereas the morphological differences between the rusts from the two species of *Agropyron* were less obvious (Table 15). These observations could indicate that the rust fungi from the two species of *Bromus* and *Agropyron* are distinct populations that may not cross-infect the other species of the same genus and

that they may be genetically distinct as well. Since only a few observations have been made, no reliable conclusions can be drawn. Observations of additional collections will be very valuable to get more insight in the various types of germling morphology observed in urediniospore collections obtained from the same and different sections of *Bromus* and *Agropyron*. Possibly the germling morphology may also be valuable to get more insight in the taxonomy of the grass hosts (Savile, 1979).

Identification of rust fungi belonging to the *Puccinia recondita* complex is very difficult, because the differences in spore features are small and the size of dimensions show a large overlap (Table 15). However, the germling morphology of the urediniospores showed remarkable differences between taxa and seems to be very valuable for the recognition of taxa belonging to *P. recondita* s.l. The suitability of germling morphology in distinguishing leaf rust taxa can be illustrated by the leaf rust fungi infecting *Agropyron repens*, *Triticum aestivum* and *Secale cereale*. On the basis of characters of urediniospores and teliospores, these rust fungi are almost identical (Table 15), but they are clearly distinct on the basis of germling morphology (Figs 13 C, A, 11E, respectively). Thus, an infection of wheat by a leaf rust from *A. repens* could easily be discovered by studying the germling morphology, whereas a distinction based on minor differences in the colour of the spore wall as used by Urban & Marková (1986) is hardly possible and subjective.

Minor differences in spore colour were also used by Cummins (1971) to distinguish *P. hordei* and *P. recondita*. Using Cummins' (1971) key, it is extremely difficult to place collections from i.a. *Trisetum*, *Arrhenatherum*, *Lolium* and *Bromus* in either *P. recondita* or *P. hordei* (Table 14). Since collections of each genus (species) studied had a diagnostic germling morphology different from that of *P. hordei* (Section 4.5), it is unlikely that any of these grass genera was infected with rust fungi belonging to *P. hordei* s.l. The rust fungi collected from *Lolium* had a germling morphology similar to that of *P. hordei*, but could be distinguished from this rust by the smaller PIH and their vertical orientation. Because of these differences and the observation that urediniospores from *Lolium perenne* could not infect *Hordeum* species (Wilkins, 1973), this rust fungus is not included in *P. hordei*.

The large differences in germling morphology of urediniospores collected from different host genera, the specialization of most *P. recondita* rusts in both aecial and uredinial stages (Table 13), and the small, but consistent, morphological differences in characters of uredinia and telia (Table 15) support a narrow species concept in *P. recondita*.

Rust taxa differing in characteristics of uredinia, telia, germling morphology and host range are considered as separate species, following Gäumann (1959) and Urban (1969). In the present case these include: *Puccinia arrhenathericola* Fischer on *Arrhenatherum elatius*, *Puccinia agropyri-juncei* Klebahn on *Agropyron junceiforme*, *P. holcina* Erikss. on *Holcus* spp., *P. loliina* Sydow on *Lolium* spp., *P. perplexans* Plowr. on *Alopecurus pratensis*, and *Puccinia triseti* Erikss. on *Trisetum flavescens*.

Rust taxa occurring on plants of *Bromus*, but restricted to a distinct species and differing in characteristics of uredinia and telia and/or germling morphology are considered infraspecific taxa, according to the concept of Urban (1969), as long as no additional collections are studied. *Puccinia bromina* Erikss. var. *bromina* includes the rust fungi collected from *B. hordeaceus* and *B. sterilis*, *P. bromina* var. *paucipora* Urban the rusts from *Bromus inermis*, and the rust collection from *B. erectus* was subsumed under *P. bromina* Erikss. s.l. since this collection could not be reliably identified due to the aberrant number of germ pores compared with the other two varieties mentioned (Table 15).

Rust fungi collected from different plant species, having similar characteristics of uredinia, telia and germling morphology, sharing the same aecial host family, and of which the urediniospores can infect each other's host species in infection experiments, are considered to be conspecific. Thus *P. recondita* rusts collected from (i) *Triticum* and *Ae. squarrosa* and (ii) *A. repens* and *H. jubatum* are included in *P. triticina* Erikss. and *P. agropyrina* Erikss., respectively. Rusts sharing the characteristics mentioned but which are probably not able to infect each other's host species, viz. *Secale* and *Ae. ovata*, are considered *formae speciales* of the same species, viz. *P. recondita* Rob. ex Desm.

In summary, this study showed that germling morphology is very useful for the identification and classification of rusts belonging to *P. recondita* s.l. It is suggested to recognize the following taxa: *P. agropyrina* Erikss., *P. agropyri-juncei* Klebahn, *P. arrhenathericola* Fischer, *P. bromina* Erikss. var. *bromina*, *P. bromina* var. *paucipora* Urban, *P. holcina* Erikss., *P. loliina* Sydow, *P. perplexans* Plowr., *P. recondita* Rob. ex Desm., *P. triseti* Erikss., and *P. triticina* Erikss. (Eriksson, 1899; Gäumann, 1959; Urban, 1969).

4.5 PUCCINIA HORDEI Otth and UROMYCES SPECIES on barley

Introduction

Worldwide, uredinia and telia of *Puccinia hordei* can be found on cultivated barley (*Hordeum vulgare*) and wild barleys (*H. spontaneum*, *H. bulbosum* and *H. murinum*), whereas in the Mediterranean area and in the USA several *Uromyces* spp. have also been reported from cultivated and wild barleys. Pycnia and aecia of *P. hordei* and *Uromyces* species from barleys are formed on several genera of the Liliaceae (Anikster & Wahl, 1966, 1979; Cummins, 1971; Clifford, 1985).

Barley leaf rust fungi (*P. hordei* and *Uromyces* spp.) do not cause severe crop losses on a widespread and regular basis, but *P. hordei* is locally important, particularly in temperate regions. The optimum temperature for germination and colonization of *P. hordei* is 10–20 °C (Clifford, 1985).

In the uredinial stage, barley leaf rusts have brownish, aparaphysate uredinia and urediniospores with 9–11 distinct germ pores with a scattered arrangement (Fig. 14A). No distinction can be made between taxa in this stage.

In the telial stage, *P. hordei* has long-covered, paraphysate telia. The teliospores are two-celled as is characteristic for the genus *Puccinia*, but one-celled teliospores characteristic for the genus *Uromyces* are usually also found. *Uromyces* spp. generally have either long-covered or early-ruptured, aparaphysate telia and exclusively one-celled teliospores (Anikster & Wahl, 1966, 1979).

All barley leaf rust fungi show some host specialization, either in the telial or in both the aecial and telial stages. Within *P. hordei* Anikster (1982) observed host specialization on the telial host only. In artificial inoculation tests basidiospores of every infected species of *Hordeum* — including *H. murinum* — were compatible with plants of the Liliaceae, viz. *Ornithogalum* spp., *Dipcadi erythraeum* and *Leopoldia eburnea*. These findings led Anikster & Wahl (1979) to incorporating the leaf rust of *H. murinum* into *P. hordei*. In the uredinial stage, however, spores from *H. bulbosum* and *H. murinum* were strictly confined to the original host species, while spores from *H. vulgare* and *H. spontaneum* were compatible in reciprocal inoculations. But these latter two host species are genetically closely related as they can be crossed easily. Between cultures derived from the various main host species, small differences were found in percentage one-celled teliospores (Table 16; Anikster & Wahl, 1979). The differences appeared to be host-specific: after inoculation of urediniospores from *H. spontaneum* on *H. vulgare*, the newly formed telia contained the same low percentage of one-celled teliospores as on *H. spontaneum* (T. Eilam, pers. comm., 1992).

Urban (1969) recognized the rust from *Hordeum murinum* as a separate species, *P. hordei-murini* Buchwald, because of the host specialization on the telial host and the lower percentage of one-celled teliospores in the rust from *H. murinum* as compared with the rust from *H. vulgare*.

Table 16. Percentage one-celled teliospores of *Puccinia hordei* collections from *Hordeum vulgare*, *H. spontaneum*, *H. bulbosum* and *H. murinum* (after Eilam, pers. comm., 1992).

host	% one-celled teliospores
H. spontaneum	3–5
H. vulgare	90
H. bulbosum	10–20
H. murinum	< 20

On species of *Hordeum* Anikster & Wahl (1966) described four species of *Uromyces* with host specialization on the aecial and telial hosts, viz. *U. viennot-bourginii* Wahl & Anikster, *U. reichertii* Anikster & Wahl, *U. christensenii* Anikster & Wahl and *U. hordeastri* Guyot. Pycnia and aecia develop on three genera of the Liliaceae, viz. *Bellevallia*, *Muscari* and *Scilla*. Generally, in the aecial stage, each *Uromyces* species is strictly specialized on one species (Table 17). In artificial inoculation tests, however, all species of *Uromyces* on barley and *P. hordei* were infectious to *Leopoldia eburnea* (Anikster & Wahl, 1966), and formed uredinia and telia on *H. spontaneum* or *H. bulbosum* (Table 17). Taxa were considered as separate species because of the strict host specialization in greenhouse experiments and the small differences in morphological characters of telia, teliospores and in the number of nuclei in the basidiospores. However, considerable overlap occurs in characters of teliospores (Table 17).

Uromyces viennot-bourginii and *U. reichertii* can be recognized by relatively long-covered telia; teliospores of *U. reichertii* are distinctly longer than those of *U. viennot-bourginii*. *Uromyces reichertii* forms four monokaryotic basidiospores, whereas *U. viennot-bourginii* forms two dikaryotic basidiospores. In single basidia of some isolates, however, one dikaryotic and two monokaryotic basidiospores can be formed (Anikster & Wahl, 1979). Moreover, these two rust species differ in aecial and telial host species (Table 17).

Uromyces christensenii and *U. hordeastri* have early-rupturing telia and are both infectious on *H. bulbosum*. *Uromyces christensenii* differs from *U. hordeastri* by the formation of two dikaryotic basidiospores, whereas *U. hordeastri* forms four monokaryotic basidiospores. Anikster & Wahl (1966) reported that teliospores from *U. christensenii* are smaller than those from *U. hordeastri*. Teliospores from *U. christensenii* and *U. hordeastri*, however, do not differ in size when *Muscari parviflorum* is the alternate host. Only teliospores of *U. hordeastri* alternating with *Bellevallia flexuosa* are somewhat larger than those from *U. christensenii* (Table 17).

Barley leaf rusts are regarded as closely related as suggested by the identical morphological characters of uredinia and urediniospores, the presence of one-celled teliospores in both genera and the occurrence on the same main host genus and on the same alternate host family (Orton, 1912, 1927). Experiments have also shown that *Uromyces* and *Puccinia* barley leaf rusts have a common host in *Leopoldia eburnea* (Anikster & Wahl, 1979).

Puccinia hordei was placed in the genus *Puccinia* only because it has a variable proportion of two-celled teliospores. However, when only urediniospores are present a reliable identification to genus and species is not possible. And even when teliospores are present it is difficult to distinguish the several *Uromyces* spp. as recognized by Anikster & Wahl (1966). In order to get more insight in the identification and classification of barley leaf rusts the germling morphology of this group of taxa was investigated.

Table 17. Aecial and telial hosts of *Uromyces* rusts and *Puccinia hordei* parasitic on barleys (*Hordeum* spp.), and some distinctive morphological characters of telia and teliospores.

species	host		telia	teliospores	
	aecial	telial		size (μm)	wall thickness (μm)
U. viennot-bourginii	*Bellevallia eigii*	*H. spontaneum*	long-covered	19.5–23 × 14.5–17	1.5–2
U. reichertii	*Scilla hyacinthoides*	*H. bulbosum*	long-covered	29–38 × 27–34	2.5–3.5
U. hordeastri	*Bellevallia flexuosa*	*H. bulbosum*	early-ruptured	19.5–22 × 14.5–19.5	1.5–2
U. hordeastri	*Muscari parviflorum*[a]	*H. bulbosum*	early-ruptured	16–21 × 13–17.5	1.5–2
U. christensenii	*Muscari parviflorum*	*H. bulbosum*	early-ruptured	16–22.5 × 12.5–17.5	1.5
P. hordei	*Ornithogalum* spp.	*H. spontaneum, H. vulgare, H. murinum, H. bulbosum*	long-covered	40–60 × 19–25(–29)[b]	1–2

Note: data are taken from Anikster & Wahl, 1966; Urban, 1969; Savile, 1984.
[a] Anikster & Wahl (1979) mention *Scilla autumnalis* as alternate host.
[b] One-celled teliospores measure 30–43(–46) × (16–)18–25(–28) μm.

Germling morphology

Description

Puccinia hordei germlings have an oblong appressorium, 25–35 × 10–15 μm (Fig. 14B). The infection peg is short. The SSV is fusiform, (30–)40–55(–60) × (7–)9–10(–12) μm, with a central septum. It is horizontally and longitudinally orientated. Frequently one or two additional septa are formed at the ends of the SSV or at the transition to the primary infection hypha (Figs 14D, 15A). At both ends the SSV tapers to form a PIH, (3–)4–5(–6) μm wide, which usually grow horizontally and longitudinally (Figs 14D, 15A), but sometimes they are deflected towards the nearest vein and grow more deeply into the leaf (Figs 14 E, F). Frequently secondary infection hyphae arise near the additional septa in the SSV. The HMC are usually unlobed with an elongated tip (Fig. 14C), 11–20 × 4–5(–6) μm. Occasionally an HMC is lobed (Fig. 15A). Secondary hyphae arising from the SSV side of the HMC septum are often present.

No differences in germling morphology were found between the 38 urediniospore collections studied of *P. hordei* collected from *Hordeum vulgare*, *H. spontaneum* and *H. murinum*, the aecial collection from *Ornithogalum*, the three uredinial collections of *U. hordeastri*, the two urediniospore collections of *U. viennot-bourginii*, the urediniospore collection of *U. christensenii* and the aecial and uredinial collections of *U. reichertii* (Appendix). Thus, it was not possible to differentiate between collections from *P. hordei* and *Uromyces* spp. occurring on barleys.

Note: In contrast one Moroccan collection (isolate 28, also described by Niks *et al.* (1989)) was observed with an aberrant germling orientation (Figs 14G, 15C). This isolate also had an unusual virulence spectrum, viz. to Pa3 and Pa7, and a relatively high aggressiveness to barley cv. Vada, known for its high level of partial resistance to *P. hordei* (Niks *et al.*, 1989). The morphology of SSV, PIH, HMC, and the presence of septa and SIH was similar to the 'regular' type of *P. hordei*. The SSV, however, was sharply bent downwards; the maximum length of the SSV before the downwards orientation was (20–)25–32(–36) μm.

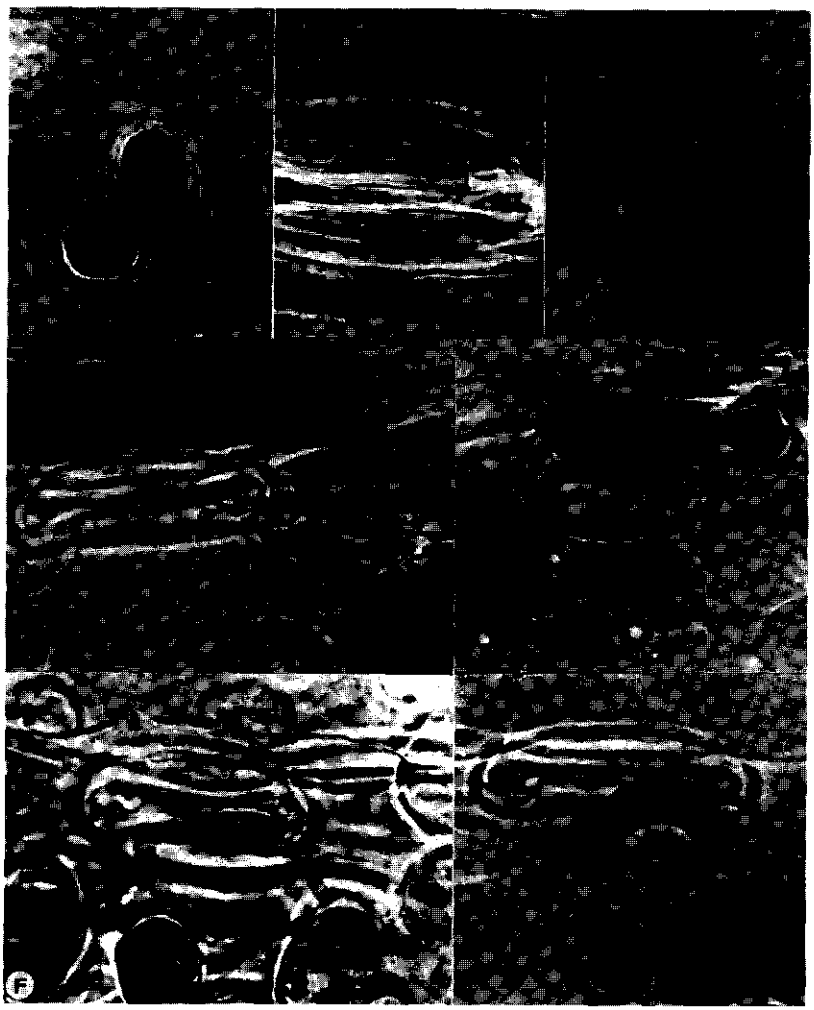


Figure 14. *Puccinia hordei*, phase contrast micrographs, scale bar = 20 μm.
A. Urediniospores collected from *Hordeum vulgare* (*P. hordei* isolate 1-2-1). **B–G.** Germlings of urediniospores collected from: **B.** *Hordeum vulgare* (*P. hordei* isolate 1-2-1), appressorium. **C.** *Hordeum vulgare* (*Uromyces viennot-bourginii* isolate Uvb-IVP), HMC. **D.** *Hordeum bulbosum* (*Uromyces hordeastri* C.S. 92.Isr41), SSV with central septum and two additinal septa (arrows) and SIH. **E.** *Hordeum vulgare* (*P. hordei* isolate 1-2-1), SSV with two vertical PIH. **F.** *Hordeum murinum* (C.S. 90.205), SSV with two PIH. Note resemblance with Fig. 14E. **G.** *Hordeum vulgare* (*P. hordei* isolate 28), SSV with two vertical PIH. Note sharply bent SSV.

Discussion

The fusiform substomatal vesicle with two primary infection hyphae, the formation of at least one central septum and occasionally a second or third septum in the SSV and the presence of secondary infection hyphae arising from the SSV discriminate barley leaf rusts from the other grass rust fungi, except *P. recondita Secale* type, subtype from *Lolium* spp. (*P. loliina*, Section 4.4). Orientation of the PIH is longitudinal and horizontal, whereas *P. recondita/Lolium* spp. has vertically-orientated PIH. Occasionally the PIH of *P. hordei* germlings may be deflected towards the nearest vein and grow more deeply into the leaf. Then the germlings of *P. recondita/Lolium* spp. may be recognized by the smaller SSV. Horizontal and vertical orientation of PIH of *P. hordei* was observed in the same slides. The germlings with vertically orientated PIH can be distinguished from isolate 28 because the SSV is not sharply bent.

Niks *et al.* (1989) discussed the taxonomic position of isolate 28, suggesting that the ineffectiveness of the Pa genes in barley to isolate 28 may be taken as evidence that isolate 28 is a distinct infraspecific taxon of *P. hordei*. Experiments conducted in Israel, however, revealed that it is possible to cross isolate 28 with 'regular' *P. hordei* isolates (Anikster *et al.*, 1992), which indicates that both isolates belong to the same species. Germling morphology of the urediniospores of these crosses was found to be very variable (own observations). 'Regular' types, isolate 28 types and intermediate forms (Fig. 15B) were found. The intermediate forms were very similar to the *P. hordei* germlings with PIH orientated towards the vein, which grow also more deeply into the leaf (Figs 14 E, F). It may thus be concluded that isolate 28 belongs to *P. hordei*. Possibly it is a locally occurring mutant (Burdon, 1992).

The host specialization on the telial host and the small differences in percentage of one-celled teliospores reported within *Puccinia* barley leaf rusts (Table 17; Anikster & Wahl, 1966, 1979) were not reflected in morphological differences in germling morphology. Therefore, *P. hordei-murini* is subsumed under *P. hordei*. Furthermore, it was not possible to differentiate between barley leaf rusts of the genus *Puccinia* and *Uromyces* nor between the formerly distinguished *Uromyces* species. In addition to the morphological similarities of uredinia and urediniospores, the presence of both one-celled and two-celled teliospores in the genus *Puccinia* and the similarities in alternate and main host families, germling morphology also supports the view that *P. hordei* and *Uromyces* species occurring on barleys are closely related taxa. Uniting all barley leaf rusts in one genus, viz. *Puccinia*, would better reflect this relationship. In any case, it is suggested to unify *Uromyces christensenii* with the *U. hordeastri* variety which has *Muscari parviflorum* as alternate host, because these taxa have the same aecial and telial hosts, and are similar in germling morpholgy, characters of uredinia, telia, and spores (Table 17).

Germling morphology of urediniospores has shown that all barley leaf rusts are closely related. The identical germling morphology and the similarities in characters of uredinia and urediniospores of barley leaf rust fungi occurring on *H. vulgare*, *H. spontaneum*, *H. bulbosum* and *H. murinum* make identification of taxa very difficult in absence of teliospores or without knowledge on the alternate host.

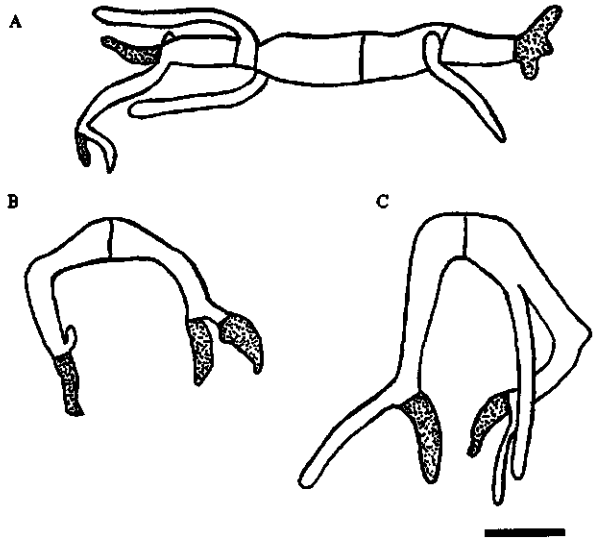


Figure 15. *Puccinia hordei*, drawings, scale bar = 20 μm.
Germlings of urediniospores collected from **A.** *Ornithogalum* spp. (aecial collection of *P. hordei*, C.S. 92.Isr7), SSV with three septa. Note lobed and unlobed HMC. **B.** cross *P. hordei* isolate 28 × 'regular' type *P. hordei*. Note resemblance with Figs 14 E, F. **C.** *Hordeum vulgare* (*P. hordei* isolate 28), sharply bent SSV with two vertical PIH.

5 EVALUATION OF MORPHOLOGICAL CHARACTERS OF INFECTION STRUCTURES OF UREDINIOSPORE GERMLINGS

The various morphological characters of the infection structures of urediniospore germlings (5.1), and several methods to observe the germling morphology (5.2) are to be evaluated.

5.1 THE VALUE OF MORPHOLOGICAL CHARACTERS OF UREDINIOSPORE GERMLINGS FOR TAXONOMY

Urediniospore germlings show a wide array of characters that might be valuable for taxonomic studies of rust fungi (Fig. 2). The potential of this approach was first considered by Pole Evans (1907). In the eighties Niks (1986, 1987, 1989) and Helfer (1987) also used germling morphology in taxonomic studies of grass rust fungi. They studied only one collection per (infraspecific) taxon. No experiments were carried out to investigate whether germling morphology is consistent according to the taxon (species, infraspecific taxon) or influenced by environmental conditions.

Appressoria

Four characters of the appressorium might be valuable for rust identification and classification, viz. its presence, size, shape, and to what extent it covers the stoma.

Generally, all Uredinales form appressoria (Emmett & Parberry, 1975), but this and other studies (Pole Evans, 1907; Niks, 1986, 1989; Helfer, 1987) have shown that *Puccinia striiformis* is an exception to this rule. The germ tube of this species enters the stoma directly. The tip of the germ tube may swell slightly before entering, but no clear appressorial vesicle is formed (this study; Pole Evans, 1907).

Commonly appressoria collapse when the contents have migrated into the substomatal vesicle (Rowell, 1984). This collapse may influence the recordings of size and shape of the appressorium and should be kept in mind when using these characters for identification. Besides, several physical or chemical factors may induce morphological variation of the appressorium (Emmett & Parberry, 1975). Staples *et al.* (1983b) found that, in general, appressoria of Wheat stem rust fungus were elongated when developed on wheat leaves or on scratched membranes, but spherical when induced on membranes by application of a heat shock or by addition of acrolein.

In the present study the effect of temperature on appressorium shape was briefly analyzed. In an experiment (see page 76) to study the effect of temperature on characters of the substomatal vesicle and primary infection hypha of Wheat leaf rust (*Puccinia recondita/Triticum*) after inoculation on the non-host species barley, appressoria were also observed. The typical oblong shape of the Wheat leaf rust

appressorium was observed on leaves incubated at different temperature regimes (night/day temperatures of: 15/19, 20/24, and 25/29 °C) which indicates that the

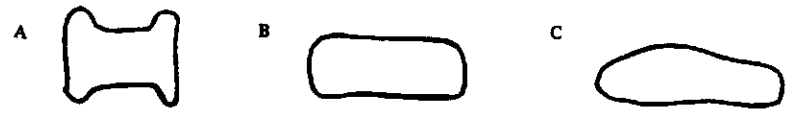


Figure 16. Schematical drawings of appressoria of grass rust fungi.
A. Square appressorium with short digitate protuberances at the corners. **B.** Oblong appressorium. **C.** Narrowly oblong appressorium.

temperature does not affect appressorium shape in these temperature ranges.

Although size of the appressorium could be rather variable within a collection, its shape (L/W ratio) and to what extent it covered the stoma (L_{app}/L_{stoma} ratio) were clearly different between several rust taxa. Three types of appressorium shape were recognized. Two-dimensional descriptions of shape are given, based on the projection of the collapsed appressorium on the leaf:

i. A relatively short ($L_{app}/L_{stoma} \approx 0.25$–$0.5$), square (L/W ratio $\approx$ 1) appressorium with short digitate protuberances at the corners (Fig. 16A). This type is commonly found in crown rusts (*Puccinia coronata* s.l.).

ii. A medium-sized ($L_{app}/L_{stoma} \approx 0.5$–$0.7$), oblong (L/W ratio $\approx$ 2) appressorium, with no or a few small digitate protuberances at the corners (Fig. 16B). This type is commonly found in leaf rusts (*Puccinia recondita* s.l., *P. hordei* s.l., *P. brachypodii* s.l. and *Uromyces* spp. from *Hordeum*).

iii. A long ($L_{app}/L_{stoma} \approx 0.8$–$1.0$), narrowly oblong (L/W ratio $\approx$ 4) appressorium without protuberances (Fig. 16C). This type is commonly found in stem rusts (*Puccinia graminis* s.l.).

The presence, shape, size, and to what extent the appressorium covers the stoma were fairly constant characters and can be used as additional tools for identification of grass rust fungi. Their value is, however, limited as they only discriminate between some large rust groups.

Infection pegs

Most rusts studied here had a short infection peg (2–4 μm long), which entered the stomatal slit and swelled to form a substomatal vesicle as soon as it extended in the substomatal cavity. Rarely, a germling had a somewhat longer infection peg. Only *P. brachypodii* s.l. from *Arrhenatherum elatius* formed an elongated infection peg which extended into the substomatal cavity. The infection peg could reach a length of 15 μm before swelling to form the SSV.

Length of the infection peg is only valuable for identification of grass rusts belonging to *P. brachypodii* s.l., as in all other rust taxa studied pegs are short.

Table 18. Length and width (μm) of the substomatal vesicles (SSV) and width (μm) of the primary infection hyphae (PIH) of germlings of *Puccinia recondita* urediniospores collected from various grasses and *P. hordei/Hordeum vulgare* after inoculation on seedling leaves of barley line L94.

host	SSV		PIH
	length	width	width
Agropyron junceiforme	(30–)45–57(–65)	(7–)10–15	(4–)5–6(–7)
Agropyron repens	(25–)29–42	9–12(–14)	(2–)4–5(–6)
Bromus hordeaceus	(22–)28–33(–37)	(10–)12–14(–16)	3–4(–5)
Bromus sterilis	(20–)25–29(–32)	(9–)11–13(–14)	(3–)4–5
Holcus lanatus	21–30	9–13(–15)	3–4(–5)
Secale cereale	(20–)25–35(–40)	(9–)10–13	(4–)5–6(–7)
Trisetum flavescens	(20–)25–28(–30)	8–10	3–4.5
Triticum aestivum	(21–)24–34(–38)	11–15(–17)	(4–)5–6
Hordeum vulgare	(30–)40–55(–60)	(7–)9–10(–12)	(3–)4–5(–6)

Substomatal vesicles

Size, shape, orientation, and septation of the substomatal vesicle (SSV) may be useful for identification and classification of rust fungi. Size of the SSV enabled a distinction between some taxa, e.g. *P. recondita/Agropyron junceiforme* and *P. recondita/ Agropyron repens* (Table 18), but this character showed a large overlap between many other taxa (Table 18).

However, the size and the shape of the ends (the narrowest part) of the SSV determined the shape of the SSV. The shape was useful for the identification of taxa as many character states were found. The SSV may be (Fig. 17)

i. fusiform (tapering towards the ends). Primary infection hyphae are formed at both ends of the SSV (e.g. *P. hordei*) or at one end end (*P. graminis*),
ii. rectangular. PIH arise at the ends of the SSV and are, in general, clearly thinner than the SSV (e.g. *P. coronata*),
iii. spheroid (*P. striiformis*) to deltoid (*P. brachypodii*),
iv. oblong (L:W = 3:2 - 2:1), with widest axis at midpoint, but margins essentially parallel (*P. bromina/Bromus sterilis*),
v. narrowly oblong (L:W = 3:1 - 6:1), with widest axis at midpoint, but margins essentially parallel (*P. bromina/Bromus erectus*),
vi. ellipsoid (L:W = 3:2 - 2:1), with widest axis at midpoint, but margins symmetrically curved (*P. recondita/Triticum*),

vii. narrowly ellipsoid (L:W = 3:1 - 6:1), with widest axis at midpoint, but margins symmetrically curved (*P. recondita/Agropyron*).

Shapes (iv) to (vii) can have acute (*P. recondita/Bromus hordeaceus*), papillate (*P. recondita/Bromus erectus*) or rounded ends (e.g. *P. recondita/Holcus lanatus*). Intermediate shapes may also occur, e.g. between acute and rounded ends (Fig. 17).

Generally, the orientation of the SSV of grass rust fungi was longitudinal and horizontal. Only in *P. brachypodii* s.l. from *Arrhenatherum elatius* and *Deschampsia cespitosa* (Section 4.3), and one isolate of *P. hordei* (Section 4.5) the SSV ends were bent downwards and grew more deeply into the mesophyll. Vertical orientation of the germling has also been described for *P. porri* (Sow.) Winter on *Allium porrum* (Davies

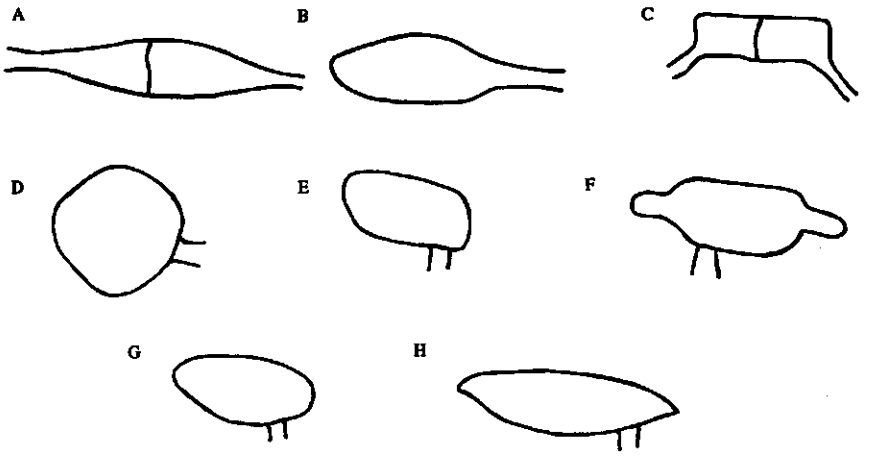


Figure 17. Schematical drawings of substomatal vesicles and orientation of primary infection hyphae of grass rust fungi.
A. Fusiform and two primary infection hyphae. **B.** Fusiform and one primary infection hypha. **C.** Rectangular. **D.** Spheroid. **E.** Oblong with rounded ends. **F.** Narrowly oblong with papillate ends. **G.** Ellipsoid with acute-rounded ends. **H.** Narrowly ellipsoid with acute ends.

& Butler, 1986). In addition, Ferreira & Rijkenberg (1989) described transversely orientated SSV for *Uromyces transversalis* (Thüm.) Winter from gladiolus.

Many urediniospore collections obtained from widely different geographic regions were studied for most grass rust taxa. Shape and orientation of the SSV was, in general, constant within a taxon. Rarely, a germling deviated from that of the majority of the germlings. Such a germling was omitted from the studies.

However, in experiments carried out at the Cereal Rust Laboratory in St. Paul, Minnesota (USA, in cooperation with Dr. A. P. Roelfs) with the Wheat leaf rust fungus, most germlings showed an aberrant morphology in shape and orientation. Instead of being ellipsoid and longitudinally orientated with one vertical primary infection hypha as was observed in greenhouse experiments of this and other studies

(Pole Evans, 1907; Niks, 1986; Helfer, 1987), the SSV were spheroid or more or less ellipsoid but vertically orientated. Besides, the PIH could be horizontally and longitudinally orientated.

As in St. Paul greenhouse temperatures were relatively high (25–35 °C), an experiment was designed to study the effect of temperature on shape and size of the SSV and on width and orientation of the PIH of urediniospore germlings of wheat leaf rust (*P. recondita/Triticum*). The experimental design was a split-plot. Temperature was the main factor with fixed effects. Five wheat leaf rust isolates which represented a random sample from the population of isolates present at the Department of Plant Breeding, Wageningen, the Netherlands, were selected for study. In each block, three barley seedling leaves were inoculated per isolate. The experiment was carried out in a controlled environment with a light intensity of 450 kLux and an 8 h night/16 h day regime at 15/19, 20/24, and 25/29 °C, respectively. Methods of inoculation, incubation and staining are described in Chapter 4. Statistical analyses were performed using PROC GLM from SAS (SAS, 1985).

Results showed that shape and orientation of the SSV was highly influenced by temperature (Table 19). At 15/19 °C most SSV had a regular shape (Fig. 18A), whereas at 25/29 °C most SSV had an irregular shape (Fig. 18 B–E). At this high temperature the SSV were spheroid, obconic, pyriform, or ellipsoid and vertically orientated and similar to the SSV observed in St. Paul. In addition, the PIH could be orientated horizontally instead of vertically (Fig. 18).

No significant effect of temperature on length and width of the SSV was found between the temperature regimes of 15/19 and 20/24 °C. Data of the 25/29 °C blocks were not included in the statistical analysis because no reliable measurements could be made on length of the irregularly shaped and vertically orientated SSV. Wheat leaf rust isolates also did not differ significantly in length of the SSV, but did differ in width of the SSV ($P = 0.0096$).

Table 19. Geographic origin and percentage of germlings with a regular type substomatal vesicle after inoculation of urediniospores of five isolates of *Puccinia recondita/Triticum* on seedling leaves of barley line L94 and incubated at three different temperature regimes.

isolate	geographic origin	temperature		
		15/19 °C	20/24 °C	25/29 °C
CPRO	Netherlands	90[a]	63	3
Flamingo	Netherlands	96	79	21
C.S. 92.038	Israel	93	67	4
P3797	Portugal	96	69	15
S5-3	Spain	93	56	1

[a] Data are means of three series: per series 20 germlings on three leaves each were assessed.

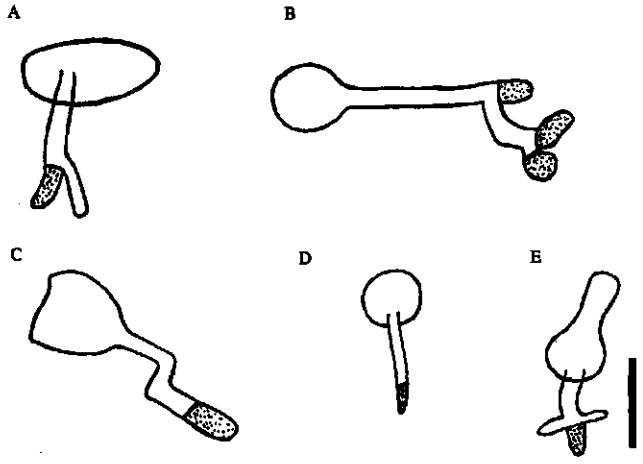


Figure 18. Drawings of substomatal vesicles of *Puccinia recondita/Triticum* after incubation at 25/29 °C in a controlled environment, scale bar = 20 μm.
A. Ellipsoid (normal morphology, isolate Flamingo). **B.** Spheroid and horizontally orientated primary infection hypha (isolate P3797). **C.** Obconical (isolate P3797). **D.** Spheroid and vertically orientated primary infection hypha (isolate C.S. 92.038). **E.** Pyriform and vertically orientated (isolate CPRO).

The results from this experiment indicate that too high a temperature may affect shape and orientation of the SSV. Therefore, incubation and growth of the Wheat leaf rust fungus germling should be conducted at moderate temperatures (10–20 °C) to obtain reliable observations. It is not necessary to incubate infected material in a greenhouse compartment as it could also successfully be done in dark rooms (own observations). Temperature optima of other rust fungi are expected to be somewhat higher (*P. graminis*) or lower (*P. striiformis*), but after incubation at 10–20 °C they always formed several well-developed germlings (own observations).

Another character that may be useful for identification is septation of the SSV. In the course of the present study three types of septation were recognized:

i. Septa were never formed (e.g. *P. brachypodii* s.l. from *Brachypodium* spp.).
ii. Septa were always present. In general, they were formed in the middle of the SSV (central, e.g. *P. hordei*), but sometimes they could be more or less eccentrical (e.g. *P. coronata*).
iii. Septa were irregularly formed. In some germlings of a collection a septum was present, whereas in other germlings it was lacking. The septum could be central or eccentrical (e.g. *P. recondita* from rye), and occasionally, a septum could be present in the PIH, especially in *P. brachypodii* s.l. from *Poa* spp., *Anthoxanthum odoratum* and *Arrhenatherum elatius*.

In some collections a germling could be observed that deviated from the majority of germlings. In *P. recondita* from *Triticum* (type i) germlings with a septum were omitted from the study as these germlings deviated from the normal type. In *P. hordei* (type ii) two or more additional septa could be formed in the SSV, likely due to a further development of the germling (Chapter 3). In type (iii) the percentage of germlings with a septum could vary largely within a collection. In collection C.S. 93.008 of *P. brachypodii* from *Poa annua* the percentage of septation of the SSV and PIH could be 16.7, 24.1, 41.7 or 55.8%, depending on the day of inoculation.

So, the type of septation and the position of the septa in the germling are useful traits for taxonomic studies. Determination of the percentage of germlings with or without a septum in type iii is not useful, since environmental conditions or other factors may influence the presence of septa.

Shape, orientation and septation of the SSV are valuable characters for taxonomic studies of grass rust fungi. It is, however, important to avoid too high a temperature during infection to minimize the occurrence of aberrant forms.

Primary infection hyphae

Characters of primary infection hyphae that may be useful for identification and classification are their number, orientation, width, and shape.

Primary infection hyphae arise from the SSV and their orientation may be horizontal and longitudinal, horizontal and transverse, or vertical. Occasionally in *P. graminis* no PIH is formed (Section 4.2). Longitudinally orientated PIH arise at one or both ends of a fusiform or ellipsoid SSV (e.g. *P. hordei*, *P. graminis*), transversely orientated PIH arise at any site of a spheroid/ellipsoid SSV (e.g. *P. recondita/Bromus hordeaceus*). Vertically orientated PIH arise at the under side of the SSV, rarely at the ends, and grow more deeply into the mesophyll (e.g. *P. recondita/Triticum*) (Fig. 17).

In general, the orientation of the PIH was constant within a taxon. In *P. recondita/Triticum* and in *P. recondita/Agropyron repens* it was occasionally observed that one of the germlings had a horizontally instead of vertically orientated PIH. In the *P. brachypodii* complex orientation of the two to five PIH could vary within germlings. Some of the PIH were vertically and others were horizontally orientated. Variation in orientation of PIH within a collection seems to be a useful taxonomic character for certain rust taxa, in which every collection studied showed this variation.

The number of PIH varied from one to six among the taxa studied. In many taxa the number of PIH was constant among germlings within a collection and within a taxon. *P. hordei* from barleys and *P. recondita* from rye, for example, always had two PIH, whereas *P. recondita* s.l. from wheat formed only one PIH. In a few taxa some of the germlings could have an additional infection hypha: *P. coronata* s.l. and *P. brachypodii/Anthoxanthum odoratum* normally had two, rarely three PIH. In other taxa the number of PIH varied considerably within a collection, but this variation was

observed in all collections from that taxon. This was characteristic for the rusts belonging to the *P. brachypodii* complex.

The width of the PIH varied largely among taxa. Germlings of *P. coronata* s.l. differed clearly from the other taxa studied by their narrow, 2–3 μm wide, PIH. The other rusts studied had intermediate, 3-6(-7) μm wide PIH, whereas *P. striiformis* s.l. had 5-19 μm wide PIH (Niks, 1986; this study). In the group of taxa with moderately wide PIH a large overlap in width of the PIH was found (Table 18). Nonetheless, in some cases the width of the PIH was useful to distinguish between taxa, e.g. *P. hordei* had somewhat narrower PIH than *P. recondita/Secale* (Table 18), taxa which both have a fusiform SSV with two horizontally orientated PIH.

However, in the experiment described on page 76 it appeared that the width of the PIH also varied significantly between Wheat leaf rust isolates ($P = 0.0395$), whereas temperature did not affect the width. These observations suggest that the width of the PIH should be used cautiously to identify taxa. It may be useful as an additional criterion in combination with other characters.

Finally, some differences in shape of the PIH were observed. Mostly PIH were straight (as in *P. recondita/Secale*), but PIH could also be hooked (as in *P. coronata*), making curves of about 90°.

Notwithstanding the variation reported in number and orientation of PIH, these characters are suitable for taxonomic studies. The width and shape of the PIH may be useful additional characters as they discern between some of the rust groups studied.

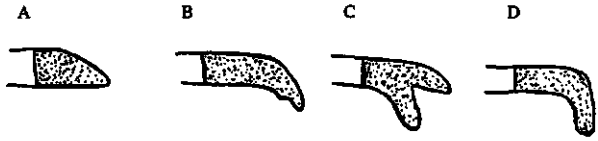


Figure 19. Schematical drawings of haustorial mother cells of grass rust fungi.
A. Straight. **B.** Straight with an elongated tip. **C.** Lobed. **D.** Hooked.

Haustorial mother cells

Characters of the haustorial mother cell include size and shape. As HMC are delimited at the tip of the PIH, width of the HMC is similar to width of the PIH. Length of the HMC, however, could vary largely within a collection and even within a germling (10 μm and 22 μm were observed in one germling of *P. graminis*). Often HMC length showed a large overlap within and among rust taxa, e.g. within and among taxa subsumed under the *P. recondita* and *P. brachypodii* complex, and within *P. graminis*.

Shape of the HMC has several character states (Fig. 19):

i. unlobed (= straight), with a rounded or acute tip,
ii. unlobed with an elongated tip,
iii. unlobed and hooked,
iv. lobed (with short digitate protuberances).

However, HMC shape was very variable within a collection. Often in one slide more than one type of HMC was found: e.g. straight, lobed, and HMC with an elongated tip in *P. hordei* s.l., or lobed and straight HMC in *P. recondita* from rye.

The HMC could be difficult to measure, especially when the germling had vertical PIH and formed its HMC more deeply in the mesophyll. Recording of the size and shape of the HMC was also difficult when the HMC themselves were vertically orientated.

However, when visible, the size and shape of the HMC can be additional criteria to confirm identification of the species complexes of grass rusts based on characters of SSV and PIH. Germlings belonging to the *P. brachypodii* complex had relatively short and unlobed HMC, those of the *P. coronata*, *P. graminis* and *P. hordei* complexes had relatively long and slender, unlobed HMC; in *P. coronata* the HMC were frequently hooked; in *P. graminis* the HMC occasionally had an elongated tip; in *P. hordei* the HMC also had an elongated tip and occasionally they were lobed. Germlings belonging to the *P. recondita* complex differed considerably in size and shape of the HMC;in the *Alopecurus* and *Secale* types the germlings mostly had lobed HMC; in the *Holcus* and *Triticum* types the HMC were unlobed or had an elongated tip.

The haustorial mother cells have only few characteristics which tend to vary within a taxon. Moreover, HMC are often difficult to observe accurately. The shape and size may be useful characters to confirm identification of the species complexes. However, this feature is unsuitable for the identification of most taxa within a species complex.

Secondary hyphae

Three types of secondary hyphae, hyphae arising after the development of the PIH and HMC, were observed in the collections studied. Most frequently secondary hyphae arose at the SSV-side of the HMC-septum (SH, Fig. 2). Only in urediniospore germlings of *Puccinia brachypodii* collected from *Brachypodium*, *P. recondita* from *Holcus lanatus*, *Trisetum flavescens* and species of *Bromus* they were rarely formed, whilst in all other rusts studied SH appeared frequently.

A second type of secondary hyphae arose along the PIH (SH-ADD, Fig. 2). They were most often observed in urediniospore germlings of *P. graminis* collected from *Avena*, but occasionally they were seen in other *P. graminis* rusts and in *P. coronata*. In *P. graminis* the presence of SH-ADD was related with length of the SSV + PIH. The longer SSV + PIH also a higher percentage of SH-ADD was observed (Table 8).

The third type of secondary hyphae was only observed in *P. hordei*; they arose near the additional septa present in the SSV. As they arise from the SSV, but after formation of PIH, HMC and septa, they are called secondary infection hyphae (SIH, Fig. 2). SIH occurred erratically (Chapter 3).

Since the formation of SIH may be influenced by environmental conditions (Chapter 3) this character should be used with caution. The presence and type of secondary hyphae can be useful for the recognition of several grass rust taxa.

Conclusions

When a normal temperature range (10–20 °C) prevails during infection of the urediniospore germling, the shape, orientation, and type of septation of the SSV, the number and orientation of PIH and the presence of SH and SIH are reliable characters to identify grass rust taxa. The presence and shape of the appressorium, the length of the infection peg, the width of the PIH and the shape of the HMC are useful additional characters. They enable the distinction of some of the (infraspecific) rust taxa studied here (Chapter 4).

5.2 Evaluation of Methods for the Microscopic Examination of Urediniospore Germlings

Several methods are available to study the morphology of infection structures of urediniospore germlings. One could study the germling morphology in sporulating plant material, either sampled from herbarium sheets or from freshly collected leaves or one could germinate urediniospores on a membrane, on host leaves or on non-host leaves and study the germling morphology after a fixed period of development. Besides these methods, some practical aspects of the methods used in the present study are discussed.

Observations in sporulating plant material

For taxonomic purposes a procedure to observe the germling morphology in sporulating plant material, usually leaves, either sampled from herbarium sheets or freshly collected in the field, would be of great value: neither viable urediniospores nor extended experimental work would be required, and type specimens, provided that sufficient material is available, can also be examined. However, experiments conducted during this study showed that staining and observation of the urediniospore germlings in sporulating leaves, especially of (wild) grasses was not satisfactory. Neither staining of the leaves with trypan blue for phase contrast microscopy (after Niks, 1986) nor with Calcofluor (Helfer, 1987) or Uvitex 2B (Jacobs, 1990) for fluorescence microscopy resulted in a satisfactory visibility of the infection structures. Both staining methods enabled better observations of germlings in freshly collected leaves than in dried (herbarium) leaves. In addition, cereal leaves, for which the staining had been developed, gave better staining results than leaves of (wild) grasses. Sectioning leaves could improve the visibility of the germlings, but to visualize a few infections many leaves had to be sectioned.

Besides the observation that germlings did not stain satisfactorily in naturally infected leaves, a further drawback of staining them in these leaves was that often only a few germlings were present that were in different stages of development. Most of the

germlings had developed a highly branched septate mycelium where it was difficult to observe the various mycelial features. Substomatal vesicles and primary infection hyphae were hardly visible in most cases because numerous secondary (infection) hyphae had emerged. The haustorial mother cells were often invisible due to the abundance of infection hyphae.

Moreover, in grass leaves the observation of the urediniospore germlings was difficult, because the germlings had to penetrate into a small substomatal cavity and between densely packed mesophyll cells, where they could not express their morphology freely. In the relatively looser mesophyll tissue of cereal seedling leaves the germlings could develop more freely and were easier to describe (Niks, 1986; this study).

So, staining of sporulating leaves appeared unsuitable for the taxonomic investigations conducted in this study. However, before drawing any taxonomic conclusions, it would be desirable to stain infection structures in host leaves of type specimens, provided sufficient material is available, to observe their morphology.

Germination of urediniospores on a substrate

Germination of urediniospores on a standard substrate, and using a standard technique to fix and stain the germlings could improve the comparison of morphological characters because the germlings can be observed at a similar developmental stage. Membranes and intact leaves (host or non-host) were considered as potentially suitable substrates.

Membranes

Several reports mention the use of membranes (Pavgi & Dickson, 1961; Maheshwari *et al.*, 1967; Heath, 1990; Deising *et al.*, 1991) or membranes placed on an agar medium (Paliwal & Kim, 1974) for the study of the development of infection structures. These studies showed that the morphology of the germlings induced on a membrane was identical to that formed in the host. They were easy to observe, extending on a surface and not in a leaf.

However, the chemical composition and physical properties of the membrane and the environment must fulfil several criteria to induce germination and development of infection structures. The optimal concentration of chemicals and the nature of physical stimuli required for differentiation seem to differ greatly between rust taxa (Pavgi & Dickson, 1961; Maheshwari *et al.*, 1967; Staples *et al.*, 1983a,b; Kaminskyj & Day, 1984a,b). This limits the use of membranes as a standard substrate for the observation of germling morphology in taxonomic studies.

Leaves

In contrast to membranes, most gramineous leaves are suited for the development of infection structures of urediniospores of grass rusts (Chapter 3). Until now, only a few

workers have studied the morphology of urediniospore germlings in order to use this character for identification of rust fungi. They observed germling morphology after inoculation of urediniospores on seedling leaves. Pole Evans (1907) and Helfer (1987) inoculated spores of, mainly, cereal rusts on a susceptible host plant. Niks (1986) inoculated spores of several grass rusts on seedling leaves of a non-host cereal. In general, the descriptions of these authors were in accordance with each other, though differences in number of septa and primary infection hyphae were apparent. These differences could be attributed to the influence of the substrate on the development of the germling and to differences in time after inoculation at which the germlings were fixed (see also Chapter 3). The differences in presence of septa and PIH, however, did not influence the overall morphology of the germlings. This indicates that both host and non-host cereal seedling leaves are suitable as a standard substrate for the observation of the germling morphology.

Some cereal accessions have hairy (seedling) leaves. When applying urediniospores with a needle onto hairy leaves (e.g. hairy *Triticale* spp. and *Avena* spp.), the spores easily fell off the leaves (this study). Therefore, the smooth barley line L94 was chosen as a standard for this study (Chapters 3 and 4). An additional advantage of this line is that the seedling leaves are relatively wide, which facilitates the application of the spores.

Practical aspects

Collecting and viability of spores

Urediniospores were collected and applied onto the seedling leaves with a needle. The advantage of this method is that spores can be taken from a few sori and that the spores can be applied densely on a specific leaf segment, which facilitates the microscopical search for appressoria and infection structures. Using a settling tower (after Eyal *et al.*, 1968) for inoculation is impractical, because it requires far more spores, at least a few milligrams, to get a sufficiently high spore density which is spread over a larger surface.

Sometimes only very low numbers of viable urediniospores reached the seedling leaves. Several reasons may account for this:

i. The viability of the spores decreased rapidly after collecting the infected leaves. Spores of freshly collected leaves germinated sufficiently; spores of leaves which had been stored at room temperature or in a refrigerator for some days had lost much of their germination capacity.
ii. When collecting spores, they could fall off the needle, particularly when the leaves were hairy (e.g. *Holcus* spp. and *Trisetum flavescens*).
iii. Collecting spores without losing spores was difficult from tiny and enrolled dry leaves (e.g. *Festuca rubra* and *F. ovina*).

iv. During transport and storage, only a few spores could remain in the sorus due to mechanical damage.

Therefore, to increase the percentage of germinating spores at inoculation it is suggested to inoculate directly after collecting the infected leaf material or to keep the infected leaves briefly in water or on agar (Helfer, 1989). It can also be useful to dig up some of the sporulating plants and transfer them to a greenhouse. This has the additional advantage that spores can be collected at any desired moment and that spores can be multiplied on the host and stored in liquid nitrogen for further research. This is especially helpful for wild grasses of which no seed stock is available. Rust fungi collected from grasses for which a susceptible host accession is available can be multiplied on this susceptible host.

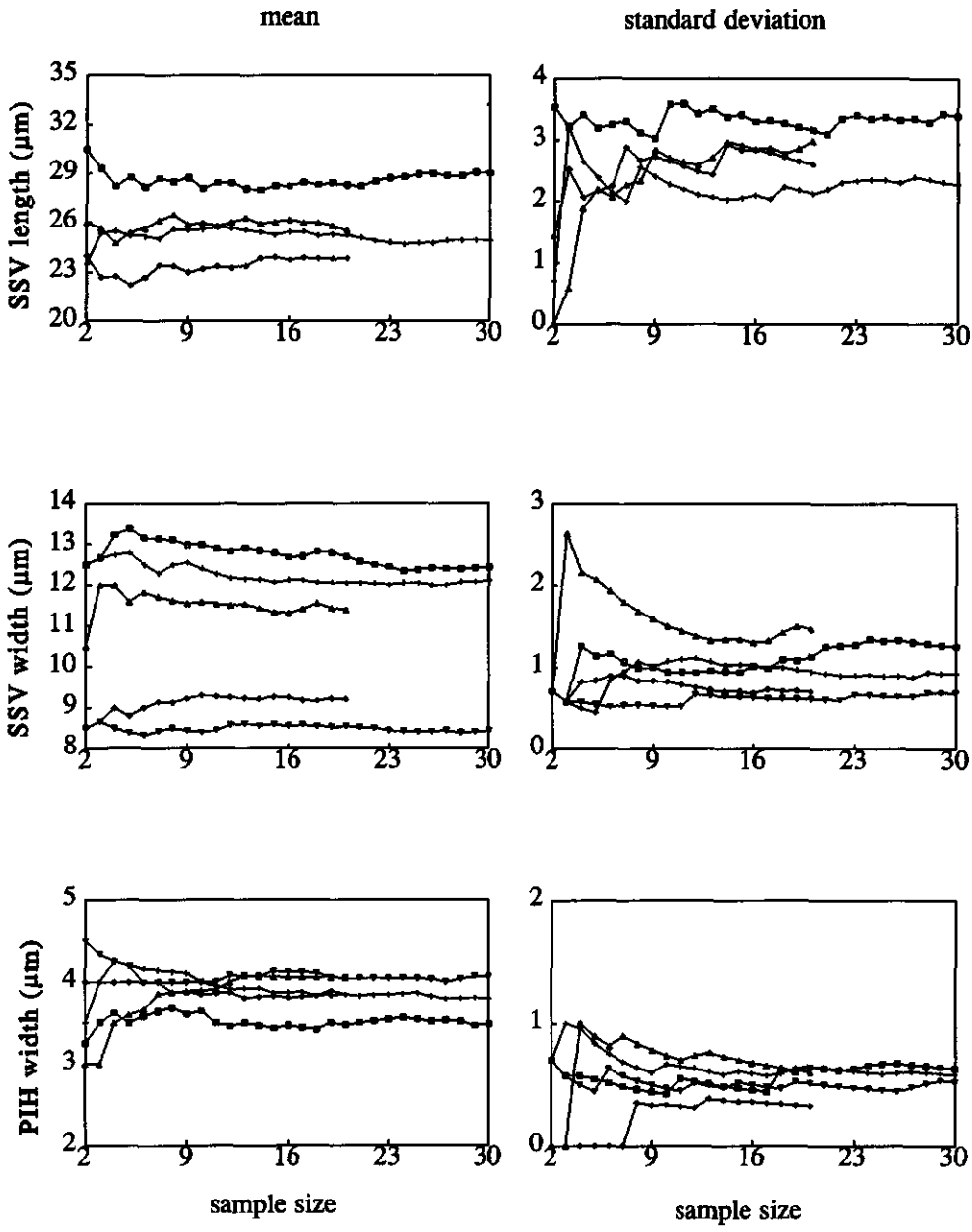
Spores taken from liquid nitrogen collections and revived following published instructions (Rowell, 1984; Staples & Macko, 1984) sometimes germinated in low percentages, too. It is, therefore, preferred to multiply the spores on a susceptible host before use.

Sample size

Under (near) optimal conditions many germlings were present on each inoculated leaf studied. To find out which sample size would be adequate, the method described by Kranz (1988) was used. For five rust taxa the length and width of the substomatal vesicles and the width of the primary infection hyphae were recorded. The mean and standard deviation, calculated for increasingly larger samples, were plotted against sample size (Fig. 20). An adequate sample size is the smallest sample from which mean and standard deviation have stabilized; this is reached here at about 15 germlings (Fig. 20).

In conclusion, for the observation of the germling morphology of urediniospores it is recommended to use freshly collected spores. It is also recommended to apply the spores onto segments of a cereal seedling leaf with a needle. After fixing, staining and embedding the leaf segments (Chapters 3 and 4) at least 15 germlings should be observed to get an adequate level of accuracy for quantitative measurements.

Figure 20. Mean and standard deviation of length and width of the substomatal vesicle, and width of the primary infection hypha for increasingly larger samples of *Puccinia recondita* s.l., collected from:
— + — *Bromus sterilis*, collection C.S. 91.015; — □ — *Bromus hordeaceus*, collection C.S. 91.019; — ▵ — *Holcus lanatus*, collection C.S. 91.042; — ◇ — *Trisetum flavescens*, collection C.S. 92.027; — ▿ — *Lolium multiflorum*, collection C.S. 91.001.


mean
standard deviation
SSV length (µm)
SSV width (µm)
PIH width (µm)
sample size
sample size

6 THE VALUE OF NUCLEAR DNA CONTENT AND ISOZYME BANDING PATTERNS FOR TAXONOMIC PURPOSES

Several techniques other than the morphological description of sorus and spore characters and host range studies are available for taxonomic studies of rust fungi. These include i.a. somatic and sexual hybridization experiments and several molecular and biochemical techniques, like DNA/DNA reassociation, restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA markers (RAPDs), DNA sequence analysis, assessment of the nuclear DNA content, and electrophoresis of total soluble proteins or specific isozymes.

In the present study an evaluation of the nuclear DNA content of the urediniospores (6.1) and isozyme banding patterns of urediniospores (6.2) for taxonomic purposes is included.

6.1 NUCLEAR DNA CONTENT OF UREDINIOSPORES

Introduction

Cytometric analysis of nuclear DNA (nDNA) contents has proven to be a valuable tool in fungal taxonomy. Cytometry has contributed to the understanding of the nuclear cycle of species, e.g. *Armillaria bulbosa* (Barla) Watl. (Peabody & Peabody, 1986) and *Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka (Hiratsuka, 1991), and to the assessment of ploidy levels of i.a. several cereal rust fungi (Williams & Mendgen, 1975; Tetsuka *et al.*, 1984), *Uromyces phaseoli* (Pers.) Wint. (Staples *et al.*, 1984) and *Itersonilia perplexans* Derx (Boekhout & Jille, 1991). It has also been used to compare the nDNA contents among species belonging to the same or different genera (i.a. Motta *et al.*, 1986; Durán & Gray, 1989; Meixner & Bresinsky, 1988; Wittmann-Meixner & Bresinsky, 1989; Eilam *et al.*, 1992a,b, 1993).

Several DNA-specific fluorochromes are available to estimate the relative DNA content of nuclei. These include Feulgen (Williams & Mendgen, 1975), propidium iodide (PI; Tetsuka *et al.*, 1984; Eilam *et al.*, 1992a,b, 1993), and 4',6-diamidino-2-phenylindole (DAPI; Staples *et al.*, 1984; Meixner & Bresinsky, 1988). Nowadays PI and DAPI are the most commonly used fluorochromes.

PI was used by Eilam *et al.* (1992a,b, 1993) to measure the fluorescence of nuclei in basidiospores of mainly cereal rust fungi. The use of basidiospores for taxonomic studies is, however, not practical, since the viability and germination of teliospores depends strongly on environmental conditions (Eilam *et al.*, 1992a). Moreover, some grass rust fungi never or rarely form telia.

Since urediniospores are the predominant spore state in rust-infected material, they may be better suited for taxonomic purposes. Therefore, the taxonomic value of the nDNA content of the — dikaryotic — urediniospores of grass rust fungi was examined.

PI stained the nuclei of urediniospores of grass rust fungi poorly (Eilam *et al.*, 1992a; own observations). Therefore, the fluorochrome DAPI was used. DAPI binds to AT-rich regions of the DNA, and the fluorescence of DAPI-stained DNA is proportional to the DNA quantity when DNAs of the same base composition are compared (Coleman *et al.*, 1981; Butt *et al.*, 1989). Studies of Coleman *et al.* (1981) have also shown that the DAPI-DNA complex is not affected by the time elapsed between staining and reading, and gives comparable values of fluorescence from slide to slide and from day to day with the same material. In addition, a constant and minimal background is present when DAPI is used at the optimal concentration.

The *Puccinia recondita* species complex was chosen for this study since most taxa included differ only slightly in morphological characters of uredinia and telia, whereas they do differ in aecial and telial host range. Authors also differ largely in the number and rank of taxa recognized within this species complex (Section 4.4).

Material and methods

Urediniospores of 12 collections classified as *Puccinia recondita*, but from five different host species, and one collection of *P. hordei* (Table 20) were multiplied on seedling leaves of the appropriate host plant in a greenhouse compartment.

Preliminary experiments showed that the nuclei in germ tubes (methods of Staples *et al.*, 1984) could be rather elongated and therefore difficult to locate in the measuring diaphragm. Nuclei in non-germinated urediniospores were mostly round and easy to locate; therefore these were used in this study, although occasionally the background fluorescence was rather high. Nuclei were only visible with fluorescence optics.

Freshly collected urediniospores were put in an Eppendorf tube and fixed in 70% alcohol overnight. After centrifugation (5 min at 13 000 rpm), the spores were placed on a slide and stained with DAPI solution (0.5 μg/ml DAPI in McIlvaine's buffer, pH 4.4; modification of the procedure by Staples *et al.*, 1984). For each collection, four replicate slides were prepared and in one of the four slides some latex drops were included to monitor consistency of the staining procedure. Slides were sealed with nail-varnish.

Quantitative DNA measurements were made using a Zeiss Axioskop, equipped with a microscope photometer MPM 200 and a microscope system processor MSP 20. The processor was adjusted according to Boekhout & Jille (1991). An automatic high voltage, measuring diaphragm of 0.25 mm and oil immersion objective 40 × / 0.9 (Planneofluar, Zeiss) were used during measuring. One of the two nuclei of each urediniospore was centred inside the measuring diaphragm by epifluorescence (1–2 s illumination). To eliminate background illumination, fluorescence measurements were made of the selected nucleus and the adjacent protoplasm. After subtracting the value obtained for protoplasm from that for the selected nucleus, the resulting value was considered proportional to the

DAPI-DNA content of the nuclei. Fluorescence was recorded in arbitrary fluorescent units (AU).

Table 20. Designation and average nuclear DNA content in arbitrary fluorescent units (AU) of DAPI-stained nuclei of urediniospore collections classified as *Puccinia recondita* s.l., obtained from five host species, and one collection of *P. hordei* (uredinial host *Hordeum vulgare*).

uredinial host species	collection[a]	average nDNA content (AU)		
		average over collections	per collection	relative to *P. recondita* from *Triticum*
Triticum aestivum		14.5 ± 1.3a[b]		1.0
	Felix		15.2 ± 0.8	
	Flamingo		14.3 ± 0.8	
	S5-1		14.1 ± 0.8	
Agropyron repens		16.8 ± 1.6a		1.2
	C.S. 92.009		17.3 ± 1.1	
	C.S. 93.009		16.2 ± 1.0	
Bromus sterilis		21.0 ± 1.4ab		1.4
	C.S. 90.025		22.3 ± 0.9	
	C.S. 91.015		20.7 ± 1.3	
	C.S. 92.013		20.0 ± 0.8	
Secale cereale		23.1 ± 1.6ab		1.6
	C.S. 89.007		23.6 ± 0.7	
	IVP		22.5 ± 0.3	
Bromus hordeaceus		35.6 ± 1.6c		2.5
	C.S. 93.011		31.5 ± 1.8	
	C.S. 93.013		39.6 ± 0.6	
Hordeum vulgare		29.3 ± 2.3bc		2.0
	1-2-1		29.3 ± 1.1	

[a] Designation of collections is as in the Appendix.

[b] Mean and standard error are given; values followed by different letters differ significantly (P = 0.05), according to Tukey's test.

In each replicate slide of each collection, the fluorescence of 25 nuclei was measured. Data were analysed with an analysis of variance using the Statistical Analytical System (SAS, 1985). Host species was considered a fixed effect and collections within a host species a random effect. Means of the host species were compared with Tukey's test ($P = 0.05$).

Results and discussion

Fluorescence measurements suggested that the nDNA content differed significantly between urediniospores collected from various host species, but differences between collections obtained from the same host species were also significant (Table 21). Fluorescence of nuclei of spores collected from *Triticum aestivum* and *Agroyron repens* was low, while nuclei of spores collected from *Hordeum vulgare* and *Bromus hordeaceus* had a high fluorescence. Fluorescence of nuclei of spores from *Secale cereale* and *Bromus sterilis* was intermediate (Table 20). Another experiment in which most of the collections studied here were also included had similar results (Swertz & Boekhout, 1992).

Eilam *et al.* (1992a,b, 1993) also reported that the fluorescence of basidiospores from *T. aestivum* was lower than that of basidiospores from *S. cereale*. The fluorescence of basidiospores of *H. vulgare*, however, was somewhat lower than that of spores of *T. aestivum* (Anikster *et al.*, 1992a,b). The values reported by Eilam *et al.* (1992a,b, 1993) and this study cannot be compared in detail due to the large differences in numbers of collections studied and experimental design.

Generally, DAPI stained the nuclei of the urediniospores properly (Fig. 21), but occasionally poorly. The hydrophobic nature of the urediniospores may hamper the uptake of the DAPI solution. To study this possibility, urediniospores were mixed with a drop of Tween 20 to reduce surface tension before adding the DAPI solution. After this treatment the spores had a higher background fluorescence than spores stained in DAPI without Tween 20, but the staining intensity of the nuclei had not increased. From these experiments it was clear that urediniospores had to be totally immersed in DAPI solution for proper staining of the nuclei.

In contrast to the observations made by Coleman *et al.* (1981) and Boekhout (pers. comm.), the fluorescence of the DAPI-stained nuclei faded gradually during storage of the slides. After four days of storage the fluorescence of the nuclei was almost nil, whereas the fluorescence of the latex drops had not faded significantly. As the slides were kept in the dark it is unlikely that the fading is due to exposure to light. Chemical or physical properties of the urediniospores may decrease the stability of the urediniospore DAPI-DNA complex over time.

The differences in fluorescence between urediniospores from the six host species were correlated with the differences in germling morphology as described in Section 4.4. Several authors consider the various taxa subsumed under *P. recondita* as separate species (Section 4.4). Results from this study also support this view.

The differences between collections from the same host species and the overlap in average nDNA content (Table 20), suggest to use the nDNA content only as an additional criterion in taxonomic studies of grass rust fungi.

Table 21. Sum of squares (SS), mean squares (MS) and expected mean squares (EMS) from analysis of variance of the nDNA content of DAPI-stained urediniospores of several collections of *Puccinia recondita* s.l., collected from various host species and *P. hordei*. df = degrees of freedom.

source of variation	df	SS	MS	EMS
host	5	2597.62	519.52*	$\sigma^2 + 4\sigma^2$(collection(host)) + Q(host)
collection(host)	7	149.41	21.34*	$\sigma^2 + 4\sigma^2$(collection(host))
error	39	152.44	3.91	σ^2

*) Significant at P = 0.001, according to Tukey's test.

Figure 21. Urediniospores of *Puccinia recondita* from *Bromus sterilis* (C.S. 90.025), stained with DAPI and viewed by epifluorescence microscopy. Scale bar = 30 μm.

6.2 Isozyme Electrophoresis of Urediniospores

Introduction

Gel electrophoresis of isozymes has proven to be a powerful tool in studying taxonomy, evolution and population genetics of plant-pathogenic fungi (e.g. Burdon & Marshall, 1981; Burdon & Roelfs, 1985a,b; Lu & Groth, 1987; Clark *et al.*, 1989; Nygaard *et al.*, 1989; Linde *et al.*, 1990; Bonde *et al.*, 1991; Koch & Köhler, 1991; Oudemans & Coffey, 1991; McCain *et al.*, 1992; Chen *et al.*, 1992). Gel electrophoresis of total soluble proteins has been used for taxonomic purposes as well, but usually there were insufficient polymorphisms to characterize infraspecific taxa (Michelmore & Hulbert, 1987).

Studies of rust fungi and powdery mildews have shown that isozyme banding patterns (isozyme phenotypes) are often highly uniform within and among populations of the same taxon (species, subspecies, *forma specialis*), even in taxa with a high variation for virulence (Burdon *et al.*, 1983; Newton *et al.*, 1985; Clark *et al.*, 1989; Linde *et al.*, 1990). Newton (1990) suggested that this isozyme uniformity may be related to the biotrophic nature of rust fungi and powdery mildews and their (often) limited host range. Nevertheless, isozyme variation has also been detected in species of sexually- (Burdon & Roelfs, 1985a,b) and asexually-reproducing rust populations (Burdon *et al.*, 1982; Park & Burdon, 1992), and in powdery mildews (Koch & Köhler, 1991).

Isozymes that show no or little variation within taxa are valuable for identification and classification of fungi (Micales *et al.*, 1986). Burdon & Marshall (1981) and Newton *et al.* (1985) reported considerable differentiation in isozyme banding patterns between rust species, whereas the differences between *formae speciales* of a species were, with the exception of *Puccinia recondita*, always less than those observed between species.

Isozyme banding patterns of the rust fungi of wild grasses have hardly been studied, except for *P. coronata* collected from *Festuca arundinacea* and *Lolium perenne* and *P. recondita* from *Bromus diandrus* (Burdon & Marshall, 1981). This may not only be due to the economic importance of cereals, but also the fact that authors had to use 25–50 mg of urediniospores per collection for starch gel and polyacrylamide gel electrophoresis. This quantity of spores is easily harvested from rust-infected cereals, but is laborious to obtain from the narrow and short leaves of most wild grasses.

Recently, the Pharmacia PhastSystem for polyacrylamide gels has been developed, which requires only small quantities of material (PhastSystem Users Manual, section Users Guide). The objectives of the present study were to determine the usefulness of PhastSystem for electrophoresis of grass rust fungi, to compare isozyme banding patterns of several rust taxa and to evaluate isozyme analysis as a tool for the identification of grass rust fungi. In a preliminary experiment, the activity and resolution of several enzyme systems were tested and some systems having no or a limited amount of isozyme variation within a taxon were selected. In

the main experiment the banding patterns of the five selected enzyme systems were studied using several grass rust taxa.

Material and methods

Preliminary experiment

Isolates

Twelve isolates of Barley leaf rust (*Puccinia hordei*) and 7 of Wheat leaf rust (*P. recondita* s.l. from *Triticum*) were multiplied on seedling leaves of a susceptible host accession. Urediniospores were collected and stored in Eppendorf tubes in portions of appr. 1 mg in liquid nitrogen or in an ultra-low temperature freezer at -80 °C until required. The isolates represented a wide range of different races and came from widely different geographic origins (Table 22). In addition, some 'house' collections, present at the Department of Plant Breeding, Wageningen, the Netherlands, of Rye leaf rust (*P. recondita* s.l., from *Secale*), Oat crown rust (*P. coronata*), and Flax rust (*Melampsora lini* (Ehrenb.) Desm.) were included.

Electrophoretic analysis of isozymes

Samples for electrophoresis were prepared by adding 25 µl of 1% 2-mercaptoethanol and 7–10 glass beads (2 mm diam) to 1 mg of urediniospores in an Eppendorf tube. Spores were ruptured by vortexing, the suspension was centrifuged for 10 min at 10 000 rpm and the supernatant was used for Phast electrophoresis.

Electrophoresis was carried out on precast polyacrylamide PhastGels (43 × 50 × 0.45 mm) as described in Application File No. 120 from Pharmacia. Four gel types were used: a homogeneous PhastGel medium of 12½% and three gradient PhastGel media, 4–15%, 10–15%, and 8–25%. The buffer system in the gels consisted of 0.112 M tris-acetate, pH 6.4. The native buffer strips used were supplied by Pharmacia and were made of 2% agarose IEF and contained 0.25 M tris and 0.88 M L-alanine, pH 8.8.

Samples were applied on the gel using 1 µl combs (8 wells). The program for the running condition was established on the control unit from the PhastSystem as follows:

1)	400 V	10 mA	2.5 W	10 Vh	(prerun)
2)	400 V	1 mA	2.5 W	10 Vh	(sample)
3)	400 V	10 mA	2.5 W	268 Vh[1]	(separation)

[1] For the homogeneous gel 12½% this was 125 Vh and for the gradient gel 4–15% 108 Vh.

The temperature was kept at 15 °C. The migration time was about 70 min for all gel types.

Table 22. Rust collections, hosts, geographic origin and electrophoretic phenotype (EP) of the *Puccinia brachypodii*, *P. coronata*, *P. hordei*, *P. recondita* and *P. striiformis* species complexes studied. NL = the Netherlands; GB = Great Britain.

collection[a]	host	origin	EP
Puccinia brachypodii			
C.S. 91.047	*Poa pratensis*	Utrecht city, NL	bra-1
C.S. 93.023	*Poa pratensis*	Rhenen, Utrecht, NL	bra-2
C.S. 93.034	*Poa pratensis*	Täsch, Mattertal, Switzerland	bra-2
C.S. 93.008	*Poa annua*	't Goy, Utrecht, NL	bra-3
C.S. 93.026	*Poa nemoralis*	Sy, Ardennes, Belgium	bra-3
C.S. 93.033	*Poa nemoralis*	Wageningen, Gelderland, NL	bra-3
C.S. 90.055	*Anthoxanthum odoratum*	Veenendaal, Utrecht, NL	bra-4
C.S. 93.010	*Arrhenatherum elatius*	Rhenen, Utrecht, NL	bra-5
C.S. 93.035	*Arrhenatherum elatius*	Täsch, Mattertal, Switzerland	bra-5
Puccinia coronata			
IVP	*Avena sativa*	Wageningen, Gelderland, NL	cor-1
95-92/3	*Avena sativa*	Czech Republic	cor-1
26-92/1	*Avena sativa*	Yugoslavia	cor-1
C.S. 93.047	*Holcus lanatus*	Wageningen, Gelderland, NL	cor-1
C.S. 93.051	*Holcus lanatus*	Rhenen, Utrecht, NL	cor-1
C.S. 92.097	*Lolium perenne*	Nideggen, Eifel, Germany	cor-1
C.S. 93.049	*Lolium perenne*	Rhenen, Utrecht, NL	cor-1
C.S. 93.040	*Lolium perenne*	Wageningen, Gelderland, NL	cor-2
C.S. 93.041	*Lolium perenne*	Wageningen, Gelderland, NL	cor-2
C.S. 93.042	*Lolium perenne*	Wageningen, Gelderland, NL	cor-2
C.S. 92.090	*Holcus lanatus*	Nideggen, Eifel, Germany	cor-3
C.S. 93.048	*Arrhenatherum elatius*	Wageningen, Gelderland, NL	cor-4
C.S. 93.050	*Arrhenatherum elatius*	Rhenen, Utrecht, NL	cor-5
Puccinia hordei			
1-2-1	*Hordeum vulgare*	Wageningen, Gelderland, NL	hor-1
3	*Hordeum vulgare*	GB	hor-1

P. hordei (continued on next page)

Table 22, continued

collection	host	origin	EP
13	*Hordeum vulgare*	Elounta, Crete, Greece	hor-1
17	*Hordeum vulgare*	Zeddam, Gelderland, NL	hor-1
18	*Hordeum vulgare*	Dwingeloo, Drenthe, NL	hor-1
22	*Hordeum vulgare*	Les Settons, France	hor-1
25	*Hordeum vulgare*	Italy	hor-1
28	*Hordeum vulgare*	Morocco	hor-1
Marakech 1-1	*Hordeum vulgare*	Morocco	hor-1
C.S. 89.230	*Hordeum vulgare*	Holetta, Ethiopia	hor-1
C.S. 90.034	*Hordeum vulgare*	Iquito, Ecuador	hor-1
Uch-Syria	*Hordeum vulgare*	Syria	hor-1
Uvb-IVP	*Hordeum vulgare*	Israel	hor-2
colour mutant 1-2-1	*Hordeum vulgare*	Wageningen, Gelderland, NL	hor-3
IVP	*Hordeum murinum*	Wageningen, Gelderland, NL	hor-4
C.S. 90.205	*Hordeum murinum*	Edinburgh, GB	hor-4
C.S. 90.223	*Hordeum murinum*	Wageningen, Gelderland, NL	hor-4
Puccinia recondita			
Flamingo	*Aegilops squarrosa*	Wageningen, Gelderland, NL	rec-1
Flamingo	*Triticum aestivum*	Wageningen, Gelderland, NL	rec-1
S5-1	*Triticum aestivum*	Spain	rec-1
S5-3	*Triticum aestivum*	Spain	rec-1
P3797	*Triticum aestivum*	Portugal	rec-1
C.S. 92.038	*Triticum aestivum*	Israel	rec-1
C.S. 92.040	*Triticum aestivum*	Israel	rec-1
CPRO	*Triticum aestivum*	Wageningen, Gelderland, NL	rec-2
C.S. 92.099	*Agropyron repens*	Wageningen, Gelderland, NL	rec-3
C.S. 93.021	*Agropyron repens*	Stokhem, Limburg, NL	rec-3
C.S. 92.009	*Agropyron repens*	Rhenen, Utrecht, NL	rec-4
C.S. 93.009	*Agropyron repens*	't Goy, Utrecht, NL	rec-4
IVP	*Hordeum jubatum*	Wageningen, Gelderland, NL	rec-4
9233	*Aegilops ovata*	Israel	rec-5

P. recondita (continued on next page)

Table 22, continued

collection	host	origin	EP
IVP	*Secale cereale*	Wageningen, Gelderland, NL	rec-6
C.S. 89.007	*Secale cereale*	Ottersum, Limburg, NL	rec-7
RMS1	*Secale cereale*	monospore culture C.S. 89.007	rec-7
RMS2	*Secale cereale*	monospore culture C.S. 89.007	rec-7
RMS3	*Secale cereale*	monospore culture C.S. 89.007	rec-7
RMS5	*Secale cereale*	monospore culture C.S. 89.007	rec-7
C.S. 92.036	*Secale cereale*	Israel	rec-8
C.S. 92.090	*Holcus lanatus*	Nideggen, Eifel, Germany	rec-9
C.S. 93.012	*Holcus lanatus*	Gameren, Gelderland, NL	rec-9
C.S. 93.022	*Holcus lanatus*	Rhenen, Utrecht, NL	rec-9
C.S. 92.097	*Lolium perenne*	Nideggen, Eifel, Germany	rec-10
C.S. 93.024	*Lolium perenne*	Wageningen, Gelderland, NL	rec-10
C.S. 93.029	*Lolium perenne*	Sy, Ardennes, Belgium	rec-10
C.S. 90.025	*Bromus sterilis*	Utrecht city, NL	rec-11
C.S. 91.015	*Bromus sterilis*	Wijlre, Limburg, NL	rec-11
C.S. 92.013	*Bromus sterilis*	Cambridge, GB	rec-11
C.S. 93.011	*Bromus hordeaceus*	Wageningen, Gelderland, NL	rec-12
C.S. 93.013	*Bromus hordeaceus*	Gameren, Gelderland, NL	rec-12
C.S. 93.017	*Bromus hordeaceus*	Schin op Geul, Limburg, NL	rec-12
C.S. 93.014	*Alopecurus pratensis*	Gameren, Gelderland, NL	rec-13
C.S. 93.019	*Arrhenatherum elatius*	Eys, Limburg, NL	rec-14
C.S. 93.030	*Arrhenatherum elatius*	Sy, Ardennes, Belgium	rec-14
C.S. 93.032	*Arrhenatherum elatius*	Wageningen, Gelderland, NL	rec-14
Puccinia striiformis			
C.S. 92.019	*Poa pratensis*	't Goy, Utrecht, NL	str-1
C.S. 93.038	*Poa pratensis*	Wageningen, Gelderland, NL	str-1
C.S. 93.039	*Poa pratensis*	Wageningen, Gelderland, NL	str-1
C.S. 93.027	*Poa pratensis*	Sy, Ardennes, Belgium	str-2
C.S. 93.036	*Dactylis glomerata*	Duddle Door, Devon, GB	str-3

Puccinia striiformis (continued on next page)

Table 22, continued

collection	host	origin	EP
C.S. 93.037	*Dactylis glomerata*	Rhenen, Utrecht, NL	str-3
C.S. 93.031	*Bromus carinatus*	Wageningen, Gelderland, NL	str-4
64008	*Hordeum vulgare*	Belgium	str-4
86018	*Hordeum vulgare*	Nepal	str-4
81550	*Hordeum vulgare*	NL	str-4
IVP	*Triticum aestivum*	Wageningen, Gelderland, NL	str-4
60018	*Triticum aestivum*	NL	str-4
66049	*Triticum aestivum*	NL	str-4

[a] Designation of collections as in the Appendix.

Enzyme staining

A total of 15 enzyme systems were tested for activity, resolution, consistent appearance of bands, optimal gel type, and spore concentration with urediniospores of the 'house' collections of the rusts listed above. Nine enzyme systems that gave good resolution and consistent appearance of bands, were tested with the 12 isolates of Barley leaf rust and the 7 of Wheat leaf rust to screen for intraspecific variation. Gels were stained using standard procedures (Vallejos, 1983).

Main experiment

Isolates

Ninety isolates (collections) representing the five large species complexes *Puccinia brachypodii*, *P. coronata*, *P. hordei* (including *Uromyces viennot-bourginii* and *U. christensenii*), *P. recondita*, and *P. striiformis* were collected from several host species. In total 30 rust species/host species combinations were studied. For each of the 30 combinations, isolates from — where possible — three locations from various countries were studied. Most isolates were field collections made in the period from 1989 to 1993. Some isolates were obtained from other institutes (Table 22). Collections made before 1993 (collection number starting with 89, 90, 91 or 92) had been stored in liquid nitrogen. In spring and summer 1993 urediniospores of the liquid nitrogen collections were multiplied on (seedling) leaves of the appropriate host species. Field collections made in 1993 were multiplied on the original host. Twice a week urediniospores were scraped off the leaves with a knife and isolates were stored in an ultra-low temperature freezer, at -80 °C, in portions of appr. 1 mg.

Table 23. Enzyme systems (abbreviation) and EC code, resolution (res), preferred PhastGel type (gel) and spore concentration (% sp) for the enzyme systems tested in this study. nr = no satisfactory results obtained (see text).

enzyme system (abbrevation)	res[a]	gel	% sp[b]
alcohol dehydrogenase (ADH) E.C. 1.1.1.1	0	nr	nr
isocitrate dehydrogenase (IDH) E.C. 1.1.1.42	0	nr	nr
acid phosphatase (ACP) E.C. 3.1.3.2	−	nr	nr
glucose-6-phosphate dehydrogenase (G6PDH) E.C. 1.1.1.49	−	nr	nr
glutamate oxaloacetate transaminase[c] (GOT) E.C. 2.6.1.1	−	nr	nr
shikimate dehydrogenase (SDH) E.C. 1.1.1.25	−	nr	nr
esterase (EST) E.C. 3.1.1.2	+	8–25	4[d]
hexokinase (HK) E.C. 2.7.1.1	+	8–25	2
malate dehydrogenase (MDH) E.C. 1.1.1.37	+	10–15	1
phosphoglucomutase (PGM) E.C. 2.7.5.1	+	8–25	2
diaphorase[e] (DIA) E.C. 1.6.4.3	++	10–15	4
phospho glucoisomerase[f] (PGI) E.C. 5.3.1.9	++	8–25	0.5
leucine aminopeptidase[g] (LAP) E.C. 3.4.11.1	++	10–15	4[h]
glutamate dehydrogenase (GDH) E.C. 1.4.1.2	++	8–25	4
6-phosphogluconate dehydrogenase (6-PGDH) E.C. 1.1.1.44	++	8–25	4

[a] 0: no activity; −: poor resolution; +: good resolution, variation in banding patterns within a taxon; ++: good resolution, no or little variation within taxa.
[b] 4% corresponding to 1 mg urediniospores in 25 µl electrophoresis buffer.
[c] Also named aspartate aminotransferase (AAT).
[d] For *Puccinia coronata* 8%; *P. recondita* from *Holcus lanatus* and *Lolium perenne* had no detectable activity.
[e] Also named menadione reductase (MR).
[f] Also named glucose phosphate isomerase (GPI).
[g] Also named aminopeptidase.
[h] For *P. coronata* and *P. striiformis* 8%.

Electrophoretic analysis of isozymes

Electrophoresis was as described for the preliminary experiment.

Enzyme staining

Five enzyme systems, diaphorase (DIA), phospho glucoisomerase (PGI), leucine aminopeptidase (LAP), glutamate dehydrogenase (GDH) and 6-phosphogluconate

dehydrogenase (6-PGDH), were tested. These enzyme systems could be tested in three separate runs on one day using the same samples. Most of the samples were tested once.

Scoring gels

Isozyme banding patterns for each of the isolates were scored by comparing to the banding pattern of the Wheat leaf rust isolate Flamingo (*Puccinia recondita/ Triticum*) which was included in every run. For each enzyme system the various banding patterns observed were numbered. Relative mobility (Rm) of the slowest and fastest bands was calculated as a percentage of the distance travelled by a band and the total length of the gel, both distances measured from the origin. Five composite photographs, one of each enzyme system, are reproduced to show some isozyme banding patterns. Isozyme phenotypes of all isolates are tabulated per species complex, and isolates having the same combination of banding patterns over the enzyme systems were designated an electrophoretic phenotype (EP). The first three letters of a species complex were used to designate an EP, e.g. 'cor-' and 'rec-' are EPs from *P. coronata* and *P. recondita*, respectively. A diagram was produced to visualize the number and name of enzyme systems that each EP shared with another EP.

Results

Preliminary experiment

Of the 15 enzyme systems tested, no activity was obtained for alcohol dehydrogenase and isocitrate dehydrogenase (tested on PhastGel gradient 8–25% with a 4 and 3.2% spore concentration, respectively). Poor resolution was obtained for acid phosphatase and glucose-6-phosphate dehydrogenase (both tested on PhastGel gradient 8–25% with a 4 and 2, and a 4% spore concentration, respectively), glutamate oxaloacetate transaminase (tested on PhastGel homogeneous 12½% and on PhastGel gradients 4–15% and 8–25% with a 4% spore concentration) and shikimate dehydrogenase (tested on PhastGel gradients 4–15% and 8–25% with a 4 and 2% spore concentration) (Table 23).

Sufficient high activity and resolution of bands were obtained with the enzymes diaphorase (DIA), esterase (EST), glutamate dehydrogenase (GDH), hexokinase (HK), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), phosphoglucomutase (PGM), phospho glucoisomerase (PGI), and 6-phosphogluconate dehydrogenase (6-PGDH) (Table 23).

Testing the latter nine enzyme systems with the 12 isolates of Barley leaf rust and the seven of Wheat leaf rust gave some variation in banding patterns among collections within these species for EST, HK, MDH, and PGM. Therefore, these enzyme systems were not used in the main experiment.

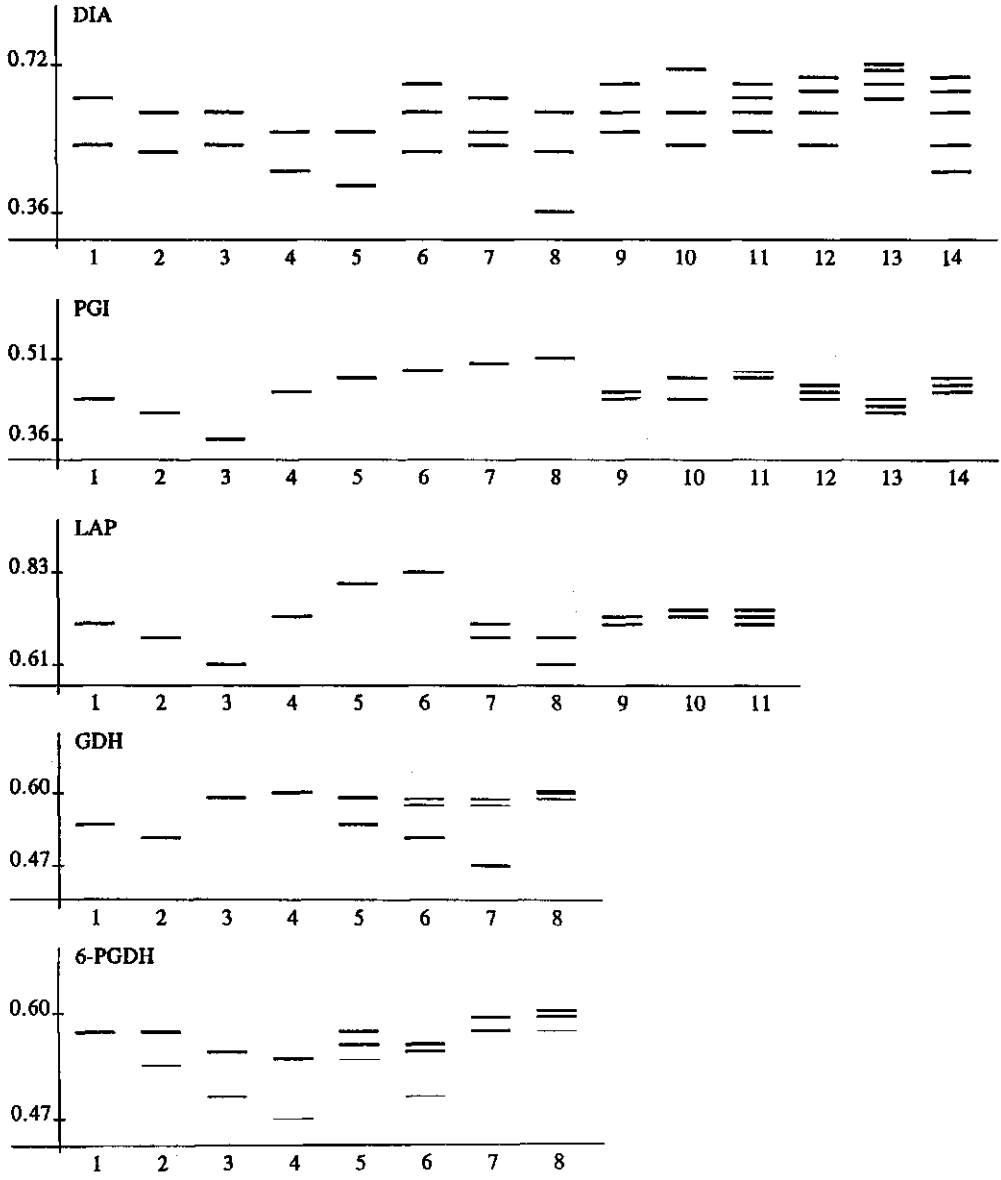


Figure 22. Schematical representation of the isozyme banding patterns of five enzyme systems observed in *Puccinia brachypodii, P. coronata, P. hordei, P. recondita* and *P. striiformis*. The anode is at the top in all cases. Rm values of the fastest and slowest bands are given (for coordination see Table 24). Banding patterns of each enzyme system are numbered consecutively. Enzyme systems are diaphorase (DIA), phospho glucoisomerase (PGI), leucine aminopeptidase (LAP), glutamate dehydrogenase (GDH) and 6-phosphogluconate dehydrogenase (6-PGDH).

The remaining five enzyme systems, DIA, PGI, LAP, GDH, and 6-PGDH, showed no or a limited amount of variation in banding patterns between collections and were selected for use in the main experiment. For GDH only the blue bands in the anodal region were screened as the blue bands in the kathodal region and the negative bands in the central region of the gel were inconsistently present (Fig. 23A).

Occasionally one of the collections of *P. hordei* or *P. recondita/Triticum* showed an isozyme banding pattern distinct from the others of the same species. Such an isozyme variation was observed for a colour mutant of *P. hordei* isolate 1-2-1 for PGI and LAP (compare EPs hor-1 and hor-3 in Table 24) and for *P. recondita/Triticum* isolate CPRO for LAP (compare rec-1 and rec-2 in Table 24).

In general, no differences were found between separate runs of the same collection. When one or all bands were missing or very faintly present, this could be attributed to too low a spore concentration (Table 23).

In the course of the experiment freshly collected urediniospores and urediniospores stored in liquid nitrogen, in an ultra-low temperature freezer at -80 °C, or for some time in an exsiccator, were used. Banding patterns observed for DIA, PGI, LAP, GDH, and 6-PGDH were identical between urediniospore samples, regardless of the method of storage.

Main experiment

A total of 56 different banding patterns was obtained with the five enzyme systems among the 90 collections (Fig. 22), and 32 electrophoretic phenotypes (EPs, collections with a distinctive combination of isozyme phenotypes) were distinguished (Tables 22 and 24).

As expected from the preliminary experiment, isozyme banding patterns were mostly identical among collections of the same rust species/host species combination, regardless of their geographic origin (Table 22; Fig. 23C, lanes 1–2). Some collections included in the same species complex but from different host species also showed identical EPs. Examples are: EP rec-1, found for collections from *Aegilops squarrosa* and *Triticum aestivum* (Table 22; Fig. 23A, lanes 7–8); EP rec-4 for collections from *Agropyron repens* and *Hordeum jubatum* (Table 22) and EP str-4, found for isolates collected from *Bromus carinatus*, *Hordeum vulgare* and *Triticum aestivum* (Table 22; Fig. 23E, lanes 6–7).

Variation in isozyme banding patterns between different collections from the same rust species/host species combination was also observed, except for 6-PGDH. Such a variation was most commonly found for LAP. Banding patterns for this enzyme system varied in six out of 30 rust species/host species combinations studied, viz. EPs cor-1 and cor-2; cor-1 and cor-3; cor-4 and cor-5; hor-1 and hor-3; rec-1 and rec-2; rec-3 and rec-4 (Figure 23E, lanes 1–2; Table 22). For the enzyme systems GDH, DIA and PGI variation within a rust species/host species combination was relatively rare. Examples are: *P. coronata/Lolium* EPs cor-1 and

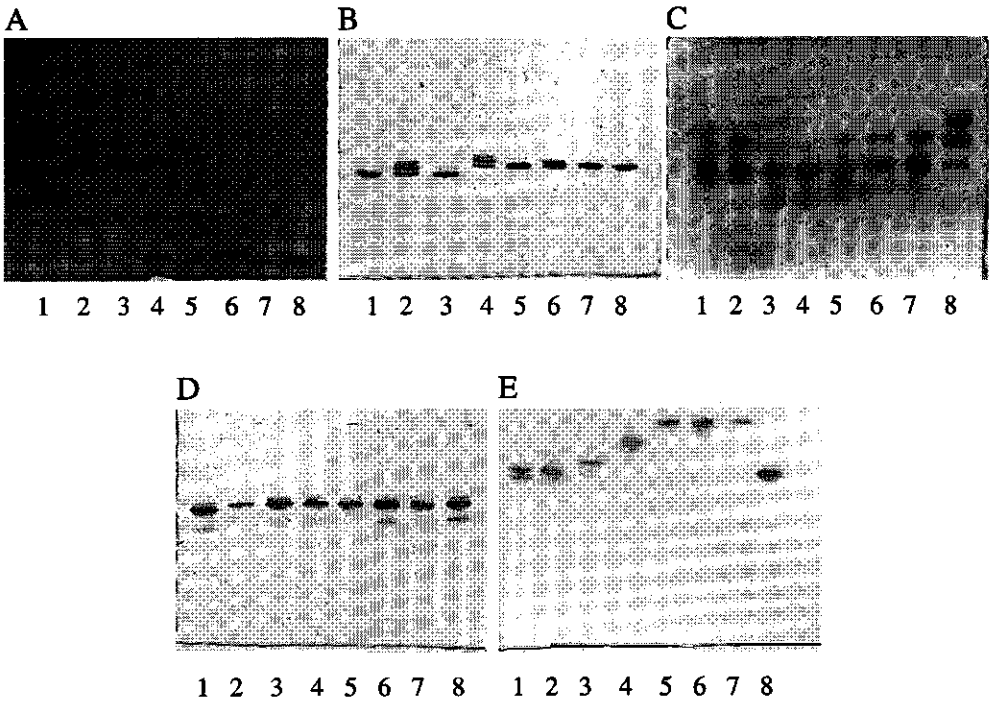


Figure 23. Photographs of selected banding patterns of five enzyme systems in *Puccinia* and *Uromyces* species. The kathode is at the top in all cases. Lane number represents collection designation. Lane 8 is *P. recondita/Triticum aestivum*, isolate Flamingo, in all cases. Refer to Fig. 22 and Table 24 for designation of isozyme banding patterns.

A. GDH. *P. recondita*: 1 = *Lolium perenne*, C.S. 93.024; 2 = *Arrhenatherum elatius*, C.S. 93.019; 3 = *A. elatius*, C.S. 93.030; 4 = *A. elatius*, C.S. 93.032; 5 = *Aegilops ovata*, 9233; 6 = *Secale cereale*, IVP; 7 = *Aegilops squarrosa*, Flamingo.

B. PGI. *P. recondita*: 1 = *Bromus sterilis*, C.S. 91.015; 2 = *B. hordeaceus*, C.S. 93.011; 3 = *Arrhenatherum elatius*, C.S. 93.030; 4 = *Secale cereale*, IVP; 5 = *Holcus lanatus*, C.S. 93.012; 6 = *Lolium perenne*, C.S. 93.024; 7 = *Agropyron repens*, C.S. 93.009.

C. DIA. 1-5, *P. hordei*: 1 = *Hordeum vulgare*, isolate 1-2-1; 2 = *H. vulgare*, isolate 28; 3 = *H. murinum*, IVP; 4 = *H. murinum*, C.S. 90.205; 5 = *H. murinum*, C.S. 90.223; 6 = *Uromyces christensenii/H. vulgare*, Uch-Syria; 7 = *U. viennot-bourginii/H. vulgare*, Uvb-IVP.

D. 6-PGDH. 1 = *P. hordei/H. vulgare*, isolate 1-2-1; 2-5, *P. brachypodii*: 2 = *Poa pratensis*, C.S. 91.047; 3 = *P. annua*, C.S. 93.008; 4 = *P. nemoralis*, C.S. 93.026; 5 = *P. nemoralis*, C.S. 93.033; 6-7, *P. coronata*: 6 = *Avena sativa*, 95-92/3; 7 = *Lolium perenne*, C.S. 93.049.

E. LAP. 1-3, *P. recondita*: 1 = *Agropyron repens*, C.S. 93.021; 2 = *A. repens*, C.S. 93.009; 3 = *Lolium perenne*, C.S. 93.029; 4-7, *P. striiformis*: 4 = *Poa pratensis*, C.S. 93.038; 5 = *Dactylis glomerata*, C.S. 93.037; 6 = *Bromus carinatus*, C.S. 93.031; 7 = *Hordeum vulgare*, 86018.

Table 24. Electrophoretic phenotype (EP) designation[a] and number of collections with a specific EP (N) for isozyme banding patterns of five *Puccinia* species complexes.

EP		enzyme system					N
		DIA	PGI	LAP	GDH	6-PGDH	
Puccinia brachypodii							
bra-1		9	14	7	4	1	1
bra-2		11	14	7	4	1	2
bra-3		9	4	8	4	1	3
bra-4		9	14	8	3	1	1
bra-5		9	5	8	8	1	2
Puccinia coronata							
cor-1		13	3	1	1	2	7
cor-2		13	3	9	5	2	3
cor-3		13	3	11	1	2	1
cor-4		13	3	8	2	2	1
cor-5		13	3	3	2	2	1
Puccinia hordei							
hor-1		2	5	5	4	3	12
hor-2		2	11	5	4	3	1
hor-3		2	11	10	4	3	1
hor-4		8	5	5	4	6	3
Puccinia recondita							
rec-1	A[b]	6	1	1	1	2	7
rec-2	A	6	1	7	1	2	1
rec-3	A	6	1	7	3	2	2
rec-4	A	6	1	1	3	2	3
rec-5	B	5	4	4	2	5	1
rec-6	B	5	12	4	2	7	1
rec-7	B	5	1	4	2	7	5
rec-8	B	5	10	4	2	7	1
rec-9	C	1	1	4	6	3	3
rec-10	D	3	9	4	6	4	3
rec-11	E	2	2	2	5	2	3
rec-12	E	2	13	8	5	2	3
rec-13	E	4	13	7	5	2	1
rec-14	F	2	2	10	7	8	3
Puccinia striiformis							
str-1		14	6	5	4	2	3
str-2		12	6	5	4	2	1
str-3		10	7	6	4	2	2
str-4		7	8	6	4	2	7

[a] Refer to Fig. 22 for enzyme abbreviations and numerical designations of isozyme banding patterns.
[b] Letters A–F refer to six subgroups within *P. recondita* (see text).

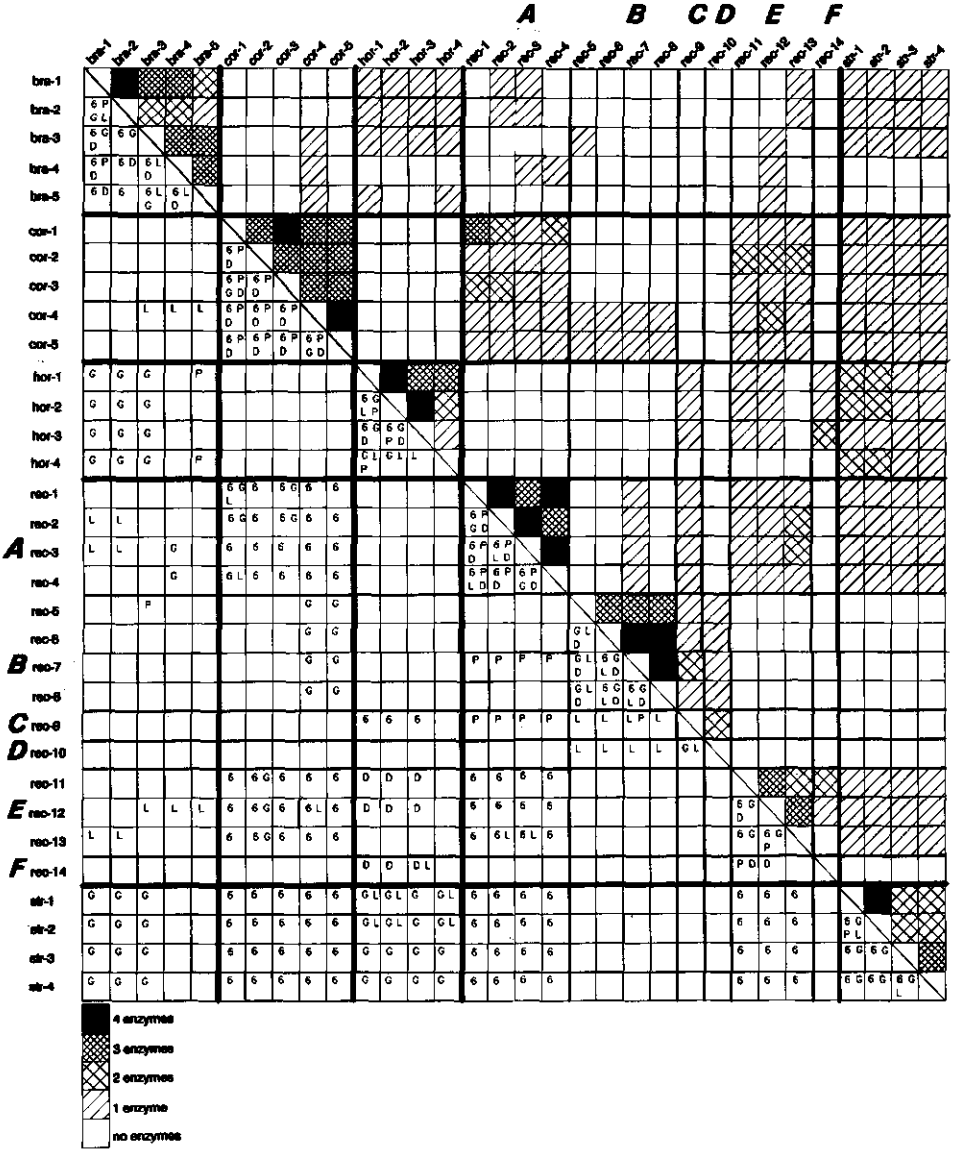


Figure 24. Comparison of electrophoretic phenotypes (EPs) of the five grass rust species complexes *Puccinia brachypodii* (bra-), *P. coronata* (cor-), *P. hordei* (hor-), *P. recondita* (rec-) and *P. striiformis* (str-). Right of the diagonal: numbers of enzyme systems having identical banding patterns between EPs; left of the diagonal: the name(s) of the enzyme system(s) having identical isozyme banding patterns. D = DIA, P = PGI, L = LAP, G = GDH, 6 = 6-PGDH. Letters A–F refer to six subgroups within *P. recondita* (see text).

cor-2 that differed in GDH and LAP banding patterns, *P. brachypodii/Poa pratensis* EPs bra-1 and bra-2, and *P. striiformis/Poa pratensis* EPs str-1 and str-2 that varied in DIA banding patterns and *P. recondita/Secale cereale* EPs rec-6, rec-7 and rec-8 that differed in PGI banding patterns (Tables 22, 24). Each rust species/host species combination that varied in banding patterns between collections never differed for more than two of the five enzyme systems tested.

Differences in isozyme banding patterns were, with the exception of *P. recondita*, always less pronounced within a rust species complex than between therust species complexes (Fig. 24). In general, the banding patterns of three or four enzymes were identical within *P. brachypodii*, *P. coronata*, *P. hordei* and *P. striiformis*. Rarely, the banding patterns of only one or two enzymes were identical. The latter was observed in EP bra-2 with EPs bra-3, bra-4 and bra-5, respectively; EP bra-1 with EP bra-5; EP hor-4 with EPs hor-2 and hor-3, respectively, and EPs str-1 or str-2 with EPs str-3 and str-4, respectively (Fig. 24).

Within *P. recondita*, a considerable variation in isozyme banding patterns was apparent. Six groups based on banding patterns were observed. Three groups (C, D, F) were specific for collections from one host species and consisted of one EP. The remaining three groups (A, B, E) were specific of collections from several host species and included several EPs (Fig. 24).

Sometimes, one of the EPs of a species complex or *P. recondita* group gave an isozyme banding pattern identical with that of other species complexes or *P. recondita* groups. An example is banding pattern 2 of 6-PGDH, that was found in *P. coronata*, *P. striiformis* and in several *P. recondita* groups (Table 24). For 6-PGDH identical banding patterns were observed in 98 out of 188 tests. For GDH, LAP, PGI, and DIA this was in 58, 33, 12 and 11 tests, respectively.

Some grass species can act as hosts for several rust species. *Arrhenatherum elatius*, for example, may be host of *P. brachypodii* s.l., *P. coronata* s.l. and *P. recondita* s.l. Urediniospores of each species complex had entirely different isozyme banding patterns, viz. EPs bra-5, cor-4, cor-5, and rec-14, respectively (Table 24).

Discussion

Usefulness of the PhastSystem

The PhastSystem proved to be very useful for isozyme electrophoresis of grass rust fungi. A pilot study had shown that the PhastGels were too small to obtain good resolution of bands for total soluble protein electrophoresis (data not shown). A small gel length also limits the resolution of total protein bands in disc electrophoresis (Shipton & Fleischmann, 1969).

One mg of urediniospores mixed with 25 μl of electrophoresis buffer (= spore concentration 4%) gave resolvable and reproducible bands for most of the enzyme systems studied. For some enzyme systems or taxa a higher or lower spore concentration was required (Table 23).

Most of the enzyme systems studied initially produced resolvable bands. The resolution of acid phosphatase, glucose-6-phosphate dehydrogenase, glutamate oxaloacetate transaminase and shikimate dehydrogenase might be improved by using gels of different pore size, pH gradient gels, isoelectric focusing gels, or by applying other staining methods.

Value of isozyme variation for identification of grass rust fungi

This is the first report on isozyme banding patterns of a large collection of grass rust fungi collected from diverse geographic locations. Among the 30 rust species/ host species combinations studied, large differences in isozyme banding patterns were observed.

Several observations indicate that the isozyme banding patterns obtained are a genuine property of the rust population examined and not influenced by the host on which the urediniospores are formed. Firstly, rust isolates known to sporulate on several host species produced identical isozyme banding patterns. This was observed for the Wheat leaf rust isolate Flamingo, collected from *Triticum aestivum* and *Aegilops squarrosa* (Fig. 23A, lanes 7–8; rec-1 in Table 22) and for Couch grass leaf rust collected from *Agropyron repens* and *Hordeum jubatum* (Table 22). Similar results were reported by Bruckart & Peterson (1991) for *Puccinia carduorum* Jacky pathogenic on both *Cynara cardunculus* and *Carduus pycnocephalus*. Shipton & Fleischmann (1969) did not find marked differences in total soluble protein patterns of several *formae speciales* of *P. graminis* growing on different host species, which again indicates that the host species has no significant effect on banding patterns. An additional study of isozyme patterns of *P. graminis* taxa collected from several host species would be required to elaborate this aspect. Secondly, when a grass species (e.g. *Arrhenatherum elatius*) was a host for different rust taxa, isozyme banding patterns largely differed between these taxa (compare EPs bra-5, rec-14, cor-4 and cor-5). Thirdly, collections within a rust taxon from various geographic regions and also from various host plant genotypes mostly showed identical banding patterns (Table 22). A similar trend was also observed in powdery mildews (Clark *et al.*, 1989; Koch & Köhler, 1991). Age of the host plant or the urediniospores did not influence the banding patterns of the enzyme systems studied. No differences were observed between urediniospores collected from seedlings, adult plants, young sori or older sori or spores stored in different ways (own observations).

Variation in isozyme banding patterns was observed in both populations with known aecial hosts (e.g. collections studied from *P. recondita/Triticum aestivum* and *P. recondita/Secale cereale*) and in taxa of which the aecial host is unknown (e.g. *P. striiformis*). The suggestion of Burdon & Roelfs (1985a) that variation in asexually-reproducing populations originates from a former sexual cycle, may be true for those cases in which the aecial host occurs in other parts of the world (*P. recondita/Triticum aestivum* and *P. recondita/Secale cereale*). However, other factors should be considered to explain the variation in asexual and sexual taxa

without local aecial hosts. Mutations, e.g. colour mutants (Samborski, 1985), may explain some of the variation. The colour mutant of *P. hordei* studied here clearly differed from the parental isolate 1-2-1 (cp. EP hor-1 and hor-3 in Table 24), but showed an identical virulence pattern (Niks & Dekens, pers. comm.).

The observed variation in isozyme banding patterns between collections from the same rust species/host species combination confirms previously published observations (Burdon *et al.*, 1982; Burdon & Roelfs, 1985a,b; Lu & Groth, 1987; Linde *et al.*, 1990; Park & Burdon, 1992), indicating that a certain degree of isozyme variation occurs among populations of rust fungi subsumed under the same taxon.

In the present study 6-PGDH proved to be a uniform enzyme system, showing no variation between collections of the same rust species/host species combination. DIA, PGI, and GDH showed a limited amount of variation and LAP showed considerable variation between collections of the same rust combination. Probably LAP is a rather variable enzyme system. Burdon & Roelfs (1985a,b) also reported variation for LAP banding patterns in sexual and asexual populations of *Puccinia graminis* f.sp. *tritici*. Nevertheless, the 11 different patterns of LAP proved useful to recognize several EPs (Fig. 22; Table 24), but due to this variability LAP will be a less distinctive enzyme system. Alhough an isozyme banding pattern of a specific enzyme system was often observed in several rust species complexes (Fig. 24), the combination of the five enzymes selected was valuable for the recognition of all species complexes and most rust species/host species combinations (Table 22).

The considerable variation in isozyme banding patterns between species complexes and the small differences between collections within a species complex, with the exception of *P. recondita*, agree with the findings of Burdon & Marshall (1981). Contrary to the findings of Newton *et al.* (1985), no differences were observed between *P. striiformis* collected from *Triticum* and *Hordeum*. However, the enzyme systems showing variation between these taxa, viz. acid phosphatase and catalase, were not studied in the main experiment. The five enzyme systems studied here may be too few to reveal differences between some *formae speciales*, and some other enzyme systems might be included for this purpose.

In conclusion, this research indicates that the PhastSystem is useful for isozyme analysis of grass rust fungi. The five enzyme systems DIA, PGI, LAP, GDH, and 6-PGDH together are valuable for the identification of most rust species/host combinations. However, the inclusion of other enzyme systems and more collections will certainly enlarge the taxonomic value of isozyme analysis.

Taxonomic implications of this study are discussed in Chapter 7.

7 GENERAL DISCUSSION

The present study aimed to assess the value of germling morphology of urediniospores for the identification and classification of grass rust fungi. It has been demonstrated that the germling morphology is a reliable taxonomic criterion, because

i. it was not influenced by environmental conditions, provided that temperatures are not extremely high (Chapters 3 and 5);
ii. it was consistent and sufficiently uniform within an (infraspecific) taxon (Chapter 4);
iii. the differences in germling morphology between taxa were more distinctive than the often minor differences in characters of sori and spores (Chapters 2 and 4). Germling morphology showed quantitative variation within a species complex, except *Puccinia recondita* s.l. and *P. brachypodii* s.l. in which the differences between included taxa were mostly qualitative. In some cases the differences could be quantitative. The differences between species complexes were, in general, qualitative;
iv. in a few cases it was observed that collections belonging to (postulated) different species had identical or similar germling morphology, viz. (i) *P. hordei* and *Uromyces* species occurring on barleys (*U. viennot-bourginii*, *U. reichertii*, *U. hordeastri* and *U. christensenii*), (ii) *P. recondita/Lolium* with *P. hordei* + the four *Uromyces* spp. on barleys and (iii) *P. recondita/Alopecurus pratensis* with *P. graminis*. The similarity in germling morphology in the first case supports the view that these species are closely related (Orton, 1912). The similarities in the latter two cases may be caused by convergent evolution, since these taxa differ in morphological characters of sori and spores and in host range.

The use of germling morphology for species delimitation of selected grass rust fungi can be further evaluated by comparing it to characters of sori and spores, isozyme banding patterns, nuclear DNA content, and literature data.

Conclusions on the taxonomy of grass rust fungi

Delimitation of species complexes

Rust fungi belonging to *P. coronata*, *P. graminis*, *P. striiformis* and *P. brachypodii* s.l. were easily distinguished from each other by germling morphology of the urediniospores (Chapter 4; Niks, 1986), by isozyme banding patterns of the urediniospores (Section 6.2), but also by morphological characters of uredinia, urediniospores, telia, and teliospores (Chapter 2). The substantial morphological differences between these species (complexes) in characters of uredinia and telia

render observations of the germling morphology and isozyme banding patterns superfluous for identification, but these and other biochemical or molecular traits may be valuable for delimitation of infraspecific taxa.

Rust fungi belonging to *P. hordei* s.l. and *P. recondita* s.l. are not reliably distinguishable on the basis of characters of uredinia and urediniospores. Only characters of telia and teliospores, which may not be present on every collection, allow a distinction between these species (Chapter 2). Germling morphology (Sections 4.4, 4.5) and isozyme banding patterns (Section 6.2) differed clearly between these two complexes. Therefore, they are very valuable for the recognition of rust collections of cereals and grasses which have brown uredinia and urediniospores with numerous distinct scattered germ pores and no telia.

The taxonomic structure of the species complexes

Puccinia coronata

In crown rusts quantitative differences were observed in size of the urediniospores and in length and width of the substomatal vesicle. Collections from oats (*Avena sativa*) had considerably longer urediniospores and substomatal vesicles than collections from several wild grasses (Table 25; Section 4.1). No clear differences between collections were apparent in characters of teliospores (Table 25).

Isozyme analysis did not clearly distinguish between collections from oats and collections from *Holcus lanatus*, *Lolium perenne* and *Arrhenatherum elatius*. Three of the five enzyme systems studied gave identical banding patterns and the other two enzymes (GDH and LAP) showed variation between collections from the same host species. The collections from *A. elatius* differ in banding patterns of the variable enzyme systems GDH and LAP from the other collections studied (Tables 22, 24, 25). The variation observed in banding patterns is considered too large to allow a further infraspecific division of *P. coronata* on the basis of isozyme electrophoresis. Burdon & Marshall (1981) also reported a high similarity between *P. coronata* collections from *Avena sativa*, *Lolium perenne* and *Festuca arundinacea*. Since in Australia the various crown rust fungi have a common host in *Lamarckia aurea*, Burdon & Marshall (1981) suggested that the high degree of isozyme uniformity within this species can be maintained by somatic hybridization and introgression on that host species. In the Netherlands *L. aurea* is rarely found (Heukels & van Ooststroom, 1962). It is unknown whether in the Netherlands or western Europe another common host for *P. coronata* occurs. Sexual recombination will not be important in Europe, since crown rust fungi presumably overwinter in the absence of the aecial host (Urban, 1969; Ullrich, 1977). Searches for a common host and additional studies on isozyme patterns will be useful to further evaluate the isozyme uniformity observed in *P. coronata*.

In crown rusts the germling morphology supports the recognition of two varieties within *P. coronata* Corda, viz. *P. coronata* var. *avenae* Fraser & Led. and *P. coronata* var. *coronata* (Section 4.1), which were distinguished by i.a. Urban (1969) on the basis of quantitative differences in urediniospores and teliospores and

Table 25. Taxonomic concepts for the combinations of rust species complex–host species based on characters of urediniospores (Ur), teliospores (Te), germling morphology (GM), isozyme banding patterns (IB), and nuclear DNA content (nD) (data from Chapters 4 and 6 and literature). – = no data available.

rust species complex	taxon recognized	Ur[a]	Te[a]	GM[a]		IB[b]	nD[a]
host species				ql	qn		
Puccinia coronata							
Avena spp.	*P. cor.* var. *avenae*	a	a		a	a	–
Holcus spp.	*P. cor.* var. *coronata*	b	a		b	a	–
Lolium spp.	*P. cor.* var. *coronata*	b	a		b	a	–
Arrhenatherum elatius	*P. cor.* var. *coronata*	b	a		b	a	–
Puccinia graminis							
Avena spp.	*P. gra.* f.sp. *avenae*	a	a		a	a[c]	–
Secale spp.	*P. gra.* f.sp. *secalis*	a	a		b	b	–
Triticum spp.	*P. gra.* f.sp. *tritici*	ab	a		bc	bc	–
Puccinia striiformis							
Triticum spp.	*P. str.* var. *striiformis*	a[d]	a[d]	a[d]		a	–
Bromus carinatus	*P. str.* var. *striiformis*	a	–	a		a	–
Hordeum spp.	*P. str.* var. *striiformis*	ab	a	a		a	–
Poa pratensis	*P. str.* var. *poae*	b	ab	b		b	–
Dactylis glomerata	*P. str.* var. *dactylidis*	b	ab	c		c	–
Puccinia brachypodii							
Arrhenatherum elatius	*P. magelhaenica*	a	a	a		a	–
Poa annua	*P. poae-nemoralis*	a	a	b	a	b	–
Poa nemoralis	*P. poae-nemoralis*	a	a	b	b	b	–
Poa palustris	*P. poae-nemoralis*	a	a	b	c	–	–
Poa pratensis	*P. poae-nemoralis*	a	a	b	d	c	–
Anthoxanthum odoratum	*P. poae-nemoralis*	a	–	b	e	b	–
Deschampsia cespitosa	*P. deschampsiae*	a	–	c		–	–
Brachypodium pinnatum	*P. brachypodii*	b	a	d	a	–	–
Brachypodium sylvaticum	*P. brachypodii*	b	a	d	b	–	–

(continued on next page)

Table 25, continued

rust species complex host species	taxon recognized	Ur	Te	GM ql	 qn	IB	nD
Puccinia recondita							
Aegilops squarrosa	*P. triticina*	a	a	a		a	–
Triticum spp.	*P. triticina*	a	a	a		a	a
Agropyron repens	*P. agropyrina*	a	a	b		a+	a
Hordeum jubatum	*P. agropyrina*	a	a	b		a+	–
Aegilops ovata	*P. recondita*	a	a	c	a	b	–
Secale cereale	*P. recondita*	a	a	c	a	b+	ab
Arrhenatherum elatius	*P. arrhenathericola*	b	a	c	b	c	–
Alopecurus pratensis	*P. perplexans*	c	a	d		d	–
Bromus hordeaceus	*P. bromina* var. *bromina*	d	a	e		e	c
Bromus sterilis	*P. bromina* var. *bromina*	d	a	f		f	ab
Holcus spp.	*P. holcina*	d	a	g		g	–
Lolium perenne	*P. loliina*	e	a	h		h	–
Bromus erectus	*P. bromina* s.l.	a	–	i		–	–
Bromus inermis	*P. bromina* var. *paucipora*	e	a	j		–	–
Agropyron junceiforme	*P. agropyri-juncei*	a	a	k		–	–
Trisetum flavescens	*P. triseti*	f	a	l		–	–
***Puccinia hordei* + *Uromyces* spp.**							
Hordeum vulgare (*P. hordei*)	*P. hordei*	a	a		a	a	–
Hordeum murinum (*P. hordei*)	*P. hordei*	a	ab		a	b	–
Hordeum vulgare (*U. viennot-bourginii*)	*P. hordei*	a	b		a	a	–
Hordeum vulgare (*U. christensenii*)	*P. hordei*	a	b		a	a	–

[a] Different letters in each column within a species complex indicate pronounced differences between hosts. qn = quantitiative differences; ql = qualitative differences in germling morphology.

[b] Different letters in each column within a rust species complex indicate differences in isozyme banding patterns of at least two enzyme systems, a "+" indicates a difference in only one enzyme system.

[c] IB data for *P. graminis* have been compiled from Burdon & Marshall (1981).

[d] Ur, Te and IB data for *P. striiformis* have been compiled from Niks (1989) and own measurements.

in host range. Germling morphology indicates that the collections of *P. coronata/A. elatius* are closer to *P. coronata* var. *coronata* than to *P. coronata* var. *avenae*.

Separating this rust fungus as *P. coronata* var. *avenae* f.sp. *graminicola* (Urban, 1967) instead of including it in *P. coronata* var. *coronata* does not seem justified (Table 25). Due to the similarity in banding patterns of 6-PGDH, PGI and DIA among all *P. coronata* collections and variation in GDH and LAP bands in collections from the same host species, these isozyme systems are not suitable for the distinction of infraspecific taxa within *P. coronata*.

In conclusion, *P. coronata* Corda is maintained as one species with two varieties, viz. var. *avenae* Fraser & Led. and var. *coronata*, because all collections studied differ only quantitatively in morphological characters of sori, spores and germling morphology. The isozyme banding patterns were very variable.

Puccinia graminis

In stem rusts some quantitative differences were observed in characters of urediniospore germlings. Collections from *Triticum* and *Secale* were closer to each other (though distinct) than to collections from *Avena* (Section 4.2; Table 25). These observations are not supported by urediniospore measurements: urediniospore dimensions of collections from *Avena* and *Secale* were similar, but somewhat distinct from those from *Triticum* (Table 25). Collections from all three hosts were similar in characters of teliospores (Table 25).

However, collections from *Triticum* and *Secale* were also closer to each other (though distinct) than to those from *Avena* in studies using various other techniques, like isozyme electrophoresis (Burdon & Marshall, 1981; Table 25), two-dimensional polypeptide mapping (Kim *et al.*, 1987), RFLPs of mitochondrial DNA (Sock & Kolmer, 1993), sequence variation of the ribosomal DNA internal transcribed spacer (Zambino & Szabo, 1993), and interfertility studies (Johnson *et al.*, 1932; Green, 1971).

Within *P. graminis* the wide host range of many isolates (Section 4.2) could facilitate cross-flow of genetic material through somatic hybridization on the telial host and through sexual recombination on the aecial hosts *Berberis* and *Mahonia*. This cross-flow could reduce the differences expressed in spore morphology between rust isolates (Savile, 1984). But small morphological differences in germling morphology between collections from the various hosts were apparent (Table 25), which are also supported by several reports on molecular and biochemical traits and interfertility studies.

The present study indicates that merging *P. graminis* from *Avena* and *Secale* in one variety, *P. graminis* subsp. *graminis* var. *stakmanii* Guyot, Massenot & Saccas ex Urban (Urban, 1967) is not justified, since they differ in germling morphology (present study) and isozyme banding patterns (Burdon & Marshall, 1981). The differences observed suggest a subdivision of the rust fungi from these

two genera and from *Triticum* in three separate infraspecific taxa. Since only a few collections of grasses and cereals were studied, additional studies on germling morphology and molecular and biochemical traits of stem rust fungi are required to evaluate the level of variation within this species and the validity to recognize infraspecific taxa within *P. graminis*. The need for additional studies was also indicated by Zambino & Szabo (1993). DNA sequences of the rDNA internal transcribed spacer regions did not show any differences between *P. graminis/Avena* and *P. graminis/Poa* and suggested their identity.

In conclusion, due to the limited data on germling morphology and molecular and biochemical traits of stem rust fungi from wild grasses, the recognition of two subspecies within *P. graminis* Pers. : Pers., viz. subsp. *graminicola* Urban occurring mainly on wild grasses and subsp. *graminis* occurring mainly on cereals, cannot yet be supported. It seems to be justified not even to recognize varieties within *P. graminis* subsp. *graminis*.

For the time being, it is suggested to assign no taxonomic rank to the collections observed from the cereals but to refer to them as *formae speciales*, viz. *P. graminis* f.sp. *avenae* Erikss. & E. Henn., *P. graminis* f.sp. *secalis* Erikss. & E. Henn. and *P. graminis* f.sp. *tritici* Erikss. & E. Henn. The distinction at a higher taxonomic level of the collections from cereals and those from wild grasses and/or the inclusion of additional taxa from wild grasses will have to be considered after obtaining additional data.

Puccinia striiformis

In *P. striiformis* s.l. Niks (1986, 1989) observed three types of germling morphology. Two types were each specific of *Poa pratensis* and *Dactylis glomerata*, respectively, and one type was found in collections of *Triticum* and *Hordeum*. Observations of the urediniospore germling morphology of the collections used in the isozyme study (Section 6.2) and of some additional collections confirmed the results of Niks (1986, 1989). Besides, a stripe rust collected from *Bromus carinatus* in Wageningen had the same germling morphology as the germlings of urediniospores collected from *Triticum* and *Hordeum* (Swertz, unpublished data). Recognition of the mainly qualitative differences between the three types of germling morphology in stripe rust collections is easier than that of the mainly quantitative differences in urediniospore and teliospore characters (Table 25).

Isozyme banding patterns were also identical for collections obtained from *Triticum*, *Hordeum* and *Bromus carinatus*. Collections from *P. pratensis* and *D. glomerata* had isozyme patterns distinct from each other and from the collections of the other hosts (Section 6.2; Table 25).

Newton *et al.* (1985) also studied isozyme patterns of stripe rust fungi collected from *Triticum* and *Hordeum*. They found that collections from *Triticum* and *Hordeum* (which they named f.sp. *tritici* and f.sp. *hordei*) differed in banding patterns of two out of 13 enzyme systems studied, viz. catalase and acid phosphat-

ase, two enzyme systems not examined in the present study. This is an argument in favour of inclusion of other enzyme systems.

So far two varieties and one *forma specialis* have been recognized within *P. striiformis* Westend., viz. var. *striiformis* and var. *dactylidis* Manners and f.sp. *poae*. This distinction was based on differences in host range (Britton & Cummins, 1956; Ullrich, 1976), temperature optimum for germination of the urediniospores (Tollenaar, 1967; Tollenaar & Houston, 1967), and urediniospore size (Urban, 1969; Manners 1960; Ullrich, 1977). The variation and similarities observed in germling morphology and in isozyme banding patterns between collections studied here supports the recognition of three separate taxa within *Puccinia striiformis*. Since the differences in germling morphology and isozyme patterns are as large between collections from *D. glomerata* and *P. pratensis* as between these two host species and collections of the other hosts it is suggested to raise the *P. striiformis* rust pathogenic on *P. pratensis* to the variety level.

In conclusion, *Puccinia striiformis* is maintained as one species, since all collections studied had linearly arranged, pale yellowish sori and the urediniospore germlings did not form an appressorium. They all formed a spheroid substomatal vesicle with some primary infection hyphae. Based on qualitative differences in germling morphology and isozyme banding patterns, it is suggested to recognize three varieties, viz. *P. striiformis* Westend. var. *striiformis*, *P. striiformis* var. *dactylidis* Manners, and *P. striiformis* var. *poae* Swertz, var. nov.

Puccinia striiformis* var. *poae Swertz, *var. nov.*

Puccinia striiformis f.sp. *poae* Tollenaar & Houston. *Can. J. Bot. 45: 291-307* (1967).

A varietatibus *striiformi* et *dactylidis* differt (i) vesiculis substomatalibus saepe collapsis corpora irregularia proferentibus e quibus (1–)2–4 hyphae inficientes primariae oriuntur; hyphae distaliter vulgo haud dilitatae, 5–8 μm latae; (ii) fractionibus isoenzymarum diaphorase, phospho glucoisomerase, leucine aminopeptidase; (iii) hospitibus ad species generis *Poae* restrictis; (iv) temperatura optima germinationis urediniosporarum 15–18 °C. Telia raro formata.
Holotypus: ad *Poam pratensem*, Sy in Belgio, 6-6-93, herb. L 990.354 083.

Puccinia striiformis var. *poae* differs from var. *striiformis* and var. *dactylidis* by (i) often collapsed substomatal vesicles from which a second irregularly shaped body is formed, which gives rise to (1–)2–4 primary infection hyphae; hyphae mostly not thickened at the ends, 5–8 μm wide (Fig. 25); (ii) isozyme banding patterns of diaphorase, phospho glucoisomerase and leucine aminopeptidase (Fig. 26; Table 24), (iii) the host specialization on species of *Poa*, and (iv) its intermediate temperature optimum for urediospore germination, viz. 15–18 °C, compared with 6–10 and 21–24 °C for var. *striiformis* and var. *dactylidis*, respectively. Telia are rarely formed.

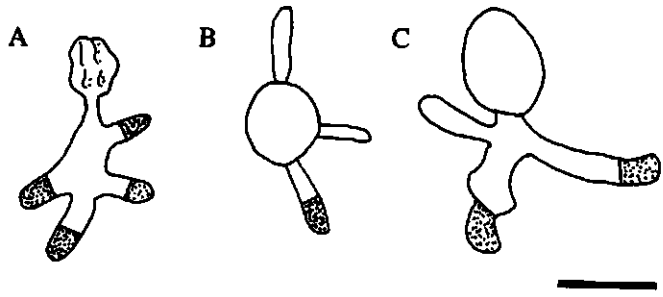


Figure 25. Germling morphology of three varieties of *Puccinia striiformis*, scale bar = 25 μm. **A.** var. *poae* from *Poa pratensis*. **B.** var. *dactylidis* from *Dactylis glomerata*. **C.** var. *striiformis* from *Triticum aestivum*. Figs 25 B, C reproduced from Niks (1986), Can. J. Bot. 64: 2976–2983.

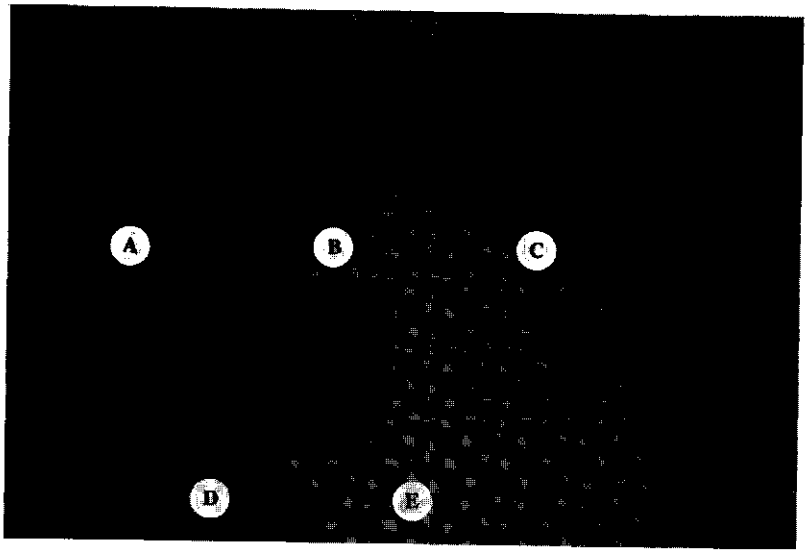


Figure 26. Isozyme banding patterns in three varieties of *Puccinia striiformis* and *P. triticina*. The kathode is at the top in all cases.
A. Diaphorase. **B.** Phospho glucoisomerase. **C.** Leucine aminopeptidase. **D.** Glutamate dehydrogenase. **E.** 6-phosphogluconate dehydrogenase. Lane designation from left to right: var. *striiformis* from *Triticum aestivum* (60018); var. *striiformis* from *Hordeum vulgare* (86018); var. *striiformis* from *Bromus carinatus* (C.S. 93.031); var. *dactylidis* from *Dactylis glomerata* (C.S. 93.037); var. *poae* from *Poa pratensis* (C.S. 93.038), (C.S. 93.027), (C.S. 93.039); *P. triticina* from *Triticum aestivum* (Flamingo).

Puccinia brachypodii

In *P. brachypodii* s.l. four qualitatively different types of germling morphology were observed. Each morphology type was specific of one host genus. Only the rusts from *Poa* and *Anthoxanthum odoratum* studied had the same germling morphology (Section 4.3; Table 25). Besides, collections from different species of either *Poa* or *Brachypodium* showed minor quantitative differences in the numbers of primary infection hyphae (Section 4.3). Differences in morphological characters of uredinia and telia were small between the host species studied, except the rusts collected from *Brachypodium* which were easily recognized by the arrangement of the sori. The germling morphology is suitable to recognize *P. brachypodii* rusts occurring on different host plants (Table 25; Section 4.3).

Isozyme analysis was only conducted with collections from *Arrhenatherum elatius, Poa* spp. and *Anthoxanthum odoratum*. Some differences in isozyme banding patterns were observed which enable a distinction of collections from *Arrhenatherum elatius* on the one hand and collections from *Poa* spp. and *Anthoxanthum odoratum* on the other hand (Table 25). Some differences in isozyme banding patterns were also found between collections from *A. odoratum* and *Poa* species, partly correlating with the differences in the number of primary infection hyphae (Table 25). Collections of *P. annua* and *P. nemoralis* combined identical isozyme patterns with minor differences in the number of primary infection hyphae (Section 6.2).

Isozyme studies of *P. brachypodii/Brachypodium* and *P. brachypodii/ Deschampsia* and of additional collections from the host plants studied here would be valuable for further evaluation of isozyme analysis for taxonomy.

Since, contrary to *P. coronata* and *P. graminis*, the observed differences in germling morphology of *P. brachypodii* collections from the four host genera studied are qualitative and the differences in isozyme banding patterns are more pronounced, it is suggested to recognize the *P. brachypodii* rusts collected from *A. elatius, Brachypodium* spp., *Deschampsia cespitosa* and *Poa* + *Anthoxanthum odoratum* as separate species as proposed by Urban (1969). The differences observed in germling morphology and isozyme banding patterns of *Anthoxanthum* and *Poa* collections are too small to warrant any infraspecific rank.

In conclusion, the germling morphology and isozyme banding patterns support the recognition of four separate species within *P. brachypodii* s.l., viz. *P. brachypodii* Otth, *P. deschampsiae* Arth., *P. magelhaenica* Peyritsch in Magn., and *P. poae-nemoralis* Otth (Urban, 1969).

Puccinia recondita

In *P. recondita* s.l. germling morphology greatly contributed to the distinction of included taxa. Fifteen different (sub)types were found. Differences between (sub)types were generally qualitative and allowed an easier identification than the

characters of urediniospores and teliospores only (Section 4.4; Table 25). Most (sub)types were specific of one host species or genus (Table 25).

Isozyme patterns were also clearly distinct between most collections from different host species. When collections from different host species had identical isozyme patterns they also had identical germling morphology and mostly were pathogenic to each other's host species, e.g. collections from *Triticum aestivum* and *Aegilops squarrosa* (Valkoun *et al.*, 1985) which are considered conspecific.

In this complex it seems justified to attribute more value to germling morphology (Section 4.4), isozyme banding patterns, and nuclear DNA content (Section 6.1; Eilam *et al.*, 1992a) than to morphological characters of uredinia and telia (or aecia when present). Collections differing both in germling morphology and isozyme banding patterns, which also may have minor morphological differences in spore features or in nuclear DNA content, can be considered as separate species. For *P. recondita sensu stricto* and *P. triticina* this view is supported by the numerous differences observed in several other molecular and biochemical traits (i.a. Zambino & Szabo, 1993; Sock & Kolmer, 1993).

Some collections having the same germling morphology showed minor differences in isozyme banding patterns, e.g. collections from *Aegilops ovata* and *Secale cereale*. These differences probably correlate with differences in aecial host genus, and the isolates may be non-pathogenic to each other's host species (Oliveira & Samborski, 1966). They differed only slightly in nuclear DNA content (Eilam *et al.*, 1992a). Considering the minor differences, it is suggested to include these collections in one species, *P. recondita* s.s. Individual collections from the respective host species can then possibly be delimited as *formae speciales*, differing in bands of one (or more) enzyme systems (Table 25) and nDNA content (Eilam *et al.*, 1992a).

On the other hand, the differences in germling morphology, uredinia and telia, and host range (Section 4.4; Table 25) suggest that rust collections from *B. hordeaceus* + *B. sterilis*, and *Bromus inermis* can be regarded as different species. The rusts from *B. hordeaceus* and *B. sterilis* also differed in isozyme banding patterns and nDNA content. Because no studies on molecular and biochemical traits of *B. inermis* have been made, it is suggested to follow the species concept of Urban (1969) and recognize two varieties in *P. bromina*, viz. *P. bromina* var. *paucipora* Urban from *Bromus inermis* and *P. bromina* Erikss. var. *bromina* from *Bromus hordeaceus* and *B. sterilis*. Within the latter variety, the rusts from *B. hordeaceus* and *B. sterilis* might be recognized as *formae speciales*, since they differ slightly in morphological characters, isozyme patterns and host range. Regarding the *P. recondita* complex worldwide a further complexity can be expected.

In conclusion, the results of the present studies on germling morphology and isozyme banding patterns suggest to recognize the following taxa within *P. recondita* s.l.: *P. agropyrina* Erikss., *P. agropyri-juncei* Klebahn, *P. arrhenathericola* Fischer, *P. bromina* Erikss. var. *bromina*, *P. bromina* var. *paucipora* Urban, *P.*

holcina Erikss., *P. loliina* Sydow, *P. perplexans* Plowr., *P. recondita* Rob. ex Desm., *P. triseti* Erikss., and *P. triticina* Erikss. (Eriksson, 1899; Urban, 1969).

Puccinia hordei and *Uromyces* species on barleys

Both germling morphology and the characters of uredinia and urediniospores were identical between *P. hordei* and *Uromyces* spp. occurring on species of *Hordeum* (Section 4.5; Table 25). Only in the telia some differences in number of cells of the teliospores were present (Table 25). Number of cells of the teliospores, however, is known to vary within a species (Chapter 2).

Considerable similarities were also observed in isozyme banding patterns. *Uromyces christensenii* was identical to *P. hordei*. *Uromyces viennot-bourginii* differs in only one enzyme system (PGI) from *P. hordei*, but the same banding pattern was also observed in a colour mutant of *P. hordei*. *Puccinia hordei* collected from *Hordeum vulgare* and *H. murinum* were found to differ from each other in two enzyme systems (Section 6.2).

Barley leaf rust fungi are not only morphologically similar, but also physiologically. All barley leaf rust basidiospores are able to infect *Leopoldia eburnea* to induce aecia. In the uredinial stage, they are all strictly confined to *Hordeum* spp., but many taxa can only infect one species of *Hordeum* (Anikster & Wahl, 1979).

The identical characters of uredinia, urediniospores and germling morphology, and the similar isozyme banding patterns and nuclear DNA contents (Eilam *et al.*, 1992a) suggest the inclusion of all barley leaf rusts into one species, *P. hordei*. Within this species two varieties might be distinguished, viz. one with one-celled (including the *Uromyces* spp.) and one with one- and two-celled (including *P. hordei*) teliospores. The rust from *Hordeum murinum* could be regarded as a *forma specialis* of the latter characterized by minor differences in isozyme banding patterns. Nevertheless, infraspecific taxa are not yet proposed here because cross-fertility of *P. hordei* and the *Uromyces* spp. has not yet been sufficiently studied.

In conclusion, the similarities in morphological, physiological, molecular and biochemical traits suggest to recognize *P. hordei* Otth as one species, including at least two infraspecific taxa. Further investigations on cross-fertility of these taxa on barleys are required.

General conclusions

Germling morphology of urediniospores is a valuable tool in taxonomic studies of rust fungi of cereals and grasses. It has shown pronounced qualitative differences between taxa some of which are hardly distinguishable on the basis of characters of uredinia and telia. Within a species or between closely related species only quantitative differences or no differences at all were seen in germling morphology. In a few cases germling morphology was similar in distantly related species, possibly due to convergent evolution.

The variation observed in isozyme banding patterns within and between species complexes tends to support the conclusions drawn from germling morphology. Taxa with similar germling morphology had only minor differences or no differences at all in isozyme banding patterns. Germling morphology is also correlated with the nDNA contents and literature data obtained with other molecular and biochemical techniques.

Since both germling morphology and isozyme electrophoresis allow a clear distinction between grass rust fungi, I therefore recommend to include descriptions of these criteria in floras and other descriptive books. Probably they will also be helpful in taxonomic studies of other rust fungi. A drawback of applying these criteria is that viable urediniospores are required, since (i) it is difficult to stain germlings present in herbarium material (Section 5.2) and (ii) isozyme activity is nil in spores stored for a long period at room temperature (Swertz, unpublished data). Of course, PCR-based techniques may also have a great potential for taxonomic studies of (grass) rust fungi and other plant-pathogenic fungi, particularly since Bruns *et al.* (1990) have succeeded in utilizing PCR for amplification of DNA from fungal herbarium specimens.

When a rust fungus is found on a plant species not yet reported as a host, or when several rather similar rust fungi can infect the same plant species (e.g. *Arrhenatherum elatius*, *Poa pratensis* or *Festuca rubra*), germling morphology is a useful tool to reliably identify the pathogen, since only few spores are required for this purpose. It is also rather easy to collect about 1 mg of urediniospores from infected leaves, enough for isozyme electrophoresis using the PhastSystem.

8 KEY TO SPECIES STUDIED

Note: At least 15 germlings per collection should be observed.

1 Appressoria absent 2
– Appressoria present 4

2 SSV spheroid with a second irregularly shaped body from which 1–4 PIH, 5–10(–19) μm wide, arise 3
– SSV spheroid, second irregularly shaped body absent; (1–)2–5 club-shaped PIH, (3–)4-5(–6) μm wide; on *Dactylis glomerata* . . . . . *Puccinia striiformis* var. *dactylidis*

3 SSV mostly collapsed; PIH mostly not thickened at the ends, 5–8 μm wide; on *Poa* . . . . . *Puccinia striiformis* var. *poae*
– SSV non-collapsed; PIH mostly thickened at the ends, (5–)6–10(–19) μm wide; on *Triticum*, *Hordeum*, *Bromus carinatus* . . . . . *Puccinia striiformis* var. *striiformis*

4 SSV ends bent downwards; PIH vertically orientated 5
– SSV ends horizontal, not bent downwards; PIH horizontally or vertically orientated 7

5 Each SSV with a central septum; two PIH; HMC often lobed; on *Hordeum vulgare* . . . . . *Puccinia hordei* 'isolate 28'
– SSV with either a central, eccentrical or no septum; two or more PIH; HMC unlobed 6

6 Elongated infection peg present, 5–7(–15) μm long; 2–3(–4) PIH; HMC 10–14 μm long; on *Arrhenatherum elatius* . . . . . *Puccinia magelhaenica*
– Elongated infection peg absent; 2 or 3 PIH; HMC 15–21 μm long; on *Deschampsia cespitosa* . . . . . *Puccinia deschampsiae*

7 SSV rectangular 8
– SSV fusiform, deltoid, (narrowly) oblong, or (narrowly) ellipsoid 10

8 Appressorium square with some digitate protuberances; SSV clearly longer than wide, with a central septum; two narrow (2–4 μm wide) horizontal, often hooked PIH; hooked HMC 9
– Appressorium oblong; SSV slightly longer than wide, occasionally septate; 4 or 5 PIH; unlobed HMC; on *Poa pratensis*, *P. nemoralis*, occasionally other *Poa* spp. . . . . *Puccinia poae-nemoralis* p.p.

9 SSV (30–)40–53(–60) × (5–)6–7(–9) μm; on *Avena* (aecia on *Rhamnus*) . . . . . *Puccinia coronata* var. *avenae*
– SSV (16–)30–38(–45) × (5–)7–9(–10) μm; on several wild grasses . . . . . *Puccinia coronata* var. *coronata*

10 SSV fusiform 11
– SSV deltoid, (narrowly) oblong, or (narrowly) ellipsoid 21

11 PIH mostly one, occasionally two, longitudinal and horizontal 12
– PIH mostly two, occasionally three, longitudinal and horizontal 14

12 Appressorium oblong; SSV 9–11(–13) μm wide; urediniospores spheroid, with scattered germ pores; on *Alopecurus pratensis* (aecia on *Ranunculus acris*) . . . . . *Puccinia perplexans*
– Appressorium narrowly oblong; SSV (7–)8–9(–12) μm wide; urediniospores ellipsoid, with equatorial germ pores 13

13 SH-ADD (35%) and second PIH (34%) frequent; SSV + PIH long, 20–95(–140) μm, mean 55.5 μm; on *Avena*. . . . . *Puccina graminis* f.sp. *avenae*
– SH-ADD (7–14%) and second PIH (19–22%) occasional; SSV + PIH shorter, 20–75(–100) μm, mean 39–47 μm; on *Triticum*, *Hordeum*, and *Secale* . . . . . *Puccinia graminis* f.sp. *tritici* and f.sp. *secalis*[1]

14 Germlings with a more or less central septum in the SSV, additional septa occasionally present 15
– Germlings with either a more or less central, an eccentrical, or no septum in the SSV 18

15 No additional septa present; PIH clearly thinner than the SSV 16
– One or two additional septa present; PIH wider near SSV than near HMC 17

16 SSV (35–)40–60 × (8–)9–11(–16) μm; SH occasionally present; urediniospores with (4-)5–6(–8) scattered germ pores; on *Bromus inermis* . . . . . *Puccinia bromina* var. *paucipora*
– SSV (20–)25–28(–30) × 8–10 μm: SH rarely present; urediniospores with (6–)8–11(–14) scattered germ pores; on *Trisetum flavescens* . . . . . *Puccinia triseti*

[1] Small quantitative differences were observed between f.sp. *tritici* and f.sp. *secalis* depending on the host species from which spores were collected. Further studies are required to evaluate the taxonomic importance of these differences (Section 5.2).

17 SSV (30–)40–55(–60) × (7–)9–10(–12) μm; PIH mostly horizontal, occasionally deflected towards the nearest vein; on *Hordeum* spp. (aecia on *Ornithogalum* and *Scilla*) . . . . . *Puccinia hordei*
– SSV (25–)30–40 × 7–9(–10) μm; PIH vertically orientated; on *Lolium perenne* . . . . . *Puccinia loliina*

18 SSV with two longitudinal and horizontal PIH 19
– SSV with two or three PIH in a variable orientation; on *Anthoxanthum odoratum*, *Poa annua*, *P. nemoralis*, *P. palustris*, and other *Poa* spp. . . . . *Puccinia poae-nemoralis* p.p.

19 SSV (20–)25–35(–40) × (9–)10–13 μm; PIH (4–)5–6(–7) μm wide; urediniospores with (6-)7–9(–10) scattered germ pores; on *Secale*, *Aegilops ovata* (aecia on *Anchusa* and *Echium*) . . . . . *Puccinia recondita*
– SSV 20–30 × (7–)8–10(–12) μm; PIH (2–)3–4(–5) μm wide; urediniospores with (8–)9–10(–13) scattered germ pores; on *Arrhenatherum elatius* . . . . . *Puccinia arrhenathericola*

20 SSV ellipsoid-oblong to deltoid, without a septum; 2–4(–5) PIH; thick-walled paraphyses in the uredinium; sori arranged in lines; on *Brachypodium* . . . . *Puccinia brachypodii*
– SSV (narrowly) oblong or (narrowly) ellipsoid, with or without a septum; PIH 1 or 2 21

21 SSV with a central septum; two vertical PIH arising at the ends of the SSV; on *Holcus*. . . . . *Puccinia holcina*
– SSV generally without a septum; PIH, one, rarely two, in a variable orientation 22

22 SSV ellipsoid-oblong to oblong, (20–)25–29(–32) × (9–)11–13(–14) μm, with rounded ends; one PIH, vertically, rarely horizontally orientated; on *Bromus sterilis* . . . . . *Puccinia bromina* var. *bromina*
– SSV (narrowly) ellipsoid, (narrowly) oblong, with acute, acuminate ends; one, occasionally two PIH, vertically and/or horizontally orientated 23

23 SSV (30–)45–57(–65) × (7–)10–15 μm; PIH (4–)5–6(–7) μm wide; on *Agropyron junceiforme* . . . . . *Puccinia agropyri-juncei*
– SSV shorter than 43 μm 24

24 SSV (narrowly) oblong, margins essentially parallel, with long papillate ends (5–8 μm long), 32–40(–43) × 10–13 μm; on *Bromus erectus* . . . . . *Puccinia bromina* s.l.

- SSV (narrowly) ellipsoid, margins symmetrically curved, with acute-rounded or short-acuminate ends (shorter than 3 μm); on other grasses 25

25 PIH horizontally and transversely orientated; SSV (22–)28–33(–37) × (10–)12–14 (–16) μm; on *Bromus hordeaceus*, *B. secalinus* . . . . . *Puccinia bromina* var. *bromina*
- PIH vertically orientated, occasionally longitudinal and horizontal 26

26 SSV (ellipsoid to) narrowly ellipsoid, (25–)29–42 × 9–12(–14) μm, with short-acuminate ends; one PIH, vertically, rarely horizontally, orientated, (2–)4–5(–6) μm wide; occasionally a second PIH; on *Agropyron repens*, *Hordeum jubatum* . . . . . *Puccinia agropyrina*
- SSV ellipsoid (to narrowly ellipsoid), with acute-rounded ends; one PIH, generally vertically orientated 27

27 Appressorium oblong; SSV (21–)24–34(–38) × 11–15(–17) μm; PIH (4–)5–6 μm wide; on *Triticum* spp., *Aegilops squarrosa* (aecia on *Thalictrum*) . . . . . *Puccinia triticina*
- Appressorium square; SSV (20–)24–30 × (7–)8–11 μm; PIH 3–4 μm wide; on *Agropyron repens* . . . . . *Puccinia coronata* s.l.

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APPENDIX - COLLECTIONS STUDIED

Collection numbers starting with 'C.S.' refer to collections made by the author. Occasionally collections made by other persons were incorporated in the 'C.S.' numbers. The collectors are mentioned within parentheses (coll.). The incubated leaf segments received from Dr J. Marková were designated 'J.M.' followed by a number.

Many collections, mainly from *Puccinia graminis*, *P. recondita* and *P. hordei*, were obtained during visits to other institutes or from the Department of Plant Breeding, Wageningen. These collections are marked by superscript letters indicating the institute from which they were obtained:

[a] Institute of Plant Protection, Praha, Ruzyně, Czech Republic (Dr J. Šebesta).
[b] Institute for Cereal Crops Improvement, Tel Aviv, Israel (Dr Y. Anikster).
[c] Department of Plant Breeding, Wageningen, the Netherlands.
[d] Agriculture Canada Research Station, Winnipeg, Manitoba, Canada (Drs D. E. Harder, J. A. Kolmer, K. Dunsmore).
[e] Cereal Rust Laboratory, St. Paul, Minnesota, United States (Drs J. Huerta-Espino, A. P. Roelfs).
[f] Research Institute for Plant Protection (IPO-DLO) Wageningen, the Netherlands (G. Kema).

PUCCINIA CORONATA Corda

PUCCINIA CORONATA Corda var. CORONATA

From *Agrostis capillaris*:

THE NETHERLANDS. Zuid-Holland: Oost-Voorne, 12 Sep. 1989, C.S. 89.129.

From *Agrostis gigantea*:

GREAT BRITAIN. Scotland: Edinburgh, Royal Botanic Gardens, 14 Nov. 1990, C.S. 90.210.

From *Agrostis stolonifera*:

THE NETHERLANDS. Friesland: Schiermonnikoog, 19 Sep. 1989 (colls R. G. Dekens & Th. Jacobs), C.S. 89.167. Limburg: Stokhem, 6 Sep. 1989, C.S. 89.103.

CZECH REPUBLIC. Central Bohemia: Pleší Vec, 7 Oct. 1989, C.S. 89.203; Martinice v. Krkonošich, 15 km W. of Semily, 11 Sep. 1990, C.S. 90.163.

GREAT BRITAIN. Scotland: Edinburgh, Warriston Cemetry, opposite Royal Botanic Gardens, 9 Nov. 1990, C.S. 90.192.

From *Agrostis tenuis*:

THE NETHERLANDS. Friesland: Gaasterland, 24 Sep. 1989, C.S. 89.176. Limburg: Haelen, Leudal, 10 Sep. 1991, C.S. 91.057; Haelen, Leudal, 10 Sep. 1991, C.S. 91.060.

FRANCE. Massif Central, Monts du Forez: Sail-sous-Couzan, road to castle St. Georges-en-Couzan, 5 July 1990, C.S. 90.093.

GREAT BRITAIN. Scotland: Edinburgh, Warriston Cemetery, opposite Royal Botanic Gardens, 9 Nov. 1990, C.S. 90.197.

GERMANY. Eifel: Nideggen, 10 Aug. 1992, C.S. 92.095.

From *Agrostis* spp.:

THE NETHERLANDS. Friesland: Gaasterland, 24 Sep. 1989, C.S. 89.179; Gaasterland, 24 Sep. 1989, C.S. 89.188. Gelderland: Wageningen, Department of Plant Breeding, 29 Sep. 1992, C.S. 92.106. Limburg: Vlodrop-station, Roote Beek, 10 Sep. 1991, C.S. 91.053. Zuid-Holland: Oost-Voorne, 12 Sep. 1989, C.S. 89.128.

FRANCE. Massif Central, Monts du Forez: Sail-sous-Couzan, road to castle St. Georges-en-Couzan, 16 July 1992, C.S. 92.068.

GREAT BRITAIN. Scotland: Edinburgh, Arthurs' seat, 18 July 1990, C.S. 90.110.

From *Alopecurus pratensis*:

THE NETHERLANDS. Gelderland: Wageningen, Bornsesteeg, 6 Nov. 1989, C.S. 89.251.

CZECH REPUBLIC. South Bohemia: Dolní Poříčí, riverside near railway, 3 Sep. 1991, J.M. IIID.

From *Alopecurus* spp.:

CZECH REPUBLIC. Central Bohemia: Dobriš, 40 km S. of Prague, 13 Oct. 1989, C.S. 89.217.

From *Arrhenatherum elatius*:

THE NETHERLANDS. Gelderland: Wageningen, Bornsesteeg, 22 Aug. 1989, C.S. 89.069; Wageningen, plant disease garden Department of Plant Pathology, 13 July 1990, C.S. 90.109; Wageningen, Department of Plant Breeding, 29 Sep. 1992, C.S. 92.101; Wageningen, Department of Plant Breeding, 30 Aug. 1993, C.S. 93.048. Limburg: Stokhem, 6 Sep. 1989, C.S. 89.107; Stokhem, 6 Sep. 1989, C.S. 89.108; Slenaken, Onderste Bos, 6 Sep. 1989, C.S. 89.122; Haelen, Leudal, 10 Sep. 1991, C.S. 91.056. Utrecht: Utrecht, Park Bloeyendael, 7 Sep. 1991, C.S. 91.052; Rhenen, 31 Aug. 1993 (coll. R. E. Niks), C.S. 93.050. Zuid-Holland: Oost-Voorne, Tenellaplas, 12 Sep. 1989, C.S. 89.145.

CZECH REPUBLIC. Central Bohemia: Prague, Botanic Gardens, 9 Oct. 1989, C.S. 89.215. South Bohemia: Dolní Poříčí, 3 Sep. 1991, J.M. IIIB.

FRANCE. Massif Central, Monts du Forez: Sail-sous-Couzan, road to castle St. Georges-en-Couzan, 5 July 1990, C.S. 90.089; Sail-sous-Couzan, road to castle St. Georges-en-Couzan, 5 July 1990, C.S. 90.091.

GREAT BRITAIN. Scotland: Edinburgh, Royal Botanic Gardens, 14 Nov. 1990, C.S. 90.214.

From *Festuca arundinacea*:

THE NETHERLANDS. Friesland: Gaasterland, on the leaves, 24 Sep. 1989, C.S. 89.185; Gaasterland, on the culms, 24 Sep. 1989, C.S. 89.186.

From *Festuca gigantea*:

THE NETHERLANDS. Limburg: Slenaken, Onderste Bos, 6 Sep. 1989, C.S. 89.123; Slenaken, Onderste Bos, 6 Sep. 1989, C.S. 89.124; Vlodrop-station, Roote Beek, 14 Aug. 1990, C.S. 90.120.

From *Festuca pratensis*:

CZECH REPUBLIC. South Bohemia: 2 km W. of Str. Hoštice, Boubin-Horaždovice, 7 Sep. 1990, C.S. 90.141.

GREAT BRITAIN. Scotland: Edinburgh, Warriston Cemetery, opposite Royal Botanic Gardens, 9 Nov. 1990, C.S. 90.198.

From *Glyceria aquatica*:

CZECH REPUBLIC. South Bohemia: Dolní Poříčí, 2 Sep. 1991, J.M. IIA.

From *Holcus lanatus*:

THE NETHERLANDS. Drenthe: Dwingeloo, 23 Oct. 1989 (coll. R. G. Dekens), C.S. 89.238. Friesland: Schiermonnikoog, 19 Sep. 1989 (colls R. G. Dekens & Th. Jacobs), C.S. 89.164. Gelderland: Wageningen, Bornsesteeg, 18 July 1989, C.S. 89.022; Lienden, 9 Aug. 1990 (coll. A. Zeven), C.S. 90.116; Wageningen, Department of Plant Breeding, 29 Sep. 1992, C.S. 92.105; Wageningen, Department of Plant Breeding, 30 Aug. 1993, C.S. 93.047. Limburg: Stokhem, 6 Sep. 1989, C.S. 89.110; Haelen, Leudal, 10 Sep. 1991, C.S. 91.058. Utrecht: 't Goy, Beusichemseweg 40, 8 Aug. 1989, C.S. 89.044; Rhenen, 31 Aug. 1993 (coll. R. E. Niks), C.S. 93.051. Zuid-Holland: Rockanje, 12 Sep. 1989, C.S. 89.140.

CZECH REPUBLIC. Central Bohemia: Rejkovice, 7 Oct. 1989, C.S. 89.210. Moravia: Skalička u. Hranice na Moravě, 5 km S. of Hranice, 13 Sep. 1990, C.S. 90.170.

ECUADOR. 30 May 1991 (coll. Th. Jacobs), C.S. 91.034.

GERMANY. Eifel, Nideggen, 10 Aug. 1992, C.S. 92.090 (also infected with *P. holcina*).

From *Holcus mollis*:

FRANCE. Massif Central, Monts du Forez: Jeansagnière near woodmills, 20 July 1992, C.S. 92.076.

From *Lolium perenne*:

THE NETHERLANDS. Gelderland: Wageningen, plant disease garden Department of Plant Pathology, 28 July 1989. C.S. 89.026; Wageningen, Bornsesteeg, 22 Aug. 1989, C.S. 89.064; Wageningen, Bornsesteeg, 22 Aug. 1989, C.S. 89.068; Wageningen, Afweg, 2 Sep. 1991, C.S. 91.050; Wageningen, Department of Plant Breeding, 29 Sep. 1992, C.S. 92.102; Wageningen, CRZ, Bornsesteeg, on cv. Blazer, C.S. 93.040; Wageningen, CRZ, Bornsesteeg, on cv. Bar RE176a, C.S. 93.041; Wageningen, CRZ, Bornsesteeg, on cv. Saione, C.S. 93.042. Limburg: Stokhem, 6 Sep. 1989, C.S. 89.111; Ottersum, fields Zelder B.V., 10 Nov. 1989, C.S. 89.009. Utrecht: 't Goy, Beusichemseweg 40, 8 Aug. 1989, C.S. 89.045; 't Goy, Beusichemseweg 40, 17 Sep. 1989, C.S. 89.155; Rhenen, 1 Sep. 1993 (coll. R. E. Niks), C.S. 93.049.

CZECH REPUBLIC. Central Bohemia: Dobriš, 40 km S. of Prague, 13 Oct. 1989, C.S. 89.220; Dobriš, 40 km S. of Prague, 13 Oct. 1989, C.S. 89.218; Zbraslav, 10 km S. of Prague, right side of Vltava river, 10 Sep. 1990, C.S. 90.149; Prague, Bečhovice, 14 Aug. 1993 (coll. Z. Urban), C.S. 93.045.

GERMANY. Eifel: Nideggen, 10 Aug. 1992, C.S. 92.097 (also infected with *P. loliina*).

From *Phalaris arundinacea*:

THE NETHERLANDS. Limburg: Haelen, Leudal, 10 Sep. 1991, C.S. 91.062; Haelen, Leudal, 10 Sep. 1991, C.S. 91.063.

PUCCINIA CORONATA var. **AVENAE** Fraser & Ledingham

From *Avena sativa*:

THE NETHERLANDS. Gelderland: Wageningen, Bornsesteeg, 22 Aug. 1989, C.S. 89.060; Wageningen, field collection of the Department of Plant Breeding, 9 Mar. 1992, isolate IVP[c]. Utrecht: 't Goy, fields Beusichemseweg 40, 19 Aug. 1990, C.S. 90.122.

CZECH REPUBLIC. isolate 954,3392/3[a].

ECUADOR. 30 May 1991 (coll. Th. Jacobs), C.S. 91.033.

ETHIOPIA. Holetta, 25 Oct. 1990 (coll. Th. Jacobs), C.S. 90.185.

ISRAEL. Golan Heights, near Qarzin, 11 May 1992, C.S. 92.Isr54.

YUGOSLAVIA. Isolate 264,3392/1[a].

From *Rhamnus* sp.:

ISRAEL. Tel Aviv, aecial collection nr. 8671 5.3, 4 May 1992, C.S. 92.Isr1[b].

PUCCINIA CORONATA Corda s.l.

From *Agropyron repens*:

CANADA. Ontario: Ottawa, 19 Aug. 1991, C.S. 91.048.

PUCCINIA GRAMINIS Pers. : Pers.

PUCCINIA GRAMINIS f.sp. **AVENAE** Erikss. & E. Henn.

From *Avena fatua*:

CANADA. Manitoba: Hugald, NA18 20[88], ACRS-661[d]. Saskatchewan: Pelly, NA29 132[84], ACRS-616[d].

From *Avena sativa*:

THE NETHERLANDS. Gelderland: field collection of the Department of Plant Breeding, 9 Mar. 1992, isolate IVP[c].

BRAZIL. NLB, APR 17.A, 1982[e].

CANADA. British Colombia: Creston, NA5 462[88], ACRS-680[d]. Manitoba: Dauphin, NA81 422[88], ACRS-697[d]. Ontario: Sudbury, NA12 122[88], ACRS-679[d]. Quebec: Ste. Hyacinthe, NA26 468[88], ACRS-681[d]. Saskatchewan: Regina, NA18 371[87], ACRS-635[d].

KENYA. RTL, APR 513.2, 1987[e].

MADAGASCAR. TLP, APR 0572.C, 1989[e].
MEXICO. NA27, APR 29.3, 1987[e].
MOROCCO. APR 13.C, 1963[e].
NEW ZEALAND. Aorangi, NA74 NZ1[90], ACRS-717[d]; Plot 1075, NA8 W2[91], ACRS-740[d].
TURKEY. DJD, APR-DHC 407.A, 1990[e].
UNITED STATES. California: NA10, APR 49.3, 1987[e]. Minnesota: race 98, APR 821.5, 1975[e].

From *Berberis* spp.:
CANADA. Ontario: Ottawa, NA25 B9[88], ACRS-664[d]; Ottawa, NA57 B10[88], ACRS-683[d]; Ottawa, NA65 B91[88], ACRS-687[d].

PUCCINIA GRAMINIS f.sp. **TRITICI** Erikss. & E. Henn.

From *Berberis* sp.:
CANADA. Ontario: Ottawa, QFM, F1307[d].

From *Hordeum jubatum*:
CANADA. Alberta: Coaldale, race C96 318[87], ACRS-F926[d]. Manitoba: Alexander, race QCC, ACRS-F1319[d]; Hunton, QCC 330[88], ACRS-F1228[d].

From *Hordeum vulgare*:
CANADA. Alberta: Zacombe, race QFC H235[90], ACRS-W224[d]. British Colombia: Creston, race QCC 189[88], ACRS-F1236[d]; Creston, race QCC 191[88], ACRS-F1231[d]. Quebec: Z'Assomption, race TMR 42[59], ACRS-W266[d].
UNITED STATES. Minnesota: QBCS, APR 357.2, 1990[e].

From *Triticum* spp.:
CANADA. British Colombia: Creston, race QCC 162[88], ACRS-F1229[d]; Creston, race QFC 277[90], ACRS-W194[d]. Ontario: Bath, race TPM, ACRS-F1321[d].
CHILE. BBHN, APR 23.A, 1981[e].
ITALY. CKCS, APR 598.2, 1985[e].
SYRIA. RKCT, APR 10.1, 1983[e].
UNITED STATES. Georgia: TNMK, APR 172.1, 1985[e]. Minnesota, colour mutants: grey brown, yellow, orange, white and wild-type spores[e]. Washington: RSHS, APR 1700.B, 1974[e].

From unspecified grass:
CANADA. Manitoba: Holland, QCC 249[90], W219[d].

PUCCINIA GRAMINIS f.sp. **SECALIS** Erikss. & E. Henn.

From *Agropyron repens*:
THE NETHERLANDS. Gelderland: Wageningen, Bornsesteeg, 22 Aug. 1989, C.S. 89.057.
CANADA. Manitoba: along Hwy 75, 40 km S. of Winnipeg, 30 July 1990.

From *Berberis* spp.:
CANADA. Ontario: B59[88], F1145[d]; B403, APR-SUR, 1986[e].

From *Hordeum jubatum*:
CANADA. Manitoba: MacDonald, 3[88], ACRS-F1182[d]; Vita, 303[88], ACRS-F1219[d]; South Manitoba: along Hwy 59, 30 July 1990; along Highway 59, near St. Pierre, 30 July 1990. Saskatchewan: Kamsock, 276[88], ACRS-F1209[d].

From *Hordeum vulgare*:
CANADA. Ontario: Appleton, 2[88], ACRS-F1183[d].

From *Secale cereale*:
THE NETHERLANDS. Gelderland: Wageningen, Bornsesteeg, 22 Aug. 1989, C.S. 89.058.
CANADA. British Colombia: Creston, 204[88], ACRS-F1223[d]. Quebec: Ste. Hyacinthe, 184[88], ACRS-F1221[d]; Ste. Hyacinthe, 185[88], ACRS-F1222[d].

UNITED STATES. Virginia: HSC, APR 64.A, 1970[e].

From *Triticum* sp.:

CANADA. Manitoba: Dauphin, 245[88], F1192[d].

PUCCINIA GRAMINIS Pers. : Pers. s.l.

From *Arrhenatherum elatius*:

THE NETHERLANDS. Gelderland: Wageningen, Bornsesteeg, 22 Aug. 1989, C.S. 89.073.

From *Dactylis glomerata*:

THE NETHERLANDS. Utrecht: 't Goy, Beusichemseweg 40, Aug. 1989, C.S. 89.080.

UNITED STATES. Idaho: BCC, APR–SUR 1027[e].

From *Deschampsia cespitosa*:

CZECH REPUBLIC. Bohemia: Jitrava n. Jablonne v Podještědí, 15 km W. of Liberec, 11 Sep. 1990, C.S. 90.155.

From *Festuca pratensis*:

THE NETHERLANDS. Gelderland: Wageningen, Wageningse Afweg, 15 Sep. 1991, C.S. 91.064.

From *Festuca* cf. *arundinacea*:

FRANCE. Alsace: Barr, near Andlau, 25 July 1992, C.S. 92.086.

From *Lolium perenne*:

THE NETHERLANDS. Gelderland: Wageningen, Wageningse Afweg, 15 Sep. 1991, C.S. 91.065; Wageningen, Department of Plant Breeding, 29 Sep. 1992, C.S. 92.100. Utrecht: 't Goy, Beusichemseweg 40, 17 Sep. 1989, C.S. 89.154.

PUCCINIA BRACHYPODII Otth species complex

PUCCINIA BRACHYPODII Otth

From *Brachypodium pinnatum*:

THE NETHERLANDS. Limburg: Stokhem, 10 June 1990, C.S. 90.073.

CZECH REPUBLIC. South Bohemia: Dolní Poříčí, 6 Sep. 1990, C.S. 90.137.

FRANCE. Massif Central, Monts du Forez: Sail-sous-Couzan, road to castle St. Georges-en-Couzan, 5 July 1990, C.S. 90.094; along D110, Sauvain to St. Georges-en-Couzan between Epinasse en Pré Chambon, 22 July 1992, C.S. 92.080; along road St. Georges-en-Couzan to Vaux, 22 July 1992, C.S. 92.081.

From *Brachypodium sylvaticum*:

THE NETHERLANDS. Limburg: Stokhem, 10 June 1990, C.S. 90.071, C.S. 90.072; Savels Bos, 11 June 1990, C.S. 90.076.

CZECH REPUBLIC. South Bohemia: Horaždovíce, 7 Sep. 1990, C.S. 90.140. West Moravia: Tlumačov n. Otrokovice, 13 Sep. 1990, C.S. 90.181.

GERMANY. Eifel: Nideggen, 10 Aug. 1992, C.S. 92.096. South Germany: Regensburg, 3 Sep. 1990, C.S. 90.126, C.S. 90.127.

FRANCE. Alsace: along road Barr to Mont St. Odile, 25 July 1992, C.S. 92.088.

PUCCINIA DESCHAMPSIAE Arth.

From *Deschampsia cespitosa*:

GREAT BRITAIN. Scotland: Edinburgh, Royal Botanic Gardens, 23 Nov. 1990, C.S. 90.213.

PUCCINIA MAGELHAENICA Peyritsch in Magn.

From *Arrhenatherum elatius*:

THE NETHERLANDS. Gelderland: Nijmegen, 26 May 1990, C.S. 90.040. Limburg: Mechelen, 9 June 1990, C.S. 90.070; Stokhem, 10 June 1990, C.S. 90.074. Utrecht: Veenendaal,

31 May 1990, C.S. 90.057; Rhenen, Maatsteeg, 27 Apr. 1993, C.S. 93.010. Zuid-Holland: 's-Gravenzande, 18 Mar. 1990, C.S. 90.007.

BELGIUM. Ardennes: Sy, 15 June 1991, C.S. 91.041.

CZECH REPUBLIC. South Bohemia: Horaždovíce, 7 Sep. 1990, C.S. 90.142. Central Bohemia: Kralupy, 10 Sep. 1990, C.S. 90.152; České středohoří Mts., Klapý p. Hazmburkem, 26 June 1991, J.M. 0C. Moravia: Bílavsko near Bystřice and Hostý, 13 Sep. 1990, C.S. 90.176.

FRANCE. Massif Central, Monts du Forez: Chalmazel, 4 July 1990, C.S. 90.082; Sail-sous-Couzan, road to castle St. Georges-en-Couzan, 5 July 1990, C.S. 90.096; Jeansagnière, along road to river Le Lignon, 6 July 1990, C.S. 90.103.

SWITZERLAND. Wallis: Folaterre, 6 June 1992, C.S. 92.031; along road Täsch to Zermatt, 23 July 1993, C.S. 93.035.

PUCCINIA POAE-NEMORALIS Otth

From *Anthoxanthum odoratum*:

THE NETHERLANDS. Drenthe: Reestdal, 7 June 1990, C.S. 90.062. Gelderland: Wageningen, Wageningse Berg, 8 Apr. 1990, C.S. 90.021; Wageningen, Bergweg, 11 June 1990, C.S. 90.077; Wageningen, Bergweg, 21 Apr. 1991, C.S. 91.006. Utrecht: Veenendaal, 31 May 1990, C.S. 90.055, C.S. 90.056.

From *Poa annua*:

THE NETHERLANDS. Gelderland: Wageningen, Lawickse Allee, 13 Mar. 1990, C.S. 90.006; Wageningen, Bornsesteeg, 27 May 1990, C.S. 90.051. Limburg: Stokhem, 9 Sep. 1989, C.S. 89.240; Ottersum, Zelder B.V., 10 Nov. 1989, C.S. 89.008. Utrecht: 't Goy, Beusichemseweg 40, 1 Apr. 1990, C.S. 90.014; 't Goy, Beusichemseweg 40, 25 Apr. 1993, C.S. 93.008.

CZECH REPUBLIC. South Bohemia: Dolní Poříčí, 6 Sep. 1990, C.S. 90.135. Central Bohemia: Březno u Postoloprty, 10 Sep. 1990, C.S. 90.154.

FRANCE. Massif Central, Monts du Forez: Sail-sous-Couzan, along road to castle St. Georges-en-Couzan, 5 July 1990, C.S. 90.092; St. Georges-en-Couzan, along road from camp site Le Mazet to castle St. Georges-en-Couzan, 16 July 1992, C.S. 92.070.

GREAT BRITAIN. Scotland: Leith, Edinburgh, 9 Nov. 1990, C.S. 90.199; Portobello Beach, 11 Nov. 1990, C.S. 90.201; Edinburgh, Royal Botanic Gardens, 14 Nov. 1990, C.S. 90.207.

From *Poa nemoralis*:

THE NETHERLANDS. Gelderland: Wageningen, Lexcesveer, 8 Apr. 1990, C.S. 90.022; Wageningen, Bergweg, 21 Apr. 1991, C.S. 91.007; Wageningen, Bergweg, 9 Mar. 1992, C.S. 92.003; Wageningen, Bergweg, 8 June 1993, C.S. 93.033. Limburg: Mechelen, 9 June 1990, C.S. 90.066; Epen, 28 Apr. 1991, C.S. 91.016. Utrecht: Rhenen, 3 Apr. 1990 (coll. R. E. Niks), C.S. 90.018.

BELGIUM. Ardennes: Sy, 15 June 1991 (coll. D.J. van der Gaag), C.S. 91.043; Sy, 6 June 1993, C.S. 93.026.

CZECH REPUBLIC. South Bohemia: Horní Poříčí, 5 Sep. 1990, C.S. 90.144.

FRANCE. Massif Central, Monts du Forez: Sail-sous-Couzan, along road to castle St. Georges-en-Couzan, 5 July 1990, C.S. 90.088.

GERMANY. Eifel: Nideggen, 4 June 1991, C.S. 91.039.

SWITZERLAND. Bern, near zoo, 4 June 1992, C.S. 92.028.

From *Poa palustris*:

THE NETHERLANDS. Drenthe: Reestdal, 7 June 1990, C.S. 90.061.

CZECH REPUBLIC. South Bohemia: Horní Poříčí, 5 Sep. 1990, C.S. 90.130; Dolní Poříčí, 6 Sep. 1990, C.S. 90.138; Strelske Hostice, 8 Sep. 1990, C.S. 90.147; Dolní Poříčí, riverside

near railway, 3 Sep. 1991, J.M. IIIC; Dolní Poříčí, right side of Otava river near railway, 4 Sep. 1991, J.M. IIIF.

SWITZERLAND. Bern, Botanic Gardens, 4 June 1992, C.S. 92.029.

From *Poa pratensis*:

THE NETHERLANDS. Gelderland: Wageningen, Nijenoord Allee, 21 Apr. 1990, C.S. 90.031; Wageningen, Bornsesteeg, 27 May 1990, C.S. 90.050; near ferry to Opheusden, 26 May 1992, C.S. 92.020. Limburg: along road Wijlre to Schin op Geul, 28 Apr. 1991, C.S. 91.014. Utrecht: Utrecht, Park Bloeyendael, 25 Aug. 1991, C.S. 91.047; 't Goy, Beusichemseweg 40, 26 May 1992, C.S. 92.018; Rhenen, Maatsteeg, 31 May 1993, C.S. 93.023.

BELGIUM. Ardennes: Sy, 15 June 1991, C.S. 91.044.

CZECH REPUBLIC. Bohemia: Novohradské Hory, Staré Hutě u Hojné Vody, 29 Aug. 1991, J.M. IA.

FRANCE. Massif Central, Monts du Forez: Jeansagnière, near the Mairie, 5 July 1990, C.S. 90.102.

GREAT BRITAIN. Scotland: Edinburgh, Warriston Cemetery, opposite Royal Botanic Gardens, 14 Nov. 1990, C.S. 90.216.

SWITZERLAND. Wallis: along road Täsch to Zermatt, 23 July 1993, C.S. 93.034.

PUCCINIA RECONDITA Rob. ex Desm. species complex

PUCCINIA AGROPYRINA Erikss.

From *Agropyron repens*:

THE NETHERLANDS. Gelderland: Wageningen, Wageningse Berg, 27 May 1990, C.S. 90.049; Wageningen, Department of Plant Breeding, 3 Dec. 1990, C.S. 90.224; Wageningen, fields CPRO, 15 Aug. 1992 (coll. D. Rubiales), C.S. 92.099. Limburg: Mechelen, Schweibergseweg, 9 June 1990, C.S. 90.069; Haelen, Leudal, 10 Sep. 1991, C.S. 91.055; Stokhem, camp site Gele Anemoon, 23 May 1993, C.S. 93.021. Utrecht: 't Goy, Beusichemseweg 40, 11 July 1989, C.S. 89.009; 't Goy, Beusichemseweg 40, 16 Aug 1989, C.S. 89.051. 't Goy, Beusichemseweg 40, 1 Apr. 1990, C.S. 90.015; 't Goy, fields Beusichemseweg 40, 19 Aug. 1990, C.S. 90.123; Rhenen, 9 Mar. 1992 (coll. R. E. Niks), C.S. 92.009; Culemborg, railway embankment, 14 June 1992, C.S. 92.044; 't Goy, Beusichemseweg 40, 25 Apr. 1993, C.S. 93.009.

CZECH REPUBLIC. Central Bohemia: Bilá Skale, 15 km NNW. of Liberec, 11 Sep. 1990, C.S. 90.158; Prague, Běchovice, 20 June 1992 (coll. Z. Urban), J.M. 92b. South Bohemia: Horní Poříčí, 10 km W. of Strakonice, on Otava river, 5 Sep. 1990, C.S. 90.131; Suchomasty, 10 km S. of Beroun, 10 Sep. 1990, C.S. 90.151.

FRANCE. Massif Central, Monts du Forez: camp site Le Mazet, St. Georges-en-Couzan, near Prachay, 18 July 1992, C.S. 92.073. Alsace: Barr, Andlau, 25 July 1992, C.S. 92.085.

GERMANY. Eifel: Nideggen, 10 Aug. 1992, C.S. 92.093.

GREAT BRITAIN. Scotland: Edinburgh, Warriston Cemetery, opposite Royal Botanic Gardens, 9 Nov. 1990, C.S. 90.195; Edinburgh, Royal Botanic Gardens, 14 Nov. 1990, C.S. 90.209.

From *Hordeum jubatum*:

THE NETHERLANDS. Gelderland: Wageningen, fields Department of Plant Breeding (coll. R. E. Niks), isolate IVP^{c}.

PUCCINIA AGROPYRI-JUNCEI Klebahn

From *Agropyron junceiforme*:

THE NETHERLANDS. Zuid-Holland: Oost-Voorne, in the dunes, 12 Sep. 1989, C.S. 89.133.

GERMANY. Langeoog: Flinthorn, 26 Aug. 1993, C.S. 93.044.

GREAT BRITAIN. Scotland: Edinburgh, Leith, Portobello Beach, 11 Nov. 1990, C.S. 90.200.

PUCCINIA ARRHENATHERICOLA Fischer

From *Arrhenatherum elatius*:

THE NETHERLANDS. Gelderland: Wageningen, Bornsesteeg, 27 May 1990, C.S. 90.054; Nijmegen, Ooy-polder, 6 June 1990, C.S. 90.060; Wageningen, Veerweg, 17 June 1993, C.S. 93.032. Limburg: Stokhem, 6 Sep. 1989, C.S. 89.109; Mechelen, Mechelse Beek, 25 May 1990, C.S. 90.035; Eyserbosschen, 21 June 1992, C.S. 92.058; Eyserbosschen, 23 May 1993, C.S. 93.019. Utrecht: Utrecht, Park Bloeyendael, 25 Mar. 1990, C.S. 90.008; Utrecht, Park Bloeyendael, 19 Apr. 1990, C.S. 90.028; 't Goy, Beusichemseweg 40, 2 June 1990, C.S. 90.059; Utrecht, Park Bloeyendael, near RW27, 17 June 1990, C.S. 90.080. Zuid-Holland: Oost-Voorne, Tenellaplas, 12 Sep. 1989, C.S. 89.148; Asperen, Put van Bullee, 12 June 1992, C.S. 92.047.

BELGIUM. Ardennes: hills around Sy, 6 June 1993, C.S. 93.030.

CZECH REPUBLIC. Central Bohemia: Zbraslav, 10 km S. of Prague, right side of Vltava river, 10 Sep. 1990, C.S. 90.148; Karlštejn, 10 Sep. 1990, C.S. 90.150. South Bohemia: Kněží hora, 1 km E. of Dolní Poříčí, on Otava river, 495 m, 6 June 1990, C.S. 90.134. Moravia: Mušlov u. Mikulova, 13 Sep. 1990, C.S. 90.180.

FRANCE. Massif Central, Monts du Forez: along road Jeansagnière–Chalmazel, 5 July 1990, C.S. 90.099; along D110, Sauvain–St. Georges-en-Couzan, between Epinasse and Pré Chambon, 22 July 1992, C.S. 92.079. Alsace: Barr, Andlau, 25 July 1992, C.S. 92.083.

GREAT BRITAIN. Scotland: Edinburgh, Warriston Cemetery, opposite Royal Botanic Gardens, 9 Nov. 1990, C.S. 90.194; Edinburgh, Royal Botanic Gardens, 14 Nov. 1990, C.S. 90.214; Edinburgh, Royal Botanic Gardens, 14 Nov. 1990, C.S. 90.215; Edinburgh, Warriston Cemetery, opposite Royal Botanic Gardens, 14 Nov. 1990, C.S. 90.217.

PUCCINIA BROMINA Erikss. var. **BROMINA**

From *Bromus hordeaceus*:

THE NETHERLANDS. Gelderland: Wageningen, Bornsesteeg, 21 Apr. 1990, C.S. 90.032; Nijmegen, Ooy-polder, Oortjeshekken, 27 May 1990, C.S. 90.044; Wageningen, Bornsesteeg, 27 May 1990, C.S. 90.052; Wageningen, Wageningse Afweg, 6 May 1991, C.S. 91.019; Wageningen, Lawickse Allee–Nude, 27 Apr. 1993, C.S. 93.011; Gameren, 17 May 1993, C.S. 93.013. Limburg: Mechelen, Mechelse Beek, 25 May 1990, C.S. 90.036; Eyserbosschen, 21 June 1992, C.S. 92.062; Schin op Geul, 23 May 1993, C.S. 93.017. Utrecht: Utrecht, Park Bloeyendael, 17 May 1991, C.S. 91.023.

FRANCE. Massif Central, Monts du Forez: Sail-sous-Couzan, road to castle St. Georges-en-Couzan, 5 July 1990, C.S. 90.087.

From *Bromus sterilis*:

THE NETHERLANDS. Gelderland: Wageningen, Costerweg, 27 May 1991, C.S. 91.026, C.S. 91.027, C.S. 91.028; Wageningen, 9 Mar. 1992, C.S. 92.004. Limburg: road Wijlre–Schin op Geul, 28 Apr. 1991, C.S. 91.015; Eyserbosschen, 21 June 1992, C.S. 92.061. Utrecht: Utrecht, Park Bloeyendael, 10 Mar. 1990, C.S. 90.002; Utrecht, Park Bloeyendael, 19 Apr. 1990, C.S. 90.025; Utrecht, Park Bloeyendael, 17 May 1991, C.S. 91.023.

CZECH REPUBLIC. Bohemia: Prague 6, Džbán (coll. Z. Urban), 1 May 1991, J.M. 0A; Prague, Dubeč, 7 June 1992 (coll. Z. Urban), J.M. 92a.

GREAT BRITAIN. Cambridge, fields of Plant Breeding Institute (coll. T. W. Hollins), Feb. 1992, C.S. 92.013.

SWITZERLAND. Wallis: Folaterre, 3 June 1992, C.S. 92.024.

GREAT BRITAIN. Cambridge, fields of Plant Breeding Institute (coll. T. W. Hollins), Feb. 1992, C.S. 92.013.
SWITZERLAND. Wallis: Folaterre, 3 June 1992, C.S. 92.024.

PUCCINIA BROMINA var. **PAUCIPORA** Urban

From *Bromus inermis*:

CZECH REPUBLIC. South Bohemia: Kněží Hora, 1 km E. of Dolní Poříčí, along Otava river, 495 m, 6 Sep. 1990, C.S. 90.136; Dolní Poříčí, 2 Sep. 1991, J.M. IID. Moravia: Tlumačov near Otrokovice, 10 km SW of Holesov, 13 Sep. 1990, C.S. 90.179.

PUCCINIA BROMINA Erikss. s.l.

From *Bromus erectus*:

SWITZERLAND. Wallis: Folaterre, 3 June 1992, C.S. 92.025. Bern, along the Aare, near the Auguet-Brücke, 4 June 1992, C.S. 92.033.

PUCCINIA HOLCINA Erikss.

From *Holcus lanatus*:

THE NETHERLANDS. Gelderland: Gameren, 15 June 1992, C.S. 92.054; Staverden, 23 June 1992, C.S. 92.064; Gameren, 17 May 1993, C.S. 93.012. Limburg: Cottese Beek, 25 May 1990, C.S. 90.037. Utrecht: Rhenen, de Dijk, 31 May 1993, C.S. 93.022; Rhenen (coll. R. E. Niks), 29 Aug. 1993, C.S. 93.043.
AUSTRIA. Gmünd, Nature Park Blockheide, 31 July 1991, J.M. IC.
BELGIUM. Ardennes: Sy, 15 June 1991, C.S. 91.042; after multiplication of C.S. 91.042 on *H. lanatus* in the greenhouse, C.S. 92.015.
FRANCE. Massif Central, Monts du Forez: along road Jeansagnière-Chalmazel, 5 July 1990, C.S. 90.098; road Sail-sous-Couzan to castle St. Georges-en-Couzan, 16 July 1992, C.S. 92.072; St. Georges-en-Couzan, camp site Le Mazet, near Prachay, 18 July 1992, C.S. 92.075; Jeansagnière, near woodmills, 20 July 1992, C.S. 92.077. Alsace: Barr, Andlau, 25 July 1992, C.S. 92.084.
GERMANY. Eifel, Nideggen, 10 Aug. 1992, C.S. 92.090 (also infected with *P. coronata*).
MADEIRA. (coll. J. E. Parlevliet), Dec. 1991, C.S. 92.001.

From *Holcus mollis*:

FRANCE. Massif Central, Monts du Forez: Jeansagnière, 4 July 1990, C.S. 90.084.
GREAT BRITAIN. Scotland: Edinburgh, Arthurs' Seat, 18 July 1990 (colls H. van Eck, R. Hutten, A. Kuipers), C.S. 90.107.

PUCCINIA LOLIINA Sydow

From *Lolium italicum*:

THE NETHERLANDS. Gelderland: Wageningen, Bornsesteeg, experimental fields CRZ, 21 May 1991, C.S. 91.025.

From *Lolium multiflorum*:

GREAT BRITAIN. Wales: Aberystwyth, field collection on *Festuca gigantea* × *Lolium* sp. hybrid multiplied on *L. multiflorum* (coll. H. Roderick), Mar. 1991, C.S. 91.001.
SWITZERLAND. Bern city, Thungasse, 4 June 1992, C.S. 92.032.

From *Lolium perenne*:

THE NETHERLANDS. Gelderland: Wageningen, Wageningse Afweg, 21 Apr. 1991, C.S. 91.009; Wageningen, Wageningse Afweg, 6 May 1991, C.S. 91.020; Wageningen, Wageningse Afweg, 6 May 1991, C.S. 91.021; Wageningen, Bornsesteeg, experimental fields CRZ, 21 May 1991, C.S. 91.024; Wageningen, Wageningse Afweg, experimental fields Phytopathology, 31 May 1993, C.S. 93.024.

BELGIUM. Ardennes: Sy, near the Tukhut, 6 June 1993, C.S. 93.029.
FRANCE. Massif Central, Monts du Forez: St. Georges-en-Couzan, along road camp site Le Mazet to castle St. Georges-en-Couzan, 16 July 1992, C.S. 92.069; camp site Le Mazet, St. Georges-en-Couzan, 16 July 1992, C.S. 92.071; St. Georges-en-Couzan, barrage near Rory, 21 July 1992, C.S. 92.078.
GERMANY. Eifel: Nideggen, 10 Aug. 1992, C.S. 92.097 (also infected with *P. coronata*).
GREAT BRITAIN. Scotland: Edinburgh, Leith, Portobello Beach, 11 Nov. 1990, C.S. 90.203.

PUCCINIA PERPLEXANS Plowr.

From *Alopecurus pratensis*:

THE NETHERLANDS. Gelderland: Gameren, 15 June 1992, C.S. 92.050; Gameren, infections obtained from aecia on *Ranunculus acris*, 17 May 1993, C.S. 93.014.
CZECH REPUBLIC. Bohemia: Novohradské Hory, between Hojná Voda and Staré Hutě, 29 July 1991, J.M. IB.

From *Ranunculus acris*:

THE NETHERLANDS. Gelderland: Gameren, 17 May 1993, C.S. 93.014.

PUCCINIA RECONDITA Rob. ex Desm.

From *Aegilops ovata*:

ISRAEL. Tel Aviv, uredinial collection, connected with aecia from *Echium* sp., 4 May 1992, C.S. 92.Isr23[b]; uredinial collection connected with aecia from *Echium tuberosum* (coll. J. Manisterski), isolate 9233[c].

From *Aegilops longissima*:

ISRAEL. Tel Aviv, uredinial collection, connected with aecia from *Anchusa* sp., 4 May 1992, C.S. 92.Isr24[b].

From *Anchusa* spp.:

ISRAEL. Tel Aviv, aecial collection nr. 9309.5.4, connected with *Aegilops longissima*, 4 May 1992, C.S. 92.Isr3[b]; Tel Aviv, aecial collection nr. 7451, connected with *Secale* sp., 4 May 1992, C.S. 92.Isr5[b].

From *Echium* sp.:

ISRAEL. Tel Aviv, aecial collection nr. 9295.12.4, connected with *Aegilops ovata*, 4 May 1992, C.S. 92.Isr4[b].

From *Secale cereale*:

THE NETHERLANDS. Gelderland: Wageningen, Bornsesteeg, 22 Aug. 1989, C.S. 89.059; Wageningen, Bornsesteeg, 22 Aug. 1989, C.S. 89.063; Bornsesteeg, experimental fields RIVRO, 27 May 1990, C.S. 90.053; Wageningen, Department of Plant Breeding, 12 Aug. 1990, C.S. 90.119; Wageningen, fields Department of Plant Breeding, isolate IVP[c]. Limburg: Ottersum, 10 Nov. 1989, C.S. 89.007; monospore culture from C.S. 89.007, Dec. 1989, RMS1; monospore culture from C.S. 89.007, Dec. 1989, RMS2; monospore culture from C.S. 89.007, Dec. 1989, RMS3; monospore culture from C.S. 89.007, Dec. 1989, RMS5.
CZECH REPUBLIC. Bohemia: Mladá Boleslav district, Obrubce (coll. Z. Urban), 3 July 1991, J.M. 0D; Hladá Boleslav district, Předměčice, Benatky n. Jiz., 27 June 1992 (coll. Z. Urban), J.M. 92c.
ISRAEL. Tel Aviv, 4 May 1992, C.S. 92.Isr25[b]; aecial collection of *Anchusa* sp. multiplied on *Secale cereale* cv. Rogo at the Department of Plant Breeding, 8 June 1992, C.S. 92.036.

From *Secale montanum*:

ISRAEL. Tel Aviv, 4 May 1992, C.S. 92.Isr10[b].

PUCCINIA TRISETI Erikss.

From *Trisetum flavescens*:

THE NETHERLANDS. Gelderland: Nijmegen, Ooy-polder, first road at the right coming from Nijmegen, 26 May 1990, C.S. 90.041; Gameren, 15 June 1992, C.S. 92.048. Limburg: Stokhem, 9 June 1990, C.S. 90.075.

CZECH REPUBLIC. South Bohemia: Horní Poříčí, 10 km W. of Strakonice, on Otava river, 5 Sep. 1990, C.S. 90.128. Bohemia: Martinice v. Krkonošich, 15 km W. of Semily, 11 Sep. 1990, C.S. 90.164. Moravia: south of Bruntal direction Uhlírský Vrch, near castle and old vulcano, 12 Sep. 1990, C.S. 90.168; Jičín district, Nadslav (coll. Z. Urban), 8 Nov. 1991, J.M. IVD.

FRANCE. Massif Central, Monts du Forez: Jeansagnière, along road to Provenchère, 4 July 1990, C.S. 90.081; Jeansagnière, 4 July 1990, C.S. 90.085; Sail-sous-Couzan, road to castle St. Georges-en-Couzan, 5 July 1990, C.S. 90.097; Road Col du Béal to Pierre-sur-Haute, 7 July 1990, C.S. 90.105; camp site Le Mazet, St. Georges-en-Couzan, near Prachay, 18 July 1992, C.S. 92.074.

SWITZERLAND. Bern: along the Aare, near the Auguet-Brücke, 4 June 1992, C.S. 92.027.

PUCCINIA TRITICINA Erikss.

From *Aegilops squarrosa*:

THE NETHERLANDS. Gelderland: Wageningen, June 93, multiplication of wheat leaf rust isolate Flamingo[c].

From *Thalictrum speciosissimum*:

CANADA. Ae 16-1[d]; Ae 8-2[d].

From *Thalictrum* spp.:

ISRAEL. Tel Aviv, aecial collection nr. 9367.26.3, connected with *Triticum* spp., 4 May 1992, C.S. 92.Isr2[b]; aecial collection nr. 2664, connected with *Triticum durum*, Tel Aviv, 4 May 1992, C.S. 92.Isr2[b].

From *Triticum aestivum*:

THE NETHERLANDS. Gelderland: Wageningen, fields Department of Plant Breeding, July 1989, C.S. 89.018; Wageningen, fields Department of Plant Breeding, isolate Flamingo[c]; Wageningen, fields Department of Plant Breeding, isolate Felix[c]; Bornsesteeg, fields CPRO (coll. D. Rubiales), isolate CPRO[c].

BANGLADESH. CBBM, 4012-2, 1989[e]; 4007-1, 1989[e].

BOLIVIA. 4126-4, 1988[e].

BRAZIL. 4078-3, 1989[e].

CANADA. British Colombia: NBB 283, 1989[d]. East Canada: PBL 394, 1987[d]. Manitoba: TBB 198, 1989[d]. Saskatchewan: MFB 190, 1989[d]; KBG 186, 1989[d].

ISRAEL. 4012-3, 1990[e]; 4016-9, 1990[e]. Tel Aviv, urediniospores from *Triticum boeoticum* 39[b], multiplied on *Triticum aestivum* cv. Little Club at the Department of Plant Breeding, 8 June 1992, C.S. 92.035; aeciospores from *Thalictrum* spp. multiplied on *Triticum vulgare* cv. Little Club, 8 June 1992, C.S. 92.038; urediniospores from *Triticum dicoccoides*, multiplied on *Triticum aestivum* cv. Little Club at the Department of Plant Breeding, 8 June 1992, C.S. 92.039; aeciospores from *Thalictrum*[a] multiplied on *Triticum aestivum* cv. Little Club at the Department of Plant Breeding, 8 June 1992, C.S. 92.040.

ITALY. 4018-1, 1988[e].

MEXICO. 4033-2, 1988[e]; 4100-1, 1989[e]; 4025-2, 1991[e].

NEPAL. 4029-4, 1988[e]; 4033-2, 1989[e].

PORTUGAL. Isolate P3797[c].

SPAIN. Isolates S5-1 and S5-3 (coll. D. Rubiales)[f].

PARAGUAY. LBBC, 96, 1989[e].

POLAND. T-52, APR-RW T52 Z, virulent on Lr26, 1985[e].
TURKEY. 4064-3, 1988[e]; CHPL, 4113-3, 1988[e].
UNITED STATES. Indiana: CLB, APR, 1969[e]; Texas: TCL, APR, 1979[e].
URUGUAY. U10-4[d]; U30-1[d].
ZAIRE. MBBM, 4029-3, 1989[e].
ZAMBIA. 4027-1, 1989[e].

From *Triticum durum*:

CHILE. 4059-3, 1988[e].
MEXICO. BBBQ, APR-JH 4061-2, 1988[e].
MOROCCO. 2-9, 1989[e]; 2-3, 1989[e]; APR, 4038-7, 1989[e].

PUCCINIA HORDEI Otth species complex

PUCCINIA HORDEI Otth

From *Hordeum murinum*:

THE NETHERLANDS. Gelderland: Wageningen, Bornsesteeg, 22 Aug. 1989, C.S. 89.066; Wageningen, along Dijkgraaf, 19 Nov. 1990, C.S. 90.223; Wageningen, Department of Plant Breeding, 9 Mar. 1992, isolate IVP[e].
GREAT BRITAIN. Scotland: Edinburgh, Portobello Beach, 11 Nov. 1990, C.S. 90.205.

From *Hordeum vulgare* or *H. spontaneum*:

THE NETHERLANDS. Drenthe: Dwingeloo, isolate 18[e]. Gelderland: Wageningen, isolate 1-2-1 and orange colour mutant of isolate 1-2-1[e]; Zeddam, isolate 17[e]. Utrecht: 't Goy, 11 July 1989, C.S. 89.012.
BRAZIL. Race 19, APR 60[e].
CANADA. Manitoba: 50 km S. of Winnipeg, along Highway 75, 30 July 1991; Beausejour, 18 km S. of Winnipeg, on barley cv. Ellice, 23 July 1991 (coll. K. Dunsmore)[d]; Beausejour, 18 km S. of Winnipeg, on barley cv. Bonanza, 23 July 1991 (coll. K. Dunsmore)[d].
ECUADOR. Iquito, Santa Catalina, 5 May 1990 (coll. J. van Leur), C.S. 90.034; Iquito, Santa Catalina, 28 May 1990 (coll. J. E. Parlevliet), C.S. 90.058.
EGYPT. Race 1, APR-MWA 40/80[e].
ETHIOPIA. Holetta, 26 Sep. 1989 (coll. Th. Jacobs), C.S. 89.230; Holetta, 25 Oct. 1990 (coll. Th. Jacobs), C.S. 90.186.
FRANCE. Les Settons, isolate 22[e].
GREAT BRITAIN. Isolate 3[e].
GREECE. Crete, isolate 13[e].
ISRAEL. Tel Aviv, 5 May 1992, C.S. 92.Isr21[b]; Golan Heights, near Qarzin, 11 May 1992, C.S. 92.Isr45 and C.S. 92.Isr46.
ITALY. Isolate 25[e].
MOROCCO. Isolate Marakech 1-1[e]; isolate 28[e]; Had Brachova, 6 June 1992 (coll. B. Ezzahiri), C.S. 92.021; Larache, 6 June 1992 (coll. B. Ezzahiri), C.S. 92.022; Airoouda, 6 June 1992 (coll. B. Ezzahiri), C.S. 92.023; collection 7311, 5 May 1992[b].
NORWAY. Race 1, APR-MWA 100/81[e].
PAKISTAN. Race 18, APR 9001.2, 1987[e].
PORTUGAL. Race 1, APR-MWA 27/80[e].
SPAIN. Race 1, APR-MWA 28/80[e].
TURKEY. Race 18, APR 9002.3, 1987[e].
UNITED STATES. Kansas: race 18, APR 9023.1, 1987[e]. Virginia; race 8, APR 197[e].

From *Ornithogalum* spp.:

ISRAEL. Tel Aviv, aecial collection 1704.6.4, 4 May 1992, C.S. 92.Isr7[b].

UNITED STATES. Kansas: race 18, APR 9023.1, 1987[e]. Virginia; race 8, APR 197[e].
From *Ornithogalum* spp.:
ISRAEL. Tel Aviv, aecial collection 1704.6.4, 4 May 1992, C.S. 92.Isr7[b].

UROMYCES CHRISTENSENII Anikster & Wahl
From *Hordeum vulgare*:
SYRIA. Bouidder, 50 km SSE of Aleppo, 1990, (coll. J. E. Parlevliet), Uch-Syria.

UROMYCES HORDEASTRI Guyot
From *Hordeum bulbosum*:
ISRAEL. Golan Heights, near Qarzin, 11 May 1992, C.S. 92.Isr41, C.S. 92.Isr42 and C.S. 92.Isr43.

UROMYCES REICHERTII Anikster & Wahl
From *Scilla hyacinthoides*:
ISRAEL. Tel Aviv, aecial collection 6385, 4 May 1992, C.S. 92.Isr6[b].
From *Hordeum bulbosum*:
ISRAEL. Tel Aviv, 5 May 1992, C.S. 92.Isr33[b].

UROMYCES VIENNOT-BOURGINII Wahl & Anikster
From *Hordeum spontaneum*:
ISRAEL. isolate Uvb-IVP[c]; Tel Aviv, 5 May 1992, C.S. 92.Isr32[b].

PUCCINIA STRIIFORMIS Westend.

PUCCINIA STRIIFORMIS Westend. var. **STRIIFORMIS**
From *Bromus carinatus*:
THE NETHERLANDS. Gelderland: Wageningen, Generaal Foulkesweg, near Jan Kopshuis, 7 June 1993, C.S. 93.031.
From *Hordeum vulgare*:
THE NETHERLANDS. 81550[f].
BELGIUM. 64008[F].
NEPAL. 86018[f].
From *Triticum aestivum*:
THE NETHERLANDS. 60018[f]; 66049[f]; Gelderland: Wageningen, isolate IVP[c].

PUCCINIA STRIIFORMIS var. **DACTYLIDIS** Manners
From *Dactylis glomerata*:
THE NETHERLANDS. Utrecht: Rhenen, Grebbeberg, near stone factory, 2 Aug. 1993, C.S. 93.036.
GREAT BRITAIN. Devon, Duddle Door, July 1993 (coll. R. E. Niks), C.S. 93.037.

PUCCINIA STRIIFORMIS var. **POAE** Swertz var. nov.
From *Poa pratensis*:
THE NETHERLANDS. Gelderland: Wageningen, Department of Plant Breeding, 2 Aug. 1993, C.S. 93.038; Wageningen, CRZ, Bornsesteeg, 9 Aug. 1993, C.S. 93.039. Utrecht: 't Goy, Beusichemseweg 40, 26 May 1992, C.S. 92.019.
BELGIUM. Ardennes: Sy, 6 June 1993, C.S. 93.027.

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SAMENVATTING

Roestige hekken, roestige kettingen, een roestvlek op de auto. Roest op metaal, veroorzaakt door oxidatie, wordt in het dagelijkse leven veelvuldig aangetroffen.

Behalve op metaal kan roest ook op planten voorkomen. Voorbeelden zijn roest op stokroos, speenkruid, koffie, boon, en grassen en granen. Roest op planten wordt veroorzaakt door een schimmel, die, om te kunnen overleven, een plant (waardplant) moet infecteren. Enige dagen na infectie vormt de schimmel een of meerdere sporenhoopjes, meestal op het blad, soms op de bladscheden, stengel of bloeiwijzen (o.a. bij grassen en granen) van de geïnfecteerde plant. Deze sporenhoopjes hebben vaak een roestbruine kleur, maar ze kunnen ook geel, oranje of zwart zijn (Hoofdstukken 2 en 4). In een sporenhoopje worden sporen gevormd die voor de verspreiding van de roest zorgen.

Enkele kenmerken van roestschimmels zijn, dat ze meerdere sporetypen kunnen produceren, een ingewikkelde levenscyclus kunnen hebben en sterk kunnen verschillen in waardplant-specificiteit (Hoofdstuk 1).

In een volledige levenscyclus kunnen roestschimmels achtereenvolgens vijf verschillende sporetypen produceren, te weten: basidiosporen, pycniosporen, aeciosporen, urediniosporen en teleutosporen, in respectievelijk de basidia, pycnia, aecia, uredinia en telia. Deze sporetypen kunnen op één plant gevormd worden zoals bij Bonenroest (*Uromyces appendiculatus*), maar een roest kan ook twee verschillende planten uit verschillende plantenfamilies nodig hebben om de levenscyclus te voltooien. Zwarte roest (*Puccinia graminis*) bijvoorbeeld vormt uredinia, telia en basidia op grassen en granen maar pycnia en aecia op zuurbes (*Berberis*). Van sommige andere roesten zijn tot nu toe alleen uredinia en telia beschreven en is het niet bekend of en op welke plant(en) pycnia en aecia gevormd worden.

Roestschimmels kunnen ook sterk verschillen in het aantal waardplantsoorten dat ze kunnen infecteren. Bonenroest kan alleen op bonen (*Phaseolus vulgaris* en *P. coccineus*) sporuleren. Dwergroest van gerst (*Puccinia hordei*) vormt uredinia en telia op gerst en pycnia en aecia op lelie-achtigen (vogelmelk, *Ornithogalum*), terwijl Kroonroest (*P. coronata*) uredinia en telia op verschillende grassen en granen en pycnia en aecia op wegedoorn en vuilboom (Rhamnaceae) vormt. Net als Kroonroest is ook de al genoemde Zwarte roest in het aeciosporenstadium meer beperkt in waardreeks (alleen op zuurbes) dan in het urediniosporenstadium (op grassen en granen).

In dit onderzoek is een studie verricht aan roestschimmels op grassen en granen. Vooral op granen en voedergrassen kunnen roestschimmels een aanzienlijke schade (opbrengst en kwaliteitsverlies) veroorzaken wanneer er geen resistente rassen verbouwd worden. In Europa zijn op grassen en granen zo'n 30–40 roestsoorten beschreven, die onderscheiden worden op morfologische kenmerken van voornamelijk de uredinia, urediniosporen, telia, teleutosporen en op waardreeks.

Het aantal onderscheiden soorten hangt sterk af van het gehanteerde soortsbegrip. Sommige auteurs hanteren een breed soortsbegrip en beschouwen alle roesten die morfologisch identiek zijn als behorend tot één soortscomplex, bijvoorbeeld het Bruine roest-complex, *P. recondita*, waarbinnen allerlei infraspecifieke eenheden (zoals variëteiten of *formae speciales*) onderscheiden kunnen worden. Binnen zo'n complex kunnen de infraspecifieke eenheden echter verschillen in urediniosporenwaard en in aeciosporenwaard, maar ook in sporekenmerken, waardoor het twijfelachtig is of het om één soort gaat. Andere auteurs hanteren een smal soortsbegrip. Elke roest voorkomend op een ander plantengeslacht, maar morfologisch bijna identiek, wordt als een aparte soort onderscheiden. Wanneer echter bijvoorbeeld bij experimenten een roest voorkomend op *Triticum* (*P. triticina* op tarwe) ook op *Aegilops*, waarvan *P. aegilopis* is beschreven, kan sporuleren, zou het beter zijn deze soorten te verenigen.

Er zijn twee problemen bij de classificatie en identificatie van roestschimmels op grassen en granen. Ten eerste zijn er weinig betrouwbare scheidende kenmerken tussen en binnen soorten. Telia, welke voor identificatie en classificatie zeer waardevol zijn, worden in het algemeen pas aan het eind van het groeiseizoen gevormd. Hierdoor zijn zij lang niet altijd op het te bestuderen materiaal aanwezig. Men is dan aangewezen op kenmerken van de uredinia en urediniosporen. De kenmerken van deze vertonen echter tussen veel soorten of infraspecifieke eenheden alleen kwantitatieve verschillen en/of een grote overlap (afmetingen, kiemporen aantal) of zij zijn subjectief of moeilijk waarneembaar (kleur van de sporen en van de sporewand, Hoofdstuk 2). Ten tweede kan de waardreeks een moeilijk hanteerbaar kenmerk zijn, omdat (i) vegetatieve grassen vaak moeilijk te determineren zijn, (ii) roesten onder bepaalde omstandigheden ook op planten kunnen voorkomen waar ze normaliter niet op kunnen sporuleren en (iii) het moeilijk kan zijn de waardreeks van een soort te bepalen (Hoofdstukken 1 en 4).

Een nieuw betrouwbaar kenmerk zou de problemen bij de identificatie en classificatie van grasroesten kunnen verminderen. Eén van de potentieel nieuwe kenmerken kan de morfologie van de primaire infectiestructuren van de urediniosporen zijn, welke in dit onderzoek nader is bestudeerd. Bij een geslaagde infectie vormt een gekiemde urediniospore een appressorium boven een huidmondje. Vanuit dit appressorium penetreert de schimmel het huidmondje en vormt in de ruimte eronder een substomataal blaasje. Vanuit dit blaasje worden een of meerdere primaire infectiehyfen gevormd die, wanneer ze tegen een mesophylcel aangroeien, een haustoriummoedercel vormen. Vanuit de haustoriummoedercel wordt in de aanliggende mesophylcel een haustorium (een voedingslichaam) gevormd, waarna er talrijke secundaire hyfen worden gevormd (Hoofdstuk 3).

De morfologie van de infectiestructuren kan (na kleuring) worden bestudeerd in het geïnfecteerde materiaal of urediniosporen kunnen eerst tot kieming gebracht worden op een geschikt substraat om vervolgens (ook na kleuring) de infecties te bestuderen. Het bleek dat wanneer urediniosporen tot kieming gebracht waren op een geschikt substraat de infecties beter gekleurd en te bestuderen waren dan wanneer geïnfecteerde bladeren of herbariumbladeren gekleurd werden. Bovendien

was in geïnfecteerd materiaal het aantal infecties veelal lager en waren ze vaak te ver ontwikkeld (er waren te veel hyfen gevormd) om ze goed waar te kunnen nemen. Tevens bleek dat zaailingbladeren beter geschikt waren als substraat dan volwassen-plant bladeren, omdat zaailingbladeren een losse weefselstructuur hebben, waardoor de infectiestructuren goed waarneembaar zijn. Verder waren onbehaarde bladeren gemakkelijker te gebruiken dan behaarde bladeren omdat de sporen bij opbreng met een naald op een behaard blad gemakkelijk wegsprongen (Hoofdstuk 5).

Als substraat kan een waardplant of een niet-waardplant worden gebruikt. Het is gebleken, dat de morfologie en ontwikkeling van de infectiestructuren tot de vorming van het haustorium identiek zijn in een waardplant en een niet-waardplant, mits de niet-waardplant een epidermis van het grassentype heeft. Vanaf de aanzet tot haustoriumvorming ontwikkelt de roestschimmel zich sneller en verder (meer secundaire hyfen en septa) in een waard dan in een niet-waard (Hoofdstuk 3).

In deze studie is een cultuurgerst (lijn L94) gekozen als standaardsubstraat voor de bestudering van de infectiestructuren, vanwege de gemakkelijke beschikbaarheid van zaad, en de brede, onbehaarde bladeren. Om infectiestructuren in een zelfde stadium van ontwikkeling te bestuderen werden bladeren met infectiestructuren van roesten die niet pathogeen op de gebruikte cultuurgerst zijn circa 40 uur na kieming van de urediniosporen verzameld en gekleurd. Infectiestructuren van roestschimmels verzameld van cultuurgerst, en dus pathogeen op het gebruikte substraat, werden bestudeerd op blad dat na 24 uur was verzameld (Hoofdstuk 3).

Er werden infectiestructuren bestudeerd van 5 verschillende roest-complexen, te weten: *Puccinia coronata, P. graminis, P. brachypodii, P. recondita* en *P. hordei*, inclusief enkele *Uromyces* soorten die pathogeen zijn op gerst. Van ieder complex werden zoveel mogelijk collecties van zoveel mogelijk verschillende waardplanten van zoveel mogelijk geografisch verschillende herkomsten bestudeerd.

De morfologie van de infectiestructuren bleek een betrouwbaar taxonomisch kenmerk te zijn. Het werd (i) niet beïnvloed door externe omstandigheden mits de temperatuur niet te hoog was (Hoofdstuk 5), (ii) er waren vele kenmerken aanwezig (Hoofdstuk 5), (iii) de morfologie was constant binnen collecties behorend tot eenzelfde soort en (iv) er waren grote verschillen tussen en soms binnen soortscomplexen (Hoofdstuk 4). Ad (iii): In twee gevallen had een individuele collectie een morfologie die afweek van die van de overige collecties van het taxon. Het eerste geval was een Marokkaans isolaat van *P. hordei*, dat ook een afwijkend virulentiepatroon had. Dit isolaat is waarschijnlijk een variant die geen taxonomische status verdient. Het tweede geval was een *P. coronata*-roest van kweekgras (*Agropyron repens*) verzameld in Canada. Over de taxonomische status van deze roest konden geen uitspraken worden gedaan omdat maar één isolaat van kweekgras is bestudeerd. Ad (iv): Het kwam slechts drie keer voor dat roesten, vermoedelijk behorend tot verschillende soorten, morfologisch dezelfde infectiestructuren hadden. Het eerste geval was *P. hordei* met de bestudeerde *Uromyces*-roesten op gerst. Aangezien deze soorten ook in sporekenmerken en in urediniosporen- en

aeciosporenwaard sterk op elkaar gelijken, zouden deze soorten tot één soort samengevoegd moeten worden. In de twee overige gevallen, een *P. recondita*-roest van vossestaart, *Alopecurus pratensis*, met *P. graminis* en een *P. recondita*-roest van raaigras, *Lolium* spp., met *P. hordei* verschilden de roesten hetzij in urediniosporekenmerken, teleutosporekenmerken, in vorm van het appressorium of in waardreeks en kan er sprake zijn van convergente evolutie (Hoofdstukken 4 en 7).

Binnen de roesten behorend tot *P. coronata* en *P. graminis* werden alleen kwantitatieve verschillen aangetroffen in morfologie van de infectiestructuren, maar binnen het *P. brachypodii*- en *P. recondita*-complex werden vele typen infectiestructuren aangetroffen. Naast deze roest-complexen zijn ook nog enkele collecties van *P. striiformis* bestudeerd. Deze bleken duidelijk in infectiestructuren morfologie te verschillen van de overige bestudeerde roest-complexen en bovendien enige kwalitatieve variatie binnen de soort te vertonen (Hoofdstuk 7).

De overeenkomsten en/of verschillen binnen een soortscomplex in infectiestructuren morfologie (Hoofdstuk 4) waren goed gecorreleerd met resultaten van de isozymelectroforese van de urediniosporen (aan roestisolaten behorend tot *P. brachypodii*, *P. coronata*, *P. hordei*, *P. recondita* en *P. striiformis*; Hoofdstuk 6.2) en de DNA-inhoud bepalingen van de kernen van de urediniosporen (alleen bepaald voor enkele soorten binnen *P. recondita* en *P. hordei*; Hoofdstuk 6.1) en met literatuurgegevens over somatische en sexuele hybridizaties en andere moleculaire en biochemische technieken (Hoofdstuk 7). Dit bevestigt nog eens de waarde van de infectiestructuren van de urediniosporen voor identificatie en classificatie van roestschimmels van grassen en granen.

Geconcludeerd wordt, dat de morfologie van de primaire infectiestructuren van de urediniosporen een betrouwbaar hulpmiddel is bij de identificatie en classificatie van grasroesten. Op basis van de morfologie van de infectiestructuren en de patronen van de isozymbanden wordt aanbevolen de *P. brachypodii*- en *P. recondita*-complexen op te splitsen in aparte soorten, binnen *P. coronata* en *P. striiformis* variëteiten te onderscheiden, waaronder de nieuw beschreven var. *poae* van deze laatste soort, *P. hordei* en *Uromyces*-soorten voorkomend op gerst te combineren tot één soort, en binnen *P. graminis* voorlopig geen verdere onderverdeling te maken.

CURRICULUM VITAE

Charlotte Ada Swertz werd geboren op 3 januari 1965 te Utrecht. Zij behaalde in juni 1983 het VWO-diploma (met latijn) aan het College Blaucapel te Utrecht en begon in september met de studie biologie aan de Rijksuniversiteit aldaar. De doctoraalfase omvatte hoofdvakken in de Plantensystematiek en Plantengeografie en de Palaeo-oecologie. Hiernaast heeft zij tijdens en na haar studie ervaring opgedaan met vegetatiekundig onderzoek. Na het behalen van het doctoraal diploma in juni 1989, werd zij per 1 juli 1989 aangesteld als onderzoeker-in-opleiding (NWO-BION) bij de vakgroep Plantenveredeling van de Landbouwuniversiteit Wageningen. Onder begeleiding van Dr. Ir. R. E. Niks was zij tot 1 oktober 1993 werkzaam aan een project getiteld 'morfologie van primaire infectiestructuren van de urediosporen als criterium voor de taxonomische indeling van roest-schimmels van grassen en granen', waarvan de resultaten verwerkt zijn in dit proefschrift. Tijdens haar promotie-onderzoek heeft zij de AIO-cursussen 'Engels in de werksituatie' (Quintrix, Wageningen) en 'Electronenmicroscopie en E.M. technieken' (Vakgroep Moleculaire Celbiologie, RU Utrecht) met succes gevolgd.