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THE RUST FUNGUS LIFE CYCLE

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INTRODUCTION

It is the intention of this paper to bring together information on the various morphological structures and physiological phenomena important in the life cycle of the rust fungi. Interjected into this discussion are sections on genetics and sexuality, and axenic culture of these fungi, in hopes of presenting a better picture of the whole life cycle. Concomitantly, it is not the intention of this paper to present taxonomic schemes, to speak to the topic of rust fungus resistance in host plants, or to review the voluminous literature on plant breeding for disease resistance. For the reader interested in keys to the rusts, I recommend the following literature: Cummins (1959, 1971), Gäumann (1959), Thirumalachar & Mundkur (1949a, b, 1950a, b), Arthur (1934), and Laundon (1973).

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The rust fungus life cycle presents perhaps the most plastic and complex such series of events, and yet, considering their economic importance, one of the better understood life cycles. I have had the uneasy feeling that were an effort not made to gather this knowledge into workable form within the very near future, the difficulty of the task would deter the will within a few short years. Undoubtedly, as coming from a relative outsider to this fungus group, some glaring gaps in the literature herein presented will be found, but it is hoped that researchers and students will find the information helpful in the correlation of material from various sectors of investigations.

The manuscript is dedicated to the late Dr. M. A. Donk, a superb taxonomist of basidiomycetous fungi, whose latest (and last) series of publications (Donk, 1972a, b, 1973a, b, c, d) caused this work to be finished.

LIFE CYCLE TERMINOLOGY

Those rust taxa which exhibit all five spore forms (occasionally without spermogonia and spermatia) are termed macrocyclic. Such forms may be restricted to a single host (autoecious), or may infect alternate hosts (heteroecious). *Puccinia graminis* Pers. on barberry and cereal grains, *Cronartium ribicola* Dietr. on pines and *Ribes* are leading examples of macrocyclic, heteroecious rusts, while *Puccinia helianthi* Schw. on sunflower and *Uromyces striatus* Schroet. on alfalfa are macrocyclic, autoecious species. Not all spore forms may be produced during a single season, or by particular strains. All macrocyclic species produce repetitive infections by means of uredospores, but not all spore forms are typical of each taxon.

A second life cycle type is termed demicyclic, and deletes the uredial stage from an otherwise macrocyclic form. Occasionally, as above, spermogonia and spermatia are also missing. Both heteroecious (*Gymnosporangium juniperi-virginianae* Schw. on apple and cedar) and autoecious (*Gymnoconia peckiana* (Howe) Trotter on *Rubus*) are included in this life cycle type.

Finally, microcyclic forms delete both aecia and uredia, usually leaving the telial and spermogonial stage, but often even deleting the latter. Because of the abbreviated life cycle, all microcyclic forms are autoecious. In some (i.e., *Puccinia malvacearum* Bert. on hollyhock), the teliospores appear as such, while in other genera the teliospores have the morphology of aeciospores but function normally in the production of basidia and basidiospores. In *Endophyllum* Lév. the telia are aecidioid (see below) and in *Kunkelia* Arth. they are caeomioid. Thus, the only means to distinguish the teliospores of *Kunkelia nitens* (Schw.) Arth. from the aeciospores of *Gymnoconia peckiana* is to observe the results of germination.

Many rust fungi are known only from the aecial stage, and these are placed in the form genera *Aecidium* Pers. and *Caeoma* Link, based on aecial morphology. Likewise, uredial structures have been found without any seeming relationship to organisms with other stages, and these have been placed in the form genus *Uredo* Pers. Although one might wish that such organisms could be linked to other life cycle stages, and thus be associated with more completely understood genera (not unlike an assumed relationship of imperfect fungi with sexually reproducing taxa), it is likely that such microcyclic forms occur naturally, having deleted all other stages from their life cycles.

A complex system of nomenclature has been developed to quickly indicate the stages found in any particular life cycle in the rusts. While easily understood by students of the group with some experience, the system at first appears bewildering. Those taxa which exhibit all five stages during their life history are called Euforms. They may be Heter-Eu- (infecting more than one host) or Aut-Eu- (occurring on a single host). In some rusts, the aecial stage is deleted, or the aecia and aeciospores are morphologically identical to uredia and uredospores, these organisms being termed Brachy-forms. All these forms are autoecious, thus enabling the "aut-" prefix to be dropped. For those organisms in which spermogonia and spermatia are missing, Maire used Cata- as a prefix, but this usage is rarely seen nowadays. When the uredial stage has been dropped, the organism is called an Opsis-form. This may be used as a prefix, such as Opsis-*Gymnosporangium*, or more commonly as a suffix, such as *Gymnosporangiopsis*. Again, forms can be Heter-Opsis-, or Aut-Opsis-. If this life cycle also deleted spermogonia, it was dubbed Catopsis- by Maire. In more general terminology, rust fungi exhibiting chiefly teliospores (with or without spermogonia) are known as Micro-forms, but Maire again specified those which exhibited both telia and spermogonia as Hypo-forms. In these forms, the teliospores are normal in that they require a resting period before germination. In some taxa, teliospore-like propagules are produced which are lighter in color, exhibit thinner walls, and more obscure germ pores, and which require no resting period before germination, often germinating *in situ*. These spores have been called leptospores, and the life cycle, otherwise identical to that of Micro-forms, is known as Lepto-form. Occasionally, only uredospores and teliospores are found (these sometimes are thought of as imperfect rusts in which other stages will hopefully be found), and these are called Hemi-forms. Finally, in some taxa the teliospores are cytologically similar to aeciospores, in which case the life cycle is called Endo-, the species with such structures often segregated in the genus *Endophyllum*.

TABLE I
STAGES IN THE LIFE CYCLE OF RUSTS

	O	I	II	III	IV
Eu-	±	+	+	+	+
Cata-	—	+	+	+	+
Brachy-	±	±*	+	+	+
Opsis-	±	+	—	+	+
Catopsis-	—	+	—	+	+
Micro-	±	—	—	+	+
Hypo-	+	—	—	+	+
Lepto-	±	—	—	+**	+
Hemi-	—	—	+	+	+
Endo-	±	—	—	+***	+

* Aecia (when present) uredioid.
** Leptospores also produced.
*** Teliospores are morphologically aeciospores.

As the enormously complex life cycle of the rusts unfolded, it occurred to several workers that patterns in these life cycles were apparent. Traditionally, numbers were assigned to the various stages (or spores) of the life cycle as follows: 0 — spermogonia and spermatia (=pycnia and pycniospores; pycnidia and pycnidiospores), I — aecia and aeciospores (=aecidia and aecidiospores), II — uredia and uredospores (=urediospores), III — telia and teliospores (=teleutosori and teleutospores), IV — basidiospores (=sporidia). Because almost all rusts which form teliospores also form basidiospores, the last figure is often deleted from the life cycle shorthand.

As with most basidiomycetous fungi, three distinct nuclear stages in the life cycle may be identified: the haploid monokaryon, the dikaryon, and the diploid. Referring to older terminology which dealt with these stages, Holm (1973) preferred the terms "haplobiontic" to microcyclic, and "diplobiontic" to macrocyclic. Furthermore, certain rust fungi (i.e., *Puccinia punctiformis* D. & H.) could be termed "facultative diplobionts."

Briefly, the macrocyclic life cycle may be summarized as follows, with more detailed discussion of portions of the life cycle fo follow.

The haploid monokaryotic stage is initiated by the germination of the basidiospore. An infectious hypha is produced, quickly expanding into an intercellular mycelium with intracellular haustoria. Dikaryotization classically takes place by nuclear fusion between a spermatium and a receptive hypha at the spermogonium neck, or by nuclear fusion between two compatible hyphae within the host tissue, although other methods have been described. The result is a dikaryotic cell which proliferates into a mycelium distinct from either parent, and which produces aecial sori and aeciospores, often in chains. Aeciospores serve as dikaryotic propagules and in heter-

forms, as infective agents to the alternate host. Aeciospores germinate into dikaryotic hyphae which usually gain entrance into host tissue through stomatal openings, forming a dikaryotic mycelium which eventually produces uredial sori and uredospores. Uredospores, like aeciospores, are dikaryotic, and serve as reinfecting agents. Occasionally, aeciospores and uredospores are morphologically hardly distinguishable. Either within the original uredium or in uredial sori produced by the reinfecting mycelium, a transition into a telium takes place, with the gradual production of more teliospores. These are usually thick-walled and serve as overwintering propagules, but in some genera similar spores are formed with thinner, lighter walls (leptospores), which germinate without a resting period. It is within the teliospore that nuclear fusion occurs, resulting in the diploid stage of the life cycle. The teliospore germinates into a short tube, the promycelium, into which the fusion nucleus migrates and undergoes two meiotic divisions. The four daughter nuclei are separated by cross-walls and on each basidial cell a sterigma is formed, surmounted by a basidiospore. Basidiospore production usually takes place in the spring, with the basidiospore infecting the alternate host to begin the cycle anew.

Tranzschel (1904) first advanced the concept that the "microtelia" (telia) of a microcyclic rust fungus seemed to occur on the same host as the aecial stage of a macrocyclic rust fungus, the telia of which were morphologically similar to the "microtelia" of the microcyclic organism. This has proven true in all cases studied thus far, and the theory has been institutionalized as "Tranzschel's law."

With this summary, the structures and stages of the rust life cycle may be discussed in more detail.

THE SPERMOGONIUM AND SPERMATIA

Persoon (1801) was the first to report these organs, and shortly thereafter Unger (1833) illustrated spermogonia, which he interpreted as distinct organisms from the rusts. Although De Bary (1853) first associated spermogonia as organs of rust fungi, he had no concept of their function. The term spermogonium has been attributed to Tyulyan (see Kuprevich & Transhel, 1957), and has recently become more popular among mycologists, although through the years these structures have been called "pycnidia" (apparently introduced by Brefeld) and "pycnia" (by Arthur, in an attempt to denote structure without obvious reference to the pycnidia of the imperfect fungi and certain Ascomycetes). Although the spores produced have been termed "pycnidiospores" or "pycniospores", they are more popularly referred to as spermatia.

Hiratsuka & Cummins (1963) summarized the various structural and developmental patterns in the spermogonia of the rusts, although several authors had concerned themselves with these organs previously (Arthur, 1904; Hunter, 1927, 1936, etc.). Arthur et al. (1929) distinguished two general spermogonial types: (*1*) subepidermal, "globoid, flask-shaped, conoidal or hemispheric", bounded peripherally, and (*2*) subcuticular or subcortical, flat and spreading, and with no differentiated peripheral boundary. These types were based more on their position within the host tissue than on their individual morphology. Dealing with the spermogonia of the Melampsoraceae only, Hunter (1936) separated seven developmental patterns, again primarily based on the position of the spermogonium within the host tissues. Subordinately, the manner of rupture (pustulate, immersed, applanate) and the shape of the organ were employed, but the system differed relatively little from that of Arthur et al. (1929).

Hiratsuka & Cummins (1963) based their system of classification of spermogonia, using taxa of all families, primarily on growth forms (determinate vs. indeterminate). Subordinate were the presence of bounding structures, and the position of the spermogonium within the host tissues. This change of emphasis to individual growth patterns allowed the emergence of eleven spermogonial types in this system of classification.

Spermogonium production is initiated by congestion of haploid mycelium in the appropriate tissue of the host. This knot of mycelium is at first without differentiated form, and may be located between the cuticle and epidermis (subcuticular), just below the epidermis but above the palisade (subepidermal), or within the deeper host tissues (subcortical). Subsequently, spermatiophores are produced, these being elongated hyphal tips usually tapering distally, and formed in the spermogonial primordium on the side nearest the host cuticle. Two general configurations of this hymenium can result: (*1*) the hymenium in a distinctly flask-shaped or subglobose configuration ("convex" of Hiratsuka & Cummins, 1963), or (*2*) more or less flat, following the contours of the host tissue. In the first instance, the spermogonium forms an ostiole through the host epidermis and/or cuticle, but the flat spermogonial types usually expose spermatia to the air by rupturing host tissues in an undifferentiated slit.

Expansion of the spermogonium can be either within definite bounds, producing an organ of limited size and shape, or can occur indefinitely, forming an irregularly-shaped organ. Moreover, spermogonia of definite shape seem distributed regularly over the host tissue, while those of indefinite form are irregularly distributed. The large genera *Coleosporium* Lév., *Cronartium* Fr., *Frommea* Arth., *Phragmidium* Link and *Triphragmium* Link in Willd. are characterized by indeterminate spermogonia, while *Puccinia* Pers., *Gymnosporangium* Hedw. f. in DC, *Uromyces* (Link) Unger, *Ravenelia* Berk., *Phakopsora* Dietel and *Uropyxis* Schroet. exhibit limited growth of spermogonia.

When bounding structures (a term used by Hiratsuka & Cummins) are present, they may take one of several forms. In the flask-shaped spermogonia of *Puccinia*, *Uromyces* and *Baeodromus* Arth., sterile periphyses are produced within the "neck" of the spermogonium, at the upper limit of the hymenium of spermatiophores and just beneath the ostiole. These periphyses may project well beyond the ostiole or may be short, terminating within the spermogonium body. In some taxa (*Gymnosporangium*, for example) periphyses are produced among the spermatiophores as well as peripherally.

In some subcuticular spermogonia, periphyses agglutinate, coalescing at the base into a more or less definite peridium layer, bounding the periphery of the organ. *Ochropsora* Diet., *Phragmidiella* P. Henn., *Prospodium* Arth. (see also Cummins, 1940), *Uropyxis* and *Phragmopyxis* Diet. exhibit this type of bounding structure composed of united periphyses.

Peridial layers of tissue often are produced when periphyses are lacking in spermogonia. In these cases, differentiated cells adhere laterally to form the peridium, which may or may not be attached to the host cuticle. The spermogonium itself may be subcuticular, sub- or intraepidermal. Of the former category, *Ravenelia*, *Diorchidium* Katch., *Tranzschelia* Arth., *Phakopsora* and *Physopella* Arth. are examples, while *Uromycladium* McAlp. and *Ravenelia* have peridial bounding structures on subepidermal spermogonia. *Gerwasia* Rac. exhibits peridial bounding structures around intraepidermal spermogonia. *Melampsora* Cast. has been reported to produce two types of spermogonia (Hiratsuka & Cummins, 1963), both with peridial tissues, but without periphyses (Hunter, 1936).

The formation of spermatia was described in some detail by Olive (1944) for *Gymnosporangium clavipes* (Cke. & Pk.) Cke. & Pk. Among others, Blackman (1904) and Hunter (1936) had described and illustrated spermatiophores producing spermatia, but had not detailed the process. In *G. clavipes*, Olive (1944) observed that each spermatiophore was apically flared into a small collar, exactly as a phialide. The spermatium primordium expanded into the collar and beyond, forming an ovoid bud at the apex of the spermatiophore. The nucleus of the spermatiophore divided, with the more apical daughter migrating into the spore primordium. The point of union between spermatium and spermatiophore constricted until the spore was separated from sporophore. Another spore primordium was initiated within the sporophore collar, and the process of spermatium development was repeated. Occasionally, several spermatia were observed in chains when no external force had separated the individual cells, but most often the spermatia became dislodged both from the sporophore apex and each other to contribute to the mass of spermatia within the spermogonial cavity. Concurrent with spermatium production in most taxa, a viscous solution, often high in sugar content, is secreted by the hyphae surrounding the spermogonial

lumen, and the whole mixture, spores and "nectar", often not only fills the spermogonial cavity, but overflows, forming either an amorphous droplet at the ostiole or hardening somewhat to form a continuing cyrrhus. In some species, under conditions of exceptional dampness, the fluid-spore mixture can be observed dripping from the leaves of the host, and Arthur et al. (1929) mentioned that in Japan, children were known to sample the "nectar" of some species for its sweetness.

Hughes (1970) has compared spermatium production to phialospore formation in the Ascomycetes (and presumably Fungi Imperfecti), concluding that only in the Ascomycetes and Uredinales are phialospores found. In those Uredinales usually considered primitive (whether those with macrocyclic life cycles by some authors, or those with simplicity of structure, such as *Pucciniastrum* Otth) spermatia are produced presumably by a single method, leading Hughes (1971) to suggest that if phialospore production has evolved from sporangial spore forms, and that if phialosporous modes of spore formation reached their zenith in the Ascomycetes, then the rusts may well be more closely related to Ascomycetes than other authors (notably Savile, 1955) had suggested. Hughes (1970) implied some reservation over the presence of phialides in spermatium production, calling for additional electron microscopic studies in these forms. Basic to his conclusions were the illustrations of Olive (1944), which showed a collar-like extension of the sporophore wall, and sequential spore formation through the collar. Although such a process is clearly phialosporous, electron microscopy has revealed that spore production through annellophores may appear almost identical. Sutton & Sandhu (1969) showed annellospore production in *Cytosporiopsis* sp., *Phoma fumosa* E. & E. and others, and the work of Brewer & Boerema (1965) indicated the same method in *Phoma exigua*. At least in *Phoma* spore production has been accepted as basically phialosporous, suggesting that further fine-structural studies on spermatium production might be profitable.[2] This is especially true in light of Olive's (1944) illustrations of the collar on the spermatiophore apex, often an indication of annellophores.

Craigie (1927) first reported a function of spermatia, briefly describing the fusion of spermatium with "receptive hypha" at the spermogonial ostiole of *Puccinia helianthi*. Receptive hyphae, rather undifferentiated hyphal tips emergent from the flask-shaped spermogonium of this species, apparently served the function of trichogyne in receiving the nucleus of the spermatium, creating a dikaryon nuclear condition in the receptor cell (and subsequently in the subtending mycelium). Craigie (1927) and Buller (1938, in similar

[2] After this manuscript was completed, the following appeared: Rijkenberg, F.H.J. & S.J. Truter. 1974. The ultra structure of sporogenesis in the pyenial stage of *Puccinia sorghi*. Mycologia **66**: 319-326.

forms) clearly separated ostiolar periphyses (paraphyses of those authors), which were marginal at the ostiole, stiff and tapering, from receptive hyphae which were thin-walled, flexuous, with blunt apices, and which emerged from the center of the ostiole. Although often of a different configuration, the spermogonia of the Melampsoraceae (Hunter, 1936) have been reported to form receptive hyphae ("flexuous hyphae" of Hunter). The distinction between periphyses and receptive hyphae was questioned by Savile (1939), who observed all intermediates between the two types identified by Craigie and Buller.

The actual fusion of spermatium with receptive hypha has been illustrated several times (Allen, 1930; Buller, 1938; Savile, 1939), with the details of the fusion and subsequent nuclear migration apparently quite similar in all species studied. The receptive hypha may be uninucleate (at least a single nucleus visible in the portion of the receptive hyphae observed, usually in parafin-sectioned preparations), or may possess two or three nuclei in its apical portion.

Because of the difficulty of tracing such hyphae to their point of origin, the length of the "receptive" hyphal tip has not been mentioned, nor the location of the basal septum of the "receptive" portion. Moreover, repeated illustrations of receptive hyphae with several spermatia adherent have been offered, but no reports have been made on multiple fertilization events. This might be especially important where a number of "physiologic races" are available as spermatium producers. Whether or not such events take place, and the possible fate of the super-dikaryotic nuclei are important points yet to be researched.

Shortly after the attachment of spermatium to receptive hypha is effectuated by the viscous substance found on both structures, the hypha forms a small peg-like protrusion, easily demonstrated if the spermatium is removed at this time (Savile, 1939). Dissolution of the walls of the spermatium and receptive hypha in juxtaposition is accomplished, presumably by enzymatic action, and the nucleus of the spermatium migrates into the cytoplasm of the receptive hypha. At this time, or prior to nuclear migration, the nucleus of the receptive hypha swells significantly. After nuclear migration, the wall of the spermatium remains attached to the receptive hypha as an empty shell. Allen (1930), among others, illustrated binucleate cells at the periphery of pycnia, indicating that they were the result of plasmogamy of spermatium and receptive hypha. Likewise, dikaryotic cells have been observed at the base of the spermogonia, explained by Wang & Martens (1939) as the result of dikaryotization and nuclear migration to the base of receptive hyphae. A similar phenomenon has been described by Sathe (1967) in *Phragmidiella heterophragmae* (Mund. & Thir.) Thir. & Mund. where nuclear migration was observed through the length of the periphyses to their base

where a dikaryotic cell was initiated. This cell proliferated into an independent dikaryotic mycelium resulting in uredia in juxtaposition to the spermogonium. In addition, hypophyllous uredia were subsequently formed.

Blackman (1904) first detected fusion of haploid vegetative cells within the host tissue, figuring nuclear migration quite clearly. Prior to his interpretation, these nuclear events had been observed, but with the implication that cell division, rather than fusion, was occurring.

Several authors have also described the presence of emergent hyphae through the stomata of the host, presumably able to act as receptors of spermatial nuclei. Olive (1953) pointed out that further proof of such a fusion is needed.

Allen (1930) illustrated germination of spermatia into narrow hyphae. Later (Allen, 1934a, b) she observed such germ tubes as mycelium within the host tissue, where it presumably could fuse with compatible hyphae to form a dikaryon. She (Allen, 1930) also observed fusion between pairs of spermatia or their germ tubes, indicating yet another method for dikaryotization.

Brown (1932) sowed uredospores in the vicinity of spermogonia and eventually observed fertile aecia, with the heterokaryotic condition presumably (but not definitely) initiated in the haploid mycelium. In hymenomycetes, this type of cross has been reported by numerous authors, and has been termed a "di-mon" cross.

AECIA AND AECIOSPORES

According to several authors, without dikaryotization incipient aecial structures are formed but do not result in aeciospore production. Fertile aecia, however, arise as a result of dikaryotization of monokaryon mycelia on or within the host tissues. The dikaryotic mycelium is intercellular, forming intracellular haustoria, which will be discussed below, for much of the research on the dikaryotic mycelium has been done with uredospores as infecting agents.

Arthur et al. (1929) recognized six forms of aecial structures, but Cummins (1959) and Wilson & Henderson (1966) narrowed the number to five. Most have been based on characters of the peridium, an almost universal feature of aecia. Not only are these peridial types distinctive, but in the absence of other spore-forming stages on which to base taxonomic placement, aecial structures have been utilized as the basis for "form-genera."

The aecium of *Puccinia* and *Uromyces* is a cupulate structure, with the peridium apparently formed of the outer ring of sporophores and spore primordia (Kuprevich & Tranchel, 1957; Singh, 1969) together with the first-formed spores themselves as the roof. As time goes on, and from the increased pressure of additional spores produced within

the aecium, the peridium ruptures irregularly into small, tooth-like repent projections, revealing the cup-like shape of the aecium. When several such aecia arise in close proximity, the term "cluster-cup" has been used. Because of the stereotypic nature of this aecial type, it has been called the "aecidioid aecium" and has been made the basis of the form-genus *Aecidium*.

Fromme (1914) traced the development of the aecial cup in several species exhibiting aecidioid aecia. From a random massing of hyphae below the epidermis of the host, additional hyphae proliferate around the initial mass in concentric fashion. Soon, however, the inner cells become oriented into a configuration perpendicular to the host surface. The upper cells of these hyphae swell considerably, become increasingly vacuolate, and eventually die. Because of their similarity to parenchymatous tissues of higher plants, Fromme described them as pseudoparenchymatous. The extent and depth of the pseudoparenchymatous tissue varied with the species, from one or two cell layers to more than twenty cell layers. The small, generally rectangular cells remaining viable at the base of the primordium constituted the fertile hymenium.

Fusion of adjacent cells in the primordium base occurs first by a small pore, then subsequently with the dissolution of almost the entire common wall, with concomitant nuclear migration and mingling of cytoplasms. The paired nuclei move up to the central portion of the elongated fertile cell, and by a process of conjugate nuclear division and spore production, aeciospores are produced.

A second peridial type is associated with the perfect genus *Gymnosporangium*, and has been made the basis of the form-genus *Roestelia* Rebent. The peridial cells are elongated, and continue to grow with the production of spores, producing a horn-like process. The apical cells of the peridium are often thicker-walled than the lateral, and dehiscence is usually by splitting of the lateral peridial wall to allow the aeciospores to sift out gradually. Farlow (1880) made some observations on the peridial cells, including the sculpturing of the side walls, but Fischer (1891, 1895) first described the differences in wall ornamentation in detail, and Kern (1910) utilized the patterns of sculpturing as taxonomic characters. Kern (1960) has reviewed the association between *Roestelia* and *Gymnosporangium*, the variations in aecial morphology of the latter, and host relationships of both. *Roestelia* was long thought to occur only on species of the tribe Pomeae (family Rosaceae), but was subsequently found on *Gillenia stipulacea* (Muhl.) Baill. (Arthur, 1909), and still later on *Philadelphus* spp. (Hydrangiaceae) (Arthur, 1912). Moreover, as reported by Kern (1960), not all species of *Gymnosporangium* exhibit roestelioid aecial stages.

Kern (1910) subdivided the roestelioid aecial groups into five subdivisions based on the sculpturing of the peridial cell walls. Aside from those in which the walls were smooth (which he did not accept

as a formal subgroup), he termed the ornamentation patterns rugose, verrucose, verruculose and spinulose. He also noted the hygroscopic nature of the peridial cells of some species (i.e., *R. pyrata*, the aecial stage of *Gymnosporangium juniperi-virginianae*), although declining to comment on the obvious effects on spore dispersal. These peridial cells remain relatively straight under conditions of low humidity, but when exposed to a saturated atmosphere become twisted and curled into subhelical configurations. Such gyrations would tend to force the spores out of the aecium and enhance dispersal during periods of changing humidity.

In another peridial form, the cells are thick-walled, and often in more than a single layer. Because the distal cells are thinner-walled than the lateral, the former were thought to form an operculum, and Arthur et al. (1929), among others, termed the aecial type an "operculate aecium." Always found on gymnospermous hosts (Wilson & Henderson, 1966), this aecial type is associated with the perfect genera *Coleosporium*, *Cronartium*, *Milesina* Magn. and *Pucciniastrum*, and forms the basis for the form-genus *Peridermium* (Link) Chev.

Some aecia develop without a well-defined peridium, and these are called caeomoid aecia. *Melampsora*, *Phragmidium* and *Xenodochus* Schlecht. exhibit these aecia, which also have served as the basis for the form-genus *Caeoma*.

Blackman (1904) described in detail the formation of the caeomoid aecium of *Phragmidium violaceum* (Schul.) Wint. The first evidence of aecium production is a massing of hyphae beneath the host epidermis, usually resulting in a series of parallel hyphae three to four cells long, perpendicular to the host surface. The cells immediately below the epidermis swell somewhat, and are divided by a transverse wall parallel to the host surface, into a lower and upper cell. The upper cell does not develop further, its nucleus eventually disintegrates, and the cell is destroyed by the upward growth of the penultimate cell. The upper cell is therefore homologous to the pseudoparenchyma cells of Fromme (1914). The lower cell elongates considerably, and in due course becomes binucleate (see below). Thereafter, through conjugate nuclear division coupled with spore production (which is also discussed in more detail below), aeciospores are produced in succession. The caeomoid aecium is perhaps the least organized aecial type, as Blackman understood, and its formation is most straightforward.

The means by which dikaryotization takes place at the base of the aecial primoridium deserves some elucidation. Christman (1905, 1907 a, b) has described a process by which two adjacent vegetative cells in the aecial primordium fused completely, with dissolution of their entire common wall. The result was complete mingling of two cytoplasms and the association of two nuclei. Blackman (1904) observed small pores in the common walls between juxtaposed cells,

with nuclear migration from one to another, but with no complete wall disintegration. These two phenomena have been termed Christman- and Blackman-type cells fusions. Earlier, Maire (1900, 1902) had observed the binucleate condition of the fertile cells of the aecial primordium, but had interpreted this condition as the result of nuclear division rather than migration, and had not concluded, therefore, that sexuality played a part in aecial production. No thorough study has been made of the connection of dikaryotized hyphae resulting from spermatium-receptive hypha fusion and the production of aecia, in part because of the difficulty of tracing the basal hyphae of the spermogonium through the host tissue to the site of aecium production. Fusion of uninucleate intercellular vegetative hyphae is common, however, and presumably may lead to the formation of fertile aecia.

Several authors have observed plurinucleate cells in the aecial primordia. Fromme (1914) summarized some of these observations, which at that time included species of *Uromyces*, *Puccinia*, *Phragmidium*, *Endophyllum* and *Melampsora*. When observed, these three- and four-nucleate cells have been interpreted as the result of fusion of a like number of uninucleate cells. In all cases, plurinucleate aeciospores have also been observed. Aside from accentuating the plasticity of the rust life cycle, such phenomena heap confusion on the genetic and sexual mechanisms in these fungi. It has been conjectured that the supernumerary nuclei in the aeciospore disintegrate, but no proof of this has been given.

A major controversy concerns the nature of "uredioid aecia," or primary uredia. Arthur, for many years a dominant force in American uredinology, followed an ontogenetic reasoning in naming the spore stages of the rusts. In his scheme, any structure which resulted from the same mycelium as the spermogonia (presumably after dikaryotization) was an aecium, regardless of its external morphology or the morphology of its spores. Thus, even though in several taxa these structures were superficially identical to uredia, and even though the spores produced were identical to uredospores, they were called aecia by Arthur, usually modified to "uredinoid aecia." Wilson & Henderson (1966) also recognized this aecial type as one of the true forms, but Laundon (1967a) discounted it. He accepted a morphological basis for classifying these spores stages, calling such structures "aecioid uredia," and pointed out several cases of shortened life cycles in which uredia and telia were absent in otherwise macrocyclic rusts. Implicit in acceptance of such uredial aecia is the fact that such aeciospores often germinate into a promycelium or basidium which produces normal basidiospores. In other words, one can either overlook obvious morphological similarity by calling such structures "aecioid uredia," or one can accept a higher plasticity of life cycle by terming them "uredial aecia." Most recently, Hiratsuka (1973) took issue with the morphological system of

classification, giving support to the "ontogenic system" summarized by Cummins (1959). It would seem that this problem will persist for some time, with continued opinions being cast. The two systems of classification were summarized by Hiratsuka (1973).

Two electron microscopic studies of the mycelium at the base of the aecium have corroborated former data. Moore (1963b) and Moore & McAlear (1961) illustrated such cells from the aecia of *Puccinia podophylli* Schw. and *Uromyces caladii*, (Schw.) Farl., both of the aecioid aecial group. In these electron micrographs the cells appeared polyhedral as expected, and often plurinucleate. Transverse wall formation apparently took place as an invagination of the plasma membrane, followed by deposition of wall material. Often separation of cells was incomplete, but no dolipore apparatus was observed. The outer mycelial wall was apparently a stratified structure, often splitting apart, with additional heterogeneous wall material being deposited between the inner and outer portions of the previous wall. This process, illustrated by Moore (1963b) in the somatic tissue below the aecium, seems precisely the same mechanism of cell wall separation figured by Moore & McAlear (1961) for aeciospore formation and disjunction.

A number of authors have described the process of aeciospore formation. Among the clearest descriptions of the phenomenon are those by Colley (1918), Moss (1929), Sappin-Trouffy (1896), and Allen (1934b). After the breakdown of the pseudoparenchymatous tissue initially formed, and after the primordial dikaryotic mycelium has become established within the aecial primordium (by whatever means), periclinal divisions of these dikaryotic hyphae produce a distal cell, often observed to contribute to the peridium layer, and a proximal cell, destined to become the aeciospore mother cell. After some elongation, this binucleate cell (now in an irregular hymenial layer) undergoes a conjugate nuclear division, followed by transverse septum formation, again to form a distal and proximal cell. The distal cell again undergoes synchronous nuclear division, but the transverse septum is formed in an inequilateral position. In some cases this cell division results in a larger aeciospore initial and a small, almost discoid disjunctor or intercalary cell, while in others the basal cell elongates to form a narrow cellular isthmus between the first and second aeciospore initial. The wall of the aeciospore thickens appreciably, with the outer portion of the wall becoming ornamented. The walls of the disjunctor cell remain thin, the nuclei usually disintegrate along with the cytoplasm, and the cell crumples, usually to be found as a small remnant attached to the aeciospore body.

The aeciospore mother cell, meanwhile, has undergone another conjugate nuclear division and subsequent septum formation to form a second aeciospore initial. This initial follows the same path as its predecessor (nuclear division and unequal cell division) to form a second aeciospore and disjunctor cell. Although some taxa produce

single aeciospores, most species exhibit aeciospores in chains, of which the more distal aeciospores are sluffed off by wind, water, insects or other vectors, while the aeciospore mother cell continues to divide to produce additional aeciospores at the chain base.

Hughes (1970) has likened this method of spore production to meristem arthrospore production exclusively found in the imperfect stages of several Ascomycetes, but in no other fungi. The possible links between the rusts and Ascomycetes are obviated by such unique inclusive behavior.

Plurinucleate aeciospores have been reported, presumably formed as the result of multiple cell fusion, although irregular nuclear divisions are also known (see Allen, 1934b), where an aeciospore initial receives three nuclei while the disjunctor cell receives only one. These nuclear irregularities simply add plasticity to an already complicated life cycle, making genetic studies all the more difficult.

The sculpturing of the aeciospore wall has not been exploited as a taxonomic character to any degree. Kuprevich & Transhel (1957) drew attention to some species formerly placed in *Peridermium*, the aeciospore walls of which appeared to contain minute rod-like particles, but the surface of which was punctate-verruculose. Accurate drawings and photographs of aeciospore surfaces are scattered through the literature. Mielke & Cochran (1952), and Hiratsuka (1970), showed the surface ornamentation of *Cronartium* spp. aeciospores to be composed of annulate warts or cogs. Their illustrations indicated that the original aeciospore wall was a stratified structure, with portions becoming dislodged, leaving the prominent but fine ornamentation. Moore & McAlear (1961), from electron micrographs of *Uromyces caladii*, concluded that cogs were left protruding from the spore wall when "intercog particles" fell out from between them. Although the cogs appeared to be composed of a different material than the spore wall itself, no comment was made concerning this apparent heterogeneity.

That aeciospores were forcibly discharged from aecial cups was first reported by Zalewski (1883), who described the phenomenon in three species of *Puccinia* and one of *Aecidium*. Dodge (1924a, b) apparently rediscovered this mechanism independently, but was informed of Zalewski's work by Buller, who also investigated this ballistospore production. Almost all of the species examined produced aecioid aecia, and most were species of *Puccinia* or *Uromyces*. In all (according to Buller, 1924), 11 species of *Puccinia* had been shown to exhibit forcible discharge of aeciospores. Dodge (1924a, b) explained the phenomenon by describing the production of germ pore plugs which appeared to cover the germ pores of the aeciospore wall. These plugs were thought to produce added tension on the already turgid aeciospore walls, which together with the pressure created by spore crowding, bounded by the peridium and host tissue,

and slowly shifting directions of this pressure due to additional spore production, finally allowed the aeciospore wall, also under turgor pressure from within the spore, to expand suddenly, throwing the spore clear of the aecial cup, up to a distance of 15 mm. Buller (1924) observed these "plugs" and thought them to be mucilaginous in consistency, but Dodge found them free in the spore print, and interpreted them as more solid in construction. This theory must be only partially accepted, however, for forcible discharge has also been reported in *Gymnoconia interstitialis* Lagerh. (Dodge, 1924b), which produces no germ pore plugs, and in which only the turgor pressure of the spores themselves could explain the discharge mechanism. In *Gymnosporangium*, forcible discharge was reported in *G. myricatum* Fromme (Dodge, 1924a) which produced aecioid aecia, but was not found in *G. clavariaeforme* (Jacq.) DC with roestelioid aecia. Thus far, therefore, the phenomenon seems limited to aecioid aecia.

Added to this is the hygroscopic nature of the peridial cells in several aecial types. Pady, Kramer & Clary (1969) attributed violent spore release to this mechanism in *Gymnosporangium juniperi-virginianae*, although failing to furnish evidence to substantiate the claim. Buller (1924) described the violent discharge of spore masses (which he called "bombs") of up to 150 spores, and this force might best be explained as a result of hygroscopic peridium qualities. The articulation, ornamentation and movement of peridial cells has been overlooked in direct investigation, but has been incidentally mentioned in the literature, especially in the roestelioid aecial types.

In a series of papers, Pady et al. (1968, 1969) described the periodicity of discharge of aeciospores in *Gymnosporangium* spp. In *G. clavipes* C. & P. and *G. globosum* Farl. spore release was correlated with declining humidity, with peaks of spore release occurring in the morning hours as relative humidity dropped, and occasionally in the late afternoon. Spore discharge was based on contraction of the spore wall in dryness rather than expansion of the spore wall as thought by Dodge (1924a, b), for spores examined after release were collapsed, but immediately swelled again in water. In these species, precipitation caused immediate spore release, but in *G. juniperi-virginianae* the hygroscopic nature of the peridium prevented spore release in water or extremely higher relative humidity. Spore production was thought to be constant, however, for abnormally high spore release was observed shortly after the peridium opened, presumably releasing the spores produced in the aecium while closed. In *G. clavipes* and *G. globosum* spore release was constant at 70°C, 90% relative humidity and 12 hour photoperiod.

Using the morphology of aecia and aeciospores, Leppik (1953) originated a phylogenetic schema for the rust fungi, especially those occurring on coniferous host plants. In this scheme, the prototype aecium exhibited a thick "pseudoperidium" to protect the aeciospores. The aecial type of *Uredinopsis* Magn., while not prototype,

was considered closest to the primitive stereotype, with roestelioid, peridermioid and caeomoid types all derived.

A key to aecial forms of rust fungi was furnished by Laundon (1967b). In it, the host was primary, and the structure of the pycnia tertiary, with the character of the aecium itself (caeomatoid vs. peridermioid) secondary.

THE DIKARYOTIC MYCELIUM

Based on observation of the vegetative state of rust fungi, Eriksson (1896, 1910, 1922) was led to propose the "mycoplasm theory," which held that at the onset of winter the hyphae of the fungus degenerated, the fungus cytoplasm mingling with that of the host cell. With winter past, the "mycoplasm" migrated into the host intercellular spaces, there reforming hyphae and haustoria. Mycoplasm was pronounced in cereal rusts, especially *Puccinia glumarum* (Schmidt) Erikss. & Henn. The theory met with immediate resistance (Bolley, 1898; Ward, 1903a, b; Klebahn, 1904a, b), was not widely accepted, and eventually fell by the wayside as more accurate observations were made on the fungus structure involved.

Allen (1934b, 1923) detailed the germination of, and subsequent infection by spermatia, but drew attention to the extremely narrow mycelium produced, contrasting it with the much broader, coarser mycelium of the dikaryon. The early literature reported on studies employing light microscopy, accurately portraying the dikaryon mycelium as intercellular, but exhibiting "interacellular" haustoria. Although the parent mycelium was typically dikaryotic, numerous reports of common plurinucleate cells were made. Haustorium formation was described in some detail, beginning with the dissolution of a small pore in the host cell wall, and the proliferation of the parent hyphal cell through the hole into the host cell cytoplasm. Almost as soon as penetration was accomplished, the haustorial portion swelled into a subspherical to lobate structure, and one or more nuclei (usually more) migrated through the narrow hyphal isthmus into the haustorial initial. Allen reported that haustoria were commonly three-nucleate. Electron micrographs (Ehrlich & Ehrlich, 1963) have shown that mitochondria and other organelles appear to gather at the point of penetration in the haustorial mother cell just before penetration, and then migrate into the enlarging haustorium after penetration is accomplished. Often deposition of wall or other extracellular material surrounds the point of penetration, and this has been termed the "collar" by Littlefield & Bracker (1970).

Light microscopic studies (Allen, 1933; Rice, 1927) as well as experiments in which the host cell was partially plasmolysed (Thatcher, 1943) had suggested that the host plasmolemma was invaginated by the intruding haustorium, and while previous electron micrographs also indicated such a phenomenon, Littlefield and Bracker (1970) first illustrated the association clearly. Not only did the host

plasmolemma generally conform to the topography of the haustorial cell wall, but often was convoluted, apparently increasing the surface area over which osmosis could take place. Unidentified particulate material was observed between the host plasmolemma and haustorium wall, and numerous lomasomes were associated with the host plasmolemma. In early host cell-haustorium associations the plasmolemma of the host is in close association with the haustorium, but in older associations a gradual encapsulation (see Shaw & Manocha, 1965) of the haustorium takes place, apparently by deposition of particulate material by the host cell external to the haustorium. Such an encapsulation might be the result of immunological phenomena.

The response of host cells to penetration by fungal haustoria varies with the susceptibility of the host. In non-resistant plants, the host plasmolemma becomes invaginated to conform with the general outlines of the haustorium, as described above. In resistant taxa, however, two responses have been reported. By far the more common is the very rapid deterioration of the host cell into which the haustorium penetrates, resulting in the death of that host cell, and the subsequent arrest of further fungus growth within that cell. Under ordinary circumstances, this leads to abatement of fungus growth generally, and the host plant is spared. Such reactions have been described widely in the literature, with summaries by Hooker (1967) and Shaw (1963). A second, apparently much less common response has been reported by Heath (1971), in which the haustorium is rapidly surrounded by a "calloselike sheath" effectively walling the haustorium off from the host cytoplasm. Although Heath stated that this sheath was an extension of the host cell wall, its separability from the encapsulation process described above is doubtful. Furthermore, with proof that biochemical substances pass through and into the encapsulation material (Ehrlich & Ehrlich, 1971), there is little evidence that this process is really effective in curbing fungus growth. Later (Heath, 1972), the ultrastructure associated with these reactions, as well as an intermediate reaction, were described.

Shaw & Manocha (1965) described the ultrastructure of haustoria. Within the fungus cells mitochondria were abundant, often fifteen or more appearing in a single cross-section. Ribosomes appeared tightly packed, and endoplasmic reticulum was common and complex. Lomasomes were commonly associated with the haustorial plasmolemma. In short, the haustorium ultrastructure gave every appearance of a cell in active metabolism. At the same time, significant changes in host cell ultrastructure were described, including formation of extensive, smooth-walled endoplasmic reticulum, fragmentation and contraction of the vacuole, increase in volume of host cytoplasm, and eventual collapse and degeneration of the host cell itself. This process is quite similar to mesophyll cell senescence on water, indicating that while the events during cell degeneration in the host-haustorium

association were not unusual, the senescence process was significantly enhanced by the presence of the fungus.

The haustoria formed by rust fungi appear quite similar to those formed by various other parasitic fungi, including *Phytophthora* (Ehrlich & Ehrlich, 1966), *Erysiphe* (Bracker, 1968; McKeen, Smith & Mitchell, 1966), *Peronospora* (Peyton & Bowen, 1963; Fraymouth, 1956) and *Albugo* (Berlin & Bowen, 1964).

In an additional electron microscope study, this time of aecial material of *Puccinia podophylli*, Moore (1963a) described the dictyosome of the Golgi apparatus. This structure conformed to earlier descriptions from animal and plant material, and was most often observed in subaecial cells in juxtaposition to host cells.

A number of electron microscope studies have shown that the normal dolipore apparatus and parenthesomes found in other basidiomycetous fungi are absent in the rust fungi. Instead, various septal pore structures have been demonstrated, and in some cases, apparent patterns of vesicles surrounding the septal pore and eventually coalescing to plug it. The most recent study, by Jones (1973) on *Uromyces dianthi* Niessl, also summarizes the preceding literature. Jones drew analogies from the septal pore itself, as well as the method of pore plugging, to structures and phenomena in the Ascomycetes, rather than to other Basidiomycetes, thus supporting other evidence suggesting the Uredinales as a more or less distinct phylogenetic line arising from ascomycetous ancestral forms, or from ancestral forms common to both the Uredinales and Ascomycetes.

UREDIA AND UREDOSPORES

According to early reports, aeciospores appear incapable of penetrating the cuticle or epidermis of the host plant, instead depending on stomatal openings as a means of entry. Once the dikaryotic mycelium has penetrated the stomate, the hyphae quickly ramify intercellularly, and infection often becomes locally chlorotic. After a period of vegetative growth, one or more small areas of compacted hyphae appear, with the hyphal tips arranged in a palisade layer. These hyphal tips elongate somewhat, and become several-septate. In some taxa, the apical cell becomes the uredospore, and the subapical cell the stalk cell, while in other taxa the uredospore is formed from a cell 2-4 layers beneath the hyphal tip.

Uredospores represent a purely asexual stage in the rust fungus life cycle. The spore is produced by dikaryon mycelium, the spore itself remains dikaryotic, and germinates into dikaryotic mycelium. Generally overlooking opportunities for parasexuality and somatic recombination, uredia and uredospores have often been used experimentally simply to enlarge a genetic pool of infective material without supposedly changing the pool itself.

As Moss (1926, 1928) described the process of uredospore production in the Melampsoraceae, the following series of events may be

summarized. From the initial "hyphal plexus" there arises a palisade layer of hyphal tips, each three-celled. The apical cell was termed peridial, the second intercalary, and the third (basal) sporogenous, and the three were easily distinguished by staining intensity. The intercalary cell soon disintegrated. The basal cell underwent synchronous nuclear division and subsequent cytokinesis, with the apical cell termed the "bud". The bud cell elongated somewhat, and again underwent nuclear division and septation. The apical cell inflated, and differentiated into the uredospore initial, while the subapical cell elongated, but remained thin-walled, forming the stalk cell. Even before maturation of the uredospore, the basal (the original sporogenous cell) cell usually branched, with nuclear division, to form a new "bud" cell, which in turn also divided and differentiated into a uredospore and stalk cell. As all these cells elongated and inflated, pressure was created against the peridial cells, which first became flattened, and then together with the host cuticle, were pushed outward, eventually rupturing to reveal the underlying sorus.

Release of individual uredospores is gained through rupture of the stalk cell, either very close to the uredospore body, in which case only a small collar is left to represent the stalk cell, or at some other point lower in the stalk cell, in which case a hyphal appendage of some length remains attached to the spore body.

Uredospores are most commonly ovoid to ellipsoid, but may be globose or depressed-ellipsoid or lobed in shape, or may be more or less isodiametric and produced in chains. These latter forms have been called aeciospores by some (Laundon, 1967b), but Hiratsuka (1973) and others retain the belief that by function such spores are uredospores.

Most uredospores exhibit ornamentation of the outer wall, and this has been variously described as echinulate, verrucose, granular or warted with scanning electron microscope studies corroborating earlier light microscope findings. While the markings on the uredospores of *Puccinia conoclinii* Seym. were found to be short, sharp, regularly scattered spines, the sculpturing of *Coleosporium helianthi* (Schw.) Arth. uredospores was seen as flattened, coalesced projections, with scattered small warts and ridges intermixed (Grand & Moore, 1970). In addition to raised ornamentation, germ pores are almost invariably present.

In the most exhaustive study of uredospore pores, Cummins (1936) distinguished between scattered, bizonate and equatorial distribution of the pores. Number of pores per uredospore ranged from 1-20. Certain correlations were made as follows: the number of pores per uredospore was higher in the Melampsoraceae than in the Pucciniaceae, scattered arrangement of pores was most common in *Puccinia* and *Uromyces*, uredospores with scattered pores were most often produced in uredia with peridia or paraphysoid hyphal tips, and spores with scattered pores were most commonly found on more

primitive host plants. Conversely, equatorial distribution of pores was correlated with the opposite of each category. Cummins suggested that the character of numerous scattered pores was a primitive condition in the rust fungi, but that reduction in size, or restriction in distribution of pores had had no effect on the phylogenetic or biological survival of the rust fungi.

Very little seems to be known about the structure of the pore itself. Although the literature on germ pores in basidiospores is growing rapidly, no concommitant literature for rust fungi seems available. Cummins (1936) observed that the pore was covered with a small cuticular cap extending beyond the normal curvature of the spore wall, and that the ornamentation of the spore wall could extend over the pore region or could be restricted from it. Likewise, a thickening of the spore wall in juxtaposition to the pore was occasionally noted, not unlike a bordered pit in coniferous xylem vessels.

In several genera, uredospores are either without color or with very small amounts of pigmentation, as in most species of Melampsoraceae, and in these taxa, pores were mostly scattered. *Bubakia* Arth. was singled out because of strong pigmentation and equatorial pore distribution. Conversely, genera of the Pucciniaceae such as *Phragmidium* and *Uropyxis* have generally colorless walls, and scattered pores, while other genera (i.e., *Pileolaria* Cast., *Tranzschelia*, *Cumminsiella* Arth., etc.) exhibit pigmented wall and equatorial pores. In *Ravenelia* and *Puccinia*, with pigmented spores, a large portion (75% in *Puccinia-Uromyces*) also have equatorial pores.

Pigmentation in uredospores of *Puccinia graminis* f. sp. *tritici* Erikss. & Henn. was shown to be composed of cytoplasmic and wall fractions, a further discussion of which may be found below. Dillen Weston (1931) found that normally pigmented spores were more resistant to ultra-violet radiation than colorless spores.

As in the development of aecia, various forms of peridia limit the uredium. In some genera (*Pucciniastrum*, *Melampsoridium* Kleb. etc.) the peridium is well developed, several cells thick peripherally, and at least one cell thick over the sorus. In these genera, an apical "ostiole" is developed either as a result of differentiation of the circumostiolar cells (*Melampsoridium*) or by degeneration of the cells in that region. Savile (1955) has ascribed the function of protection from insects to such outward-pointed cells. In some species of *Cronartium* a similar peridial structure is formed, but this often weathers away during spore discharge. In other genera (*Hyalopsora* Magn., *Uredinopsis*, *Milesia* White, etc.) the uredial peridium is very thin over the sorus, but somewhat thicker and composed of isodiametric cells peripherally. In *Melampsora* the peridial structure is even more delicate, and in most taxa with uredial peridia as a unicellular layer, the peridium is extremely fugacious, almost indiscernible in mature material. For this reason, the peridial structure has not been emphasized in taxonomic systems, except in those genera in which it is unique.

In some rust fungi, notably *Uredinopsis*, the uredospores are dimorphic. In such taxa, the uredospores produced in spring and early summer are uniformly thin-walled, while those produced toward late summer and early fall are often verrucose and with thicker walls. These latter spores have been termed "amphispores," and were thought to aid the fungus in overwintering. These are not to be confused with "leptospores," thin-walled spores produced in telial sori with otherwise thick-walled teliospores.

Uredospores are often interspersed with "sterile" hyphal tips, and these have been termed paraphyses. In some taxa, these paraphysoid hyphae are differentiated in some way, either flexibly lanceolate, capitate or urtiform. In some forms, such as *Phakopsora vignae* (Arthur, 1929), the thick-walled, inflated paraphysoid hyphae form a canal through which the uredospores pass after pedicel rupture. In other unique forms, the hyphae of the uredial primordium protrude through a stomate, then diverge to produce a saucer-shaped apothecial structure, on the upper surface of which are developed uredospores (Cummins, 1937). In most forms, the uredium is located beneath the cuticle and epidermis of the host plant, becoming erumpent when spore and stalk cell development forces a rupture of host tissues.

Uredospores serve as reinfecting agents, and as such, are unique in the rust fungus life cycle. Because of the rapid development of uredia after initial infection, several crops of uredospores may be produced in a single growing season, with wind-blown uredospores being dispersed over long distances. Thus the common wheat rust fungi can overwinter in the uredial state in more moderate climates, and by successive crops of new uredospores on host plants quickly spread northward each summer into areas otherwise uninhabitable by these fungi. Such overwintering in frigid climates could well be accomplished by teliospores, and undoubtedly occurs, but concerted eradication programs for alternate hosts have eliminated much of the substrate for possible infection by resultant basidiospores, making this form of overwintering less economically important.

Early studies on longevity of uredospores indicated that weather conditions largely controlled the ability of the spores to overwinter or oversummer. Laboratory experiments (Rosen & Weetman, 1940) corroborated these data, indicating that at low temperatures (5-10° F) and moderate to low humidity (25-50%) uredospores retained high infectivity for at least 300 days, but that the ability to infect was reduced substantially if not totally at higher temperatures and/or higher humidities.

The use of a continual spore counter (Kramer & Pady, 1965) has shown that uredospores of three species of *Melampsora* and *Puccinia* are liberated increasingly toward midday, and spore liberation is decreased thereafter (Pady, 1971). This corroborates earlier findings of such periodicity in air-borne uredospores (Pady et al., 1965).

Although not often used as a source of primary taxonomic character fields, although often as secondary, uredospores have been employed by Cummins (1971) in a key including *Puccinia*, *Uromyces* and *Uredo*. As used by Cummins, the various groups are as follows:

Group I. Uredia with paraphyses; uredospores echinulate; germ pores equatorial or rarely basal.
Group II. Uredia with paraphyses; uredospores echinulate; germ pores scattered.
Group III. Uredia with paraphyses; uredospores verrucose; germ pores equatorial. *No species known.*
Group IV. Uredia with paraphyses; uredospores verrucose; germ pores scattered. One species of *Uredo*.
Group V. Uredia without paraphyses; uredospores echinulate; germ pores equatorial or rarely basal.
Group VI. Uredia without paraphyses; uredospores echinulate; germ pores scattered.
Group VII. Uredia without paraphyses; uredospores verrucose; germ pores equatorial.
Group VIII. Uredia without paraphyses; uredospores verrucose; germ pores scattered.
Group IX. Uredia not produced (-opsis-forms), or unknown; species of uncertain affinities.

Hughes (1970) used uredospore production as a model to which to apply terminology usually reserved for classic "imperfect fungi" (although surely uredospores are asexual, and therefore must be considered to belong to an "imperfect" generation of the rust fungi). In these terms, the production of pedicellate uredospores in succession from a single sporogenous cell was strictly analogous to "sympodioconidia."

UREDOSPORE PHYSIOLOGY

Of total uredospore mass, Shu et al. (1954) found 21.9% carbohydrate, 1.4% chitin, 25.9% protein and 19.7% lipid, and Yarwood (1950) found water content ranging from 10-30%. The spore wall includes chitin (Shu et al., 1954), glucomannan (Prentice et al., 1959) and hemicellulose (Evtushenko, 1960). The major sugar of resting uredospores is trehalose (Syamananda et al., 1962), which may be a storage form, converted into more useable forms on germination.

As might be expected, most normal pathways have been identified and found functional in uredospores. Enzymes of the Embden-Meyerhof-Parnas pathway (Caltrider & Gottlieb, 1963), the hexose-monophosphate pathway (Caltrider & Gottlieb, 1963; Staples et al., 1961) and the tricarboxylic acid cycle have been found, although the TCA cycle was most difficult to prove (Staples & Wynn, 1965).

Electron transport system (White & Ledingham, 1961; Caltrider & Gottlieb, 1963) and protein synthesis were also identified as more or less normal, although resting uredospores were found to synthesize protein largely from pre-existing proteins rather than from incorporation of new substrate.

As might be expected, central to several pathways was acetate. In one series of experiments (Staples, 1962), acetate carbon was rapidly incorporated into a number of cell constituents, including protein, but most rapidly into the organic acid pool, including malonate and glutamate. Incorporation into these acids indicated that both the tricarboxylic acid cycle and glyoxylate pathways were functional. Moreover, uredospores are able to convert propionate into pyruvate and then to acetate (Reisener et al., 1963), but the exact pathway appears different from the same conversion in higher plants (McConnell & Finlayson, 1964). Assimilation of short-chain fatty acids such as valerate and pelargonate was rapid, and degradation was to propionate, pyruvate and acetate (Reisener et al., 1964). Much of this information was gained from experiments utilizing radioactive labelling of substrates, which also showed that synthesis of certain proteins proceeded from glutamate and acetate, as would be expected.

Staples & Wynn (1965) made the interesting and somewhat frustrating point that discrepancies in results using uredospores for physiological studies might be accounted for, in part, by likely contamination of uredospores by bacteria or fungal spores, especially where relatively large quantities of uredospores were needed for experimental purposes. Moreover, variation in growth conditions for host plants caused concomitant variation in the "quality" of uredospores, perhaps leading to experimental results not in agreement across broad lines.

Among the significant changes in physiology occurring at germination are synthesis of chitin, decrease in lipid content, and increase in soluble carbohydrates (Caltrider et al., 1963; Shu et al., 1954). Conflicting data on protein synthesis are available (cf. Sussman & Douthit, 1973), and virtually no change in rates of endogenous or exogenous gas exchanges (Shu et al., 1956) occur. Increases in carbohydrate metabolism are accompanied by changes in numbers, size and configurations of mitochondria (Williams & Ledingham, 1964).

A number of workers have experimented with variables in the germination process, including physical forces such as light, temperature and moisture (Emge, 1958) and contact phenomena (Dickenson, 1949, 1955), and in some instances "fusion bodies" were reported (Rodenhiser & Hurd-Karrer, 1947). These closely resembled the substomatal vesicles found in axenic cultures. Strangely, however, none of these experiments led to axenic cultures of the organisms involved.

Conflicting conclusions have been drawn regarding respiratory rate changes in dormant versus germinating uredospores. It is commonly accepted that respiratory rates are reduced in dormant stages, and that upon germination, significant increases in respiratory rates are measureable. Shu et al. (1954) concluded that no such dramatic differences were detectable in germinating uredospores of *Puccinia graminis*. The more recent data of Maheshwari & Sussman (1970) indicate that although the kinetics are complex, an increase of respiratory capacity in germinating uredospores is detectable. Correlation of phospholipid synthesis with respiratory rate changes in germinating uredospores has also been made (Langenbach & Knoch, 1971a, b).

Some time ago (Allen, 1955, 1957) a volatile inhibitor of uredospore germination was found to be produced by uredospores themselves. One report (Forsyth, 1955) indicated a possible identification as trimethylethylene, but more recent data identify the compound as methyl ferulate (Macko et al., 1971). These data and others are summarized by Fries (1973). The inhibiting substance is apparently lost when uredospores are soaked in water.

The nature of the self-inhibitor of germination produced by uredospores has also been postulated as an inhibitor of respiration (Naito & Tani, 1967). Various sets of data have related spore population density and effective inhibition, while others have found no correlation. This literature was briefly summarized by Sussman & Douthit (1973).

Uredospore germination is strongly enhanced by certain aldehydes, terpenes and related compounds (French & Gallimore, 1971, 1972), and n-nonanal, a strong germination inducer, is produced by uredospores themselves (French & Weintraub, 1957).

GENETICS AND SEXUALITY

Until very recently, no attempt could be made to aseptically cross rust strains, for these fungi had not been grown on artificial substrates. Nevertheless, several studies on mutagenesis and genetics were accomplished, summarized most recently by Day (1972).

In general, two mutants types could be exploited in crossings. First, mutants for virulence could be detected naturally, or produced by use of an accepted mutagen. For the most part, increased virulence was detected, and these mutants harvested for genetic studies, but in some cases (Flor, 1950; Johnson & Newton, 1938) mutants with decreased virulence were identified. Such forms could act as adequate markers in genetic studies as well. Moreover, as might be expected, resistence or susceptibility to virulent mutants may be detected in both hosts of heteroecious rusts. Other factors, some of which are surely not subject to genetic analysis, also govern virulence

and resistence, and these have been termed "variations in aggressiveness" (Day, 1972). Together, genetic pathogenicity and aggressiveness have been discussed by van der Plank (1968), and Zadoks (1972).

Second, morphological markers have been detected or produced. In the best known of these, uredospore color mutants may be noted in nature or may be induced. In *Puccinia graminis*, uredospore cytoplasm is normally orange due to a carotenoid pigment, while the uredospore wall is brownish. When both pigments are present, a rich reddish-brown effect is created, but single mutants may be either "orange" (colorless wall, normal cytoplasm), or "brown" (normal wall, colorless cytoplasm), and selfing between F_1 progeny produced occasional double mutants, which were white (Newton & Johnson, 1927; Newton et al., 1930). Both mutants were found to be recessive. Similarly, white spermogonia were observed as a result of single basidiospore inoculation of barberry (Green, 1964). Using ethyl methane sulphonate, Baker & Teo (1966) were able to produce the usual uredospore color mutants, as well as mutants in which teliospores were produced much more rapidly than usual.

Induction and selection of mutants is far more difficult in a dikaryotic or diploid system than in a haploid. When genetic studies must be carried on through the intermediary of a host plant, in addition to the vagaries of the dikaryotic system, however, rather little can be established which is not open to significant question. Two general methods have been utilized to select for mutant types: 1) use of uredospores known to be heterozygous for a trait, with selection for those in which the trait is expressed, even though recessive; and 2) use of uredospores with mixed or unknown pedigree, and selection against hosts normally exhibiting resistance. Day (1972) has suggested that treatment of dormant or germinating teliospores, with selection of infections produced from single basidiospores might be more productive, although this has not been purposefully attempted as yet.

Two means of genetic analysis are open to investigation. 1) Meiotic recombination, resulting from normal diploidization and concomitant meiosis in the promycelium, is exhibited in the basidiospores produced. If such a population of basidiospores is properly diluted and applied to the host plant, often single-spore inoculations can be made. Genotype purity is assumed to be maintained, but the inoculum greatly increased, by spermogonium formation from single basidiospore inoculation. Spermatia may be used to make reciprocal crosses (Flor, 1942; Patton, 1962), or pooled (Johnson, 1946, 1954). In such crosses, the spermatium contributes virtually no cytoplasm, while the receptive hypha contributes both a nucleus and cytoplasm. It is possible, therefore, to establish that certain traits of virulence are cytoplasmically inherited (Johnson, 1946, 1954). In all such crosses, the resultant aecia may be analysed, or the aeciospores used as inoculum, and the resultant uredospores or teliospores analysed.

2) Mitotic recombination was predictable when fusion between dikaryons, between dikaryons and monokaryons, and between monokaryons was observed. Genetic evidence was not forthcoming until parasexual behavior was observed in *Aspergillus*, prompting investigators to attempt similar experiments with rust fungi on host plants. Nelson et al. (1955) appear to have first established the concept in these organisms, while Watson (1957), Watson & Luig (1968) and Ellingboe (1961), among others, experimentally demonstrated the phenomenon of diploidization and somatic recombination.

AXENIC AND TISSUE CULTURE DOMESTICATION

Williams et al. (1966) reported that uredospores of *Puccinia graminis* f. sp. *tritici* could be germinated on artificial medium containing yeast extract, especially if the density of spores was high. Germination was followed by subsequent growth as sterile binucleate mycelium to form small colonies, which eventually formed uredospores and teliospores. Later (Williams et al. 1967), sporulation in artificial culture was enhanced, and the spores used in infection experiments. Mycelium so grown was also successful in producing infections under sterile conditions, and the causal organism was again isolated from sori, fulfilling Koch's postulates for the isolation of disease-causing organisms. Suspension of uredospores in gelatin (Coffey et al., 1969) was found to enhance germination and subsequent growth over spores spread in a water suspension.

Perhaps most intriguing was a report by Bushnell (1968) that, although the original Australian strain of *P. graminis* used by Williams et al. (1966) had been manipulated successfully in a North American laboratory, 11 North American strains of the same species had failed to grow under identical conditions. Moreover, an additional three strains of Australian fungus had been artificially cultured during the same time. Unless some peculiar technical error was at fault (hardly the case since the original strain had performed as expected), North American strains of *P. graminis* f. sp. *tritici* had uniformly resisted culture while four strains of Australian fungus had succumbed.

Melampsora lini has recently been domesticated on similar media (Turel, 1969a, b; Coffey, Bose & Shaw, 1970). Attempts were made to culture three strains of the fungus, and all three responded favorably. In similar experiments (Harvey & Grasham, 1970a, b, c) *Cronartium ribicola* was grown on cellophane membranes covering tissue cultures of *Pinus monticola* Dougl. Tissue cultures of western white pine were subsequently infected using germinating basidiospores of the fungus, and still later by infected tissue cultures.

For many decades the rusts were assumed to be obligate parasites unable to be domesticated on synthetic media. Although for the most part the definition was expressed correctly, there was a distres-

sing tendency to assume that the reason for the obligate nature of the parasitism was the inability to be cultured, and not the opposite which was really the case. First, a number of investigators dealing exclusively with higher plants found that such plants could be placed in culture on artificial media, forming callus tissue in such situations. Using the information thus gained, research on the co-culturing of rusts on plant tissue cultures was undertaken, and the results published (Hotson & Cutter, 1951; Cutter, 1951, 1952, 1959, 1960; Hotson, 1953). *Gymnosporangium juniperi-virginianae* was so grown, followed by *G. globosum* and *G. clavipes.* Later, *Uromyces aritriphylli* (Schw.) Seeler was similarly domesticated. In this process, plant tissue cultures were obtained under sterile conditions from the host plant, either already infected with rust mycelium, or without infection. Infected calluses occasionally produced characteristic rust spores, but usually exhibited only a sterile mycelium in the nuclear condition appropriate to that stage of the life cycle from which the isolate had been taken. Infection experiments were undertaken in tissue cultures and field-grown plants, and these met with generally uniform success.

In some instances, the rust mycelium growing intercellularly on plant callus was observed to grow into the agar medium beneath the callus. This phenomenon was almost always preceded by a degeneration of the callus tissue, but the resulting "saprophytic" mycelium could be subcultured onto artificial media without the presence of the host plant. While growth was reported as slow under such conditions, isolates were subcultured for some years on modified Gautheret's medium. Because of difficulties in identifying the original isolate of *Gymnosporangium*, (see Cutter, 1959) the total findings of these reports were cast into some doubt for several years. Few other attempts to culture rusts were made until some years later.

A cytological study of vegetative hyphae and spore production of saprophytically grown *Puccinia graminis* was made by Rajendren (1972). He surmised that nuclear migration occurred through septal pores into younger, anucleate hyphal tips. Uredospores were formed both on diffuse mycelia and in deep locule-like cavities.

Nuclear staining of axenic cultures resulting from germination of uredospores of *Puccinia graminis* f. sp. *tritici* (Williams, 1971) has shown that both dikaryotic and monokaryotic mycelia are produced. The production of dikaryotic mycelium from germinating uredospores was found to be correlated with the formation of infective structures, namely, the substomatal vesicle. In most instances, an appresorium was also formed, but in its absence, dikaryotic hyphae proliferated from the substomatal swelling. Conversely, monokaryotic mycelium apparently resulted from a monokaryotization of binucleate uredospores somehow influenced by the absence of the substomatal vesicle of the germling. The highest percentages of substomatal

vesicle production were obtained on nutrient agar (vs. water agar), after heat shock (30°C for two hours), using uredospores from recently opened uredia. Allen (1957) had demonstrated production by uredospores of a volatile substance when the spores were germinated on the surface of water or dilute buffer. This substance stimulated the production of infective structures (appresorium and substomatal vesicle).

The substomatal vesicle has been postulated as the seat of biochemical processes which govern infectivity and the hyphal type to be produced subsequently (Williams, 1971). Dickenson (1949, 1955) had theorized such governance, with the reports of Dunkle et al. (1970) showing that nucleoli appeared in the vesicle, Dunkle et al. (1968) and Ramakrishnan & Staples (1970) on the effect of inhibitors, new protein synthesis, and RNA synthesis, supporting such an influential role for this structure. Coffey & Allen (1973), conversely, found no spontaneous production of vesicles or appresoria in axenic cultures of *Melampsora lini* or *Puccinia helianthi.*

In the few papers dealing with nutritional requirements of rust fungi grown saprophytically, most common sugars (glucose, mannose, fructose, etc.) were found to support growth, one or more amino acids were required, including in some cases a sulphur amino acid, and in *Puccinia graminis* (Coffey et al., 1969; Kuhl et al., 1971; Scott & McLean, 1969) and *P. helianthi* (Coffey & Allen, 1973) bovine serum albumin seemed a requirement.

TELIA AND TELIOSPORES

Typically in the macrocyclic rust fungi, late in the growing season, a second spore type appears within the old uredial sorus, or in separate sori scattered toward the periphery of the infection. The spores produced are often significantly thick-walled, and form the over-wintering mechanism for the rust fungus. Teliospores also act as the location for karyogamy.

The shape and location of the telium vary considerably. In some genera usually considered as primitive (Laundon, 1973), teliospores are sessile and embedded within the host tissues, either in the mesophyll (*Uredinopsis*), or in the epidermal region. In the latter category, spores may be found in a monolayer (*Melampsora*), or in more than one rank (*Phakopsora*). In all these genera, the sessile teliospore has been used as a primary character to segregate the Melampsoraceae (sessile) from the Pucciniaceae (pedicellate) (cf. below, under suprageneric classification).

Formation and germination of intraepidermal teliospores of members of the Pucciniastraceae have been reported by several authors. Pady (1946) described the process in *Melampsorella cerastii* (Pers.) Schroet., and compared this process with similar events described by other authors. In *M. cerastii* the dikaryotic mycelium in the host

plant leaf (*Cerastium arvense* L.) ramified intercellularly, but just above the lower epidermis hyphae became more densely packed, until a more or less pseudoparenchymatous layer of hyphae was formed in that region. From this layer, certain cells were identifiable as "primordial cells" by their brick-like shape. From these cells, slender penetration tubes were produced, penetrating the host lower epidermal cells in a manner not unlike penetration to form normal dikaryotic haustoria. The protuding hyphal tip in the host epidermal cell was initially elongate, but quickly swelled. Nuclear migration from the primordial cell into the teliospore initial cell followed rapidly, with the nuclei becoming very long and slender in passing through the isthmus between parent cell and teliospore initial. Almost as soon as the two nuclei passed into the teliospore initial, karyogamy occurred, and at this point the teliospore initial could be termed the teliospore. Teliospores remained thin-walled, and germinated without a dormancy period. Occasionally the teliospore initial became divided into two or more cells, but each cell apparently acted as an independent teliospore.

Although successive "budding" from the primordial cell aggregate and subsequent penetration and teliospore initial formation were described, successive nuclear division within the primordial cells was not reported, nor was the development of more than one teliospore initial from a single primordial cell. Upon germination of the teliospore, the old teliospore became evacuated and collapsed, allowing space for the formation of new teliospore initials. In most infections, virtually the entire host lower epidermis was involved in teliospore production.

In the Pucciniaceae, teliospore production is almost identical to that of uredospores. Teliospores are pedicellate, the stalk cell becoming evacuated during maturation of the spore, and eventually rupturing to free the spore, or remaining intact, and the spores largely remaining in the sorus on the dead or moribund plant material. In most species the stalk cell is nonfunctional except as an anchoring device, but in some taxa (i.e., *Gymnosporangium*) the stalk gelatinizes, extending the spore into the air for possible liberation or more probably to germinate, producing wind-blown basidiospores *in situ*.

In *Puccinia* teliospores are produced in gradually increasing numbers within the old uredial sorus, now more properly called the telium or telial sorus. In this way, the original "meristematic" cells of the palisade which produced uredospores are converted into the production of teliospores. In *Gymnosporangium*, however, uredia and uredospores are commonly not present, and telia are produced *de novo*. Dodge (1918) described the organization of the telium in this genus, as well as the ontogeny of the individual teliospore. A palisade of hyphal tips, each 4-6 cells long, was formed beneath the epidermis of the host cell, much as that described for the organiza-

tion of most uredial sori. The apical cell of each chain was sacrificed as an enlarged, more or less bladder-like cell, which Dodge referred to as a "buffer cell," for its seeming function was to act as a buffer between the sporogenous cells below and the host epidermis and cuticle above. When these cells had inflated and evacuated, the penultimate cell of the chain began to elongate into the "spore bud." A paired nuclear division took place, followed quickly by septum formation to form the basal cell, which apparently remained meristematic, and the spore bud itself. Although Dodge did not explain the differentiation into spore and stalk cell, other authors report the subsequent division of the bud into an upper and lower cell, just as in uredospore production.

From the basal, meristematic cell of the chain, new hyphal branches are produced, destined to differentiate into additional spore-stalk combinations.

In the normal long life cycle, when either aecia or uredia or both are produced, the telium arises directly from preceding dikaryotic mycelium. In rust fungi where no aecial or uredial stage exists, telia are produced from the union of monokaryotic mycelia. In *Galloway a pinicola* Arth. (Dodge, 1925), teliospore production is initiated by fusion of juxtaposed cells in the telial primordium, which occurs in the haploid state. These fusions occur between cells in the third or fourth rank in the chains of cells composing the primordium. After cell fusion to form the dikaryon, the apical, uninucleate cells elongate, eventually rupturing the host epidermis and revealing the developing telial sorus. The teliospores are produced in chains, separated by evacuated, elongate cells, not unlike those produced in *Didymopsora* Diet. (Cunningham, 1968).

Allen (1933) traced the nuclear behavior and development of individual teliospores in *Puccinia malvacearum* Bert. The dikaryotic primordial cells begin differentiation with elongation of the cells toward the outer surface of the host, usually with two terminal cells elongating simultaneously. Usually before any discernible differentiation of the teliospore initial itself, a branch hypha is produced just below the terminal septum, eventually to develop into a second teliospore-stalk combination. In this species, only single teliospore-stalk combinations are produced from each hyphal tip. The teliospore initial begins to swell and eventually develops the characteristic thick wall with germ pore, while the stalk cell slowly elongates, remaining slender and thin-walled. The contents of the stalk cell eventually degenerate, and the cell generally evacuates, and finally, from a combination of weather action and pressure from underlying cell growth, the stalk cell ruptures and the teliospore is liberated.

Nuclear fusion may take place at various times during the maturation or even the germination of the teliospore. In *Puccinia malvacearum* (Allen, 1933) the teliospore initial is binucleate, remaining so until the thick wall of the teliospore begins to develop. The stalk cell apparently remains binucleate throughout its life. In

Didymopsora paraguayensis (Speg.) Cunn. (Cunningham, 1968), the teliospores of which are formed in chains, the lower individual teliospores are binucleate, with nuclear fusion occurring during the period in which the teliospores are pushed out by growth of underlying cells. Apparently by the time the teliospore is liberated, it is in the diploid state. This sequence seems to agree with that found in *Gymnosporangium juniperi-virginianae*, (Weimer, 1917), where the young teliospores are binucleate, but by the time of gelatinization of the stalk cell the teliospores are uninucleate and diploid.

Using terminology borrowed from more classical imperfect spore production, Hughes (1970) summarized the three major means of teliospore production as follows: a) meristem arthospores — basically the same process leading to aeciospore production, with spores in chains interspersed by intercalary cells; b) sympodioconidia — basically the same process leading to pedicellate uredospore production; and c) a simple budding system characteristic of the Melampsoraceae. These three methods of teliospore production are more or less congruent with well-accepted taxonomic groups, namely, *Cronartium*, *Puccinia* and *Melampsora*.

In the formation of some teliospores, notably of species of *Scopella* Mains, *Chaconia* Juel and *Olivea* Arth., the sporogenous cell is easily identified, and teliospores may be produced in succession from it. Some investigators found the presence of such cells valuable as taxonomic characters, but Thirumalachar & Cummins (1949) discounted their significance in taxonomy, and distributed these organisms over already established genera. Although apparently often difficult to observe, such a mode of teliospore production insures the succession of teliospores of different ages, and acts as a repetitive sporogenous device, quite possibly an advantage over the production of a single crop of teliospores as usually found in the Melampsoraceae.

Little function has been ascribed to the pedicel of the teliospore. In several species of *Gymnosporangium* the pedicel imbibes water and swells to rather enormous proportions. When the aggregate of many such pedicels swell concurrently, the typical "spore horn" of the genus is produced. Some species of *Puccinia* have also been reported with swelling pedicel, and in *Uropyxis amorphae* (Cart.) Schroet. the inner wall of the teliospore itself expands with the pedicel. Pady (1948) reported on a spore dispersal mechanism of *Puccinia tumidipes* Pk. in which the pedicel is ruptured at spore maturity, with the teliospores generally lying loosely within the telium. When the pedicel comes in contact with water, it suddenly evaginates, quickly gelatinizes and surrounds the teliospores itself. The result is first to throw the spore a short distance (1-5 mm) from the telium, and second to prohibit the teliospores from becoming clumped, at least when wet. Finally, the gelatinized pedicel dries to a horny mass, with the aggregated teliospores becoming crust-like,

presumably an additional over-wintering advantage.

Such an adventure into teliospore forceable dispersal should not be confused with basidiospore dispersal, and therefore be taken as an additional mechanism for forceable discharge of meiospores. Instead dispersal of teliospores is not unlike dispersal of peridioles in some gastromycetous fungi, where this is independent of basidiospore formation and dispersal altogether. Moreover, the rare occurrence and the short distance of actual dissemination indicate the relative lack of advantage accruing to this particular mechanism.

Production of the teliospore itself is, of course, an asexual event, unlike the germination of the teliospore and production of basidiospores. As such, the septation of the teliospore cannot be directly related to the septation of the basidium, unless internal germination takes place (see below, under teliospore germination). Likewise, little homology may be found between the longitudinal septation of some teliospores (*Sphenopsora* Diet., *Diorchidium*, *Anthomyces* Diet., etc.) and the cruciately septate basidium of the Tremellaceae, for presumably such septation in the incipient teliospore take place before karyogamy, whereas such septation in the Tremellaceae takes place after meiosis. Moreover, distinction must be made between those teliospores which become longitudinally septate, and those which may basically be unicellular, but which are adherent into small clusters. Presumably, the structure of the pedicel should serve to reveal such compound teliospores, as in *Anthomycetella* H. & P. Sydow, where the small cluster of teliospores and basal cells are supported by a compound pedicel consisting of individual adherent pedicels in like number to the basal cells to which they are attached.

Likewise, transverse septation of the teliospore is common. Of the nonpedicellate types, *Gambleola* Mass., *Pucciniosira* Lager. and *Didymopsora* all produce teliospore columns, and apparently no transversely septate teliospores are found embedded in host tissue. Of the pedicellate group, many well-known genera form transversely septate teliospores, including *Puccinia*, *Gymnosporangium*, *Frommea* and *Phragmidium*.

In some genera, teliospores are unicellular, but are adherent either laterally, breaking up into plates of spores as in *Alveolaria* Lager., or vertically, and then breaking up irregularly, as in *Baeodromus*, and *Crossopsora* H. & P. Sydow. In *Cronartium* the adherence is so strong that the column of teliospores does not break up, but germination takes place *in situ*. In *Masseeola* Diet. the unicellular teliospores are embedded in a gelatinous matrix, which is extruded from the telium in a hair-like column, and in *Gymnosporangium* the elongated, gelatinized teliospore stalk cells produce a long horn-like column of teliospores.

In several genera of rust fungi, teliospores germinate without a dormancy period, and in these groups the teliospore wall is often pale and thin. Of the latter, some genera are *Chrysocelis* Lager. &

Diet., *Aplopsora* Mains and *Ochropsora*. *Goplana* Racib. also belongs here, but Donk (1972b) drew attention to similarities between this genus and the Auriculariales, especially in view of the erumpent fructification in *Goplana* and its lack of highly differentiated teliospores. Conversely, the teliospores of many common rust fungi require a period of dormancy, usually prolonged cold treatment followed by some degree of heat shock, before germination can take place. Such organisms include *Puccinia graminis* and *Melampsora lini*.

TELIOSPORE GERMINATION

Before discussing the events of teliospore germination, it is unfortunately necessary to review some conflicting sets of terminology as they relate to the parts of the basidium. Three systems of terminology have been widely used, and these, together with several others, were reviewed by Talbot (1954). More recent commentaries include those by Martin (1957) and Donk (1958, 1973a-d). Basically, these systems are as follows. All systems at some time have agreed that the teliospore may be termed the probasidium, but in an attempt to homologize this structure with the saccate protuberance found in some members of the Auriculariaceae, Martin and Rogers replaced "probasidium" with "hypobasidium." For Linder (1940), the entire reproductive organ was the basidium, regardless of its shape, septation or origin. The basidial body (probasidium) produced sterigmata, on the tips of which were borne the basidiospores. For Neuhoff (1924), and later for Martin (cf. 1957 and earlier) and Rogers (1934, 1971), the probasidium (in the case of the rust fungi, the teliospore) is extended into an epibasidium, which, in the Uredinales, is potentially four-celled (the promycelium or basidium of most authors). Each cell of the epibasidium produces a single tube-like emanation (called by other terminologies the sterigma), still part of the epibasidium, on which the basidiospores are borne. For Donk (1931, 1973a-d) and Talbot (1954), the probasidium of all basidiomycetes is the seat of karyogamy (the teliospore of the rust fungi), while meiosis takes place in the metabasidium. In the holobasidiomycetes and in those rust fungi in which internal germination occurs, the organ remains morphologically the same: the teliospore is both the probasidium and the metabasidium. In those fungi where karyogamy and meiosis are separated into distinct structures, the probasidium and metabasidium may be distinguished (i.e., *Puccinia*, etc.). The metabasidium eventually becomes typically pluricellular (but see below for exceptions). The tube-like extension of each cell of the metabasidium is termed the sterigma, divided into the tube itself (the protosterigma of Donk), and the tapered tip bearing the basidiospore (the spiculum).

The details of nuclear fusion and meiosis in the rusts were uncovered slowly. Dangeard & Sappin-Trouffy (1893) first found that

preceding teliospore development in *Puccinia buxi* DC. the mycelium was dikaryotic, but that nuclear fusion took place in the teliospore. Although the origin of the binucleate mycelium was not explained until sometime later, the process of nuclear fusion and subsequent division suggested a sexual mechanism, and this led to further investigations, especially during the decades of the twentieth century when rusts were under particular scrutiny for plant pathological purposes. Much of the investigation into rust life histories centered around nuclear behavior, especially in the development and germination of the teliospore into the basidium (promycelium of some authors). Because of the apparent obligate parasitism of these organisms, genetic experiments were difficult to perform, and often the only clue to genetic phenomena could be gained from cytological observations.

Through many years of experimentation and synthesis of data, one fact became clear: nuclear behavior in the rusts was exceedingly variable, with several patterns emergent, and aberrancies found in almost all. Although the variations in morphological development of the teliospore and the telium are used as the primary characters on which families are based, the production of the promycelioid basidium seems universal in sexually reproducing rusts. Most of the macrocyclic rusts follow what has become a classic nuclear pattern (most often exemplified to beginning students by *Puccinia graminis* or *Puccinia malvacearum*), while the microcyclic forms often exhibit abbreviated nuclear patterns, sometimes with significant results. Most authors agree that the pattern followed by the above species constitutes the most primitive, although the most complex, with variations derived from it.

Nuclear behavior in basidia of these species follows an almost identical pattern to that of other phragmobasidial types, excluding the smuts. The teliospore represents the probasidium, the organ in which nuclear fusion takes place, and arises from the generally binucleate vegetative mycelium of the dikaryotic stage of the rust.

At the outset, the hyphal tip destined to become the teliospore, or probasidium, is binucleate, each nucleus assumed to be in the haploid condition, and the combination to be heterokaryotic. In the Uredinaceae, a semi-conjugate nuclear division takes place, with subsequent transverse septation to produce a stalk cell and a teliospore initial. The stalk cell extends somewhat, becoming slender and evacuated eventually, while the teliospore initial swells, and wall material is deposited. In *Puccinia* the teliospore initial again undergoes simultaneous nuclear division and transverse septation, resulting in a two-celled, thick-walled teliospore with a single germ pore evident in each cell.

The nuclei in the teliospore are quite small and condensed at first, but soon swell in size, with some apparent differentiation into an inner, transparent area (the endosphere of Savile, 1939), and the

outer portion in which the staining chromosomes appear (the ectosphere of Savile). It is in this condition that nuclear pairing takes place. Prior to karyogamy, the nucleolus stains quite clearly, but in nuclear pairing the nucleoli are seen to remain separate for a time, finally coalescing. A single nucleolus is clearly seen in the resultant diploid nucleus of each cell of the teliospore. During or after karyogamy, germination of the probasidium (teliospore) is undertaken, and a short tube is produced from each germ pore, this being termed the promycelium by most older workers, or the metabasidium (Martin, 1957). The fusion nucleus migrates into the promycelium some distance from the teliospore, and there the chromosomes proceed to condense, shorten, and become more independently visible. It is at this stage (just prior to Metaphase I of meiosis) that chromosome counts have been taken, with the estimates in *P. malvacearum* of a haploid number of "about 5" (Allen, 1933) or 4 (Savile, 1939). During or before Prophase I (Olive, 1947), the nucleoli (plainly visible during interphase) disintegrate at the same time as the nuclear membrane. In most cases, spindle fibers are oriented along the long axis of the basidium, with prominent astral rays often present. The presence of centrioles has been postulated, but these organelles have not actually been observed.

Anaphase I proceeds rapidly, as does Telophase I. Most authors report little or no distinct Prophase II period before reorganization for Metaphase II. The formation of a cross wall between daughter nuclei may occur after Anaphase I, or may be delayed until both meiotic divisions have taken place, to be formed along with the cross walls between second division daughter nuclei.

Meiosis II follows the normal pattern, with the spindle fibers usually oblique in the basidial lumen, and the daughter nuclei scattered and small. Cross wall formation separates the basidial cell into four daughter cells. Each daughter cell of the basidium produces a narrow hyphal appendage, tipped with a tapering sterigma. These hyphal outgrowths have been termed secondary protosterigmata by Donk, but have been accepted merely as sterigmata of variable length by most authors. The length of the sterigma seems dictated by the distance of the basidium from the air environment, with growth continued until air-substrate interface is reached, at which time a sterigmatal point is produced, surmounted by a basidiospore initial.

The four daughter nuclei resulting from meiosis migrate through the sterigmata into the basidiospore initials (also known as sporidia by some authors). In *P. malvacearum* and *P. lobata* B. & C. (Berkson & Britton, 1969) another nuclear division (assumed to be mitotic) takes place within each initial, rendering the basidiospore binucleate at the time of liberation, but in *P. graminis* the basidiospore remains uninucleate until germination. Olive (1947, 1949a, b) and others have emphasized the nuclear condition of the basidiospore as a clue to the production of infectious hyphae upon its germination. The normal basidiospore of *P. malvacearum*, produced in the process

summarized above, while binucleate, is homokaryotic and, in a truly heterothallic species, could not produce heterokaryotic hyphae or normal aecia and aeciospores but would require a "mating" with another compatible homokaryotic haplont.

One common variation in this developmental sequence involves a less accurate partitioning of the basidium into separate cells. In *Uromyces aloes* (Thirumalachar, 1946), the basidium is usually divided into a single uninucleate and another trinucleate cell, with no subsequent basidiospore production, but the development directly of infectious hyphae. In *Sphenospora kevorkianii* Linder (Olive, 1947) only two cross walls are produced in the basidium as a rule, with the middle cell usually binucleate. The basidiospore produced by the proximal and distal cells are uninucleate, but the single spore formed on the binucleate cell is binucleate, and presumably heterokaryotic. Two and four-celled basidia are also produced. Almost identical events have been observed in *Gymnosporangium clavipes* (Olive, 1949b), but are rare in that species, and presumably aberrant.

Several authors have reported homothallism in macrocyclic rusts, but in a number of these reports the percentage of single-basidiospore infections producing aecia was very low. Olive (1949b) has argued that with the occasional production of binucleate basidial cells, and concomitant binucleate, heterokaryotic basidiospores, such "homothallic" behavior may be explained in an otherwise heterothallic fungus. Such heterokaryotic basidiospores would produce infections, imitating homothallic taxa. Almost identical nuclear behavior has been ascertained in *Neurospora tetrasperma*, which appears homothallic, but in which the ascospores are binucleate and heterokaryotic.

Variations in nuclear behavior and development of the promycelium were reviewed by Moreau & Moreau (1919), Dodge & Gaiser (1926), Dodge (1929), Hiratsuka (1973), Jackson (1931, 1935), and Olive (1953) offered a diagram of the various configurations which may be summarized as follows, with some revisions.

1. The classical pattern described in detail above, followed by *Puccinia malvacearum* as outlined by Allen (1933), and *Gymnoconia nitens* Kern & Thurston (Kunkel, 1914). In *Endophyllum sempervivi* (A. & S.) deBary the germinating units are morphologically aeciospores, although acting the part of teliospores.

2. Probasidia arise from binucleate cells. No nuclear fusion takes place, and the two nuclei migrate into the promycelium, there dividing once to form four daughters. The basidium is four-celled, and each basidiospore receives a single nucleus. Although the pattern is almost identical to the first, no karyogamy or meiosis occurs, and the entire process may be viewed as essentially asexual. *Gymnoconia (Caeoma) nitens* (Dodge & Gaiser, 1926) and *Endophyllum euphorbiae-sylvaticae* (DC.) Wint. (Sappin-Trouffy, 1896) exhibit this pattern.

3. Nuclear fusion takes place in the teliospore. After the first meiotic division in the promycelium a transverse cross-wall is formed, separating the two daughter nuclei. Although a second nuclear division is performed, no concomitant cytokinesis is undertaken, resulting in a two-celled basidium, each cell of which is binucleate. Each basidiospore receives two nuclei, with the possibility that these nuclei may be heterokaryotic or homokaryotic for any given gene, dictated by the segregation of the gene over the two divisions. *Puccinia arenariae* (Schum.) Wint. (Lindfors, 1924) follows this sequence, as apparently do others (Jackson, 1935).

4. In the binucleate young teliospore, one nucleus disintegrates, leaving a single (presumably haploid) nucleus to migrate into the promycelium. This nucleus undergoes a single division (presumably mitotic), and a cross-wall divides the basidium into two cells, in the lower of which the single nucleus degenerates. The basidiospore formed from the distal cell receives the surviving single nucleus. Again, as in the second pattern above, no karyogamous or meiotic events occur, and the process can be interpreted as asexual. According to Jackson (1931), *Endophyllum valerianae-tuberosae* (associated with *Puccinia commutata* P. & H. Sydow by Wilson & Henderson, 1966) exhibits such behavior.

5. The mycelium and teliospore are uninucleate. After nuclear migration into the promycelium a single division (presumably mitotic) is completed, and a single cross-wall formed. The basidium is two-celled, each cell producing a single basidiospore, which receives a single (presumably haploid) nucleus. Dodge (1924c) discovered this behavior in *Endophyllum (Caeoma) nitens* Kern & Thurston, and later (Dodge, 1929) contrasted the small nucleus in the teliospore of this organism to the larger nucleus found in *Endophyllum euphorbiae* Plowr. (later called *E. euphorbiae-sylvaticae*), surmising that the teliospore nucleus in *E. nitens* was haploid. If this is so, then again no karyogamy or meiosis takes place, all the nuclei are haploid, and the process is essentially asexual.

6. The mycelium and teliospore are uninucleate, the nucleus migrating into the promycelium, there to divide twice (presumably meiotically), with concomitant formation of three cross-walls to form four basidial cells. Each cell produces a single basidiospore which receives a single nucleus. Dodge (1929) drew attention to the teliospore nucleus size, conjecturing that this nucleus was diploid, thus explaining the double division in the promycelium as meiosis. Such behavior is found in *Endophyllum euphorbiae* (renamed *E. uninucleatum* Moreau, which has been associated with *E. euphorbiae-sylvaticae* (Wilson & Henderson, 1966) and presumably of which it is an aberrant strain.

7. Nuclear fusion occurs normally in the teliospore, the fusion nucleus migrating into the promycelium. There two nuclear divisions occur, but instead of three cross-walls being formed to separate the basidium into four daughter cells, only two septa materialize, with

the formation (usually) of two uninucleate cells and one binucleate cell. Subsequently, three basidiospores are produced, two uninucleate, the third binucleate. Such behavior is apparently common in *Sphenospora kevorkianii* (Olive, 1947), and present but rare in *Gymnosporangium clavipes* (Olive, 1949b). Whether the binucleate basidiospore were heterokaryotic for any given gene would depend on two factors: a) which of the four daughter nuclei were included in the binucleate basidial cell, and b) the segregation pattern of the gene involved.

In significant numbers of taxa production of an external promycelium from the teliospore is reduced or eliminated. In most such instances, at the time of germination, the diploid nucleus within the teliospore undergoes meiotic divisions, and the resultant four haploid daughter nuclei become arranged in more or less longitudinal order in the teliospore cytoplasm. Subsequently, three transverse septa are formed, separating these nuclei in individual cells. The teliospore wall is autolysed in local areas, and narrow hyphal protrusions extend through the wall and elongate into sterigmata. These sterigmata can be of definite length if the teliospore germinates under conditions of relative dryness, but may be quite long if the teliospore germinates in water or in a gelatinous matrix (the latter situation has been described for *Goplana* by Cummins, 1935).

Perhaps the largest and most widely spread genus with such internal germination is *Coleosporium* (on which the family Coleosporiaceae was based), with around 100 species and world-wide distribution. In this genus, the telia are dry and the teliospores are thin-walled but often exhibit a significant wall thickening of the distal tip. Transverse septation is internal, and the sterigmata are produced through the teliospore wall, extending to the surface of the sorus. Germination takes place without the usual resting period. Because of this structure, developmental pattern and germination time, Olive (1949a) chose to use the term "basidial sorus" for the more commonly accepted telial sorus, and the new usage was also suggested by Wilson & Henderson (1966). Olive also called the teliospore in this genus a probasidium, a term originated by Martin (1938), but expanded to include all basidial cells in which nuclear fusion takes place (thus the basidial primordium in most sexually-reproducing basidiomycetes).

In observations on *Coleosporium vernoniae* B. & C., Olive (1949a) noted that in addition to the typical transverse septation of the thin-walled teliospore, several other patterns of septation also could be found. Each cell, regardless of its position or the configuration of the cell quartet, formed a sterigmatal outgrowth surmounted by a basidiospore. As usual, nuclear fusion took place in the young teliospore, followed by meiosis. After basidiospore formation, a mitotic nuclear division took place, resulting in binucleate basidiospores, but one of the daughter nuclei degenerated shortly thereafter.

Olive drew attention to the irregular septation pattern in *C. vernoniae* (as Weir, 1912, had for *C. pulsatillae* (Str.) Weir) as a possible phylogenetic link to the tremellaceous, "cruciately-septate" basidium, drawing support for the theory that the Tremellales were to be derived from rust-like ancestral forms. Conversely, he equated the mitotic nuclear division within the basidiospores with the third division prior to formation of ascospores within the ascus, supporting the theory of Linder that the rusts were to be derived from Ascomycetous forms.

Gallowaya was originally described as a microcyclic form of *Coleosporium*, but Dodge (1925) showed that the teliospores of this genus are produced in chains, in sequence, while those of *Coleosporium* are produced singly on individual sporophores.

Thirumalachar (1945) described *Acervulopsora*, in which the thin-walled teliospore begins germination by elongation of the distal end. Elongation is short, however, and internal transverse septation occurs normally, but utilizing only the distal portion of the teliospore. In most cases, these individual cells then disarticulate, acting as basidiospores, but not as ballistospores. In *Maravalia* Arth. essentially the same process initiates germination, but elongation is more prolonged, and the resultant promycelium does not utilize the teliospore shell, being entirely external.

An interesting "intermediate" situation occurs in *Zaghouania* Pat. and *Cystopsora* Butl. where the promycelium is external, but partially evacuates the teliospore shell, the cell contents migrating into the promycelium. In *Zaghouania* a four-celled promycelium produces four basidiospores, while in *Cystopsora* both two- and four-celled promycelia are found, with appropriate numbers of basidiospores produced.

Internal germination has been used as a family character, forming the diagnostic basis for the Coleosporiaceae. Most modern authors, however, have found it more convenient to drop this character at that rank, redistributing the taxa throughout the other families. This disposition seems more natural, for such forms seem to exhibit most other combinations of characters found in other families.

The growth of the promycelium is apparently regulated in part by external physiological factors. If teliospore germination takes place in air or on the meniscus of water, the promycelium is definite in length, producing transverse septa normally, and the four constituent cells develop sterigmata and basidiospores. If germination takes place while immersed in water, however, the promycelium usually grows indefinitely, and usually without the production of transverse septa and spores (see Colley, 1918). Under certain conditions, traditionally associated with dry conditions during or just after teliospore germination, the promycelium has been observed to break apart into its constituent cells, each cell acting separately as a "conidium-like" body (see Spaulding & Rathbun-Gravatt, 1926), or producing a single sterigma with a basidiospore. Such events have been observed in *Gymnosporangium* (Cramer, 1876; Farlow, 1880; Barclay, 1890a), *Uromyces*, and *Caeoma* (Barclay, 1890b).

Most discussions of homothallism and heterothallism in these organisms took place before sexual incompatibility had been investigated in depth. Blackman's (1904) discovery that the binucleate condition in mycelia could be initiated by nuclear migration between compatible uninucleate mycelia seemingly solved the problem of the mechanism of dikaryotization. Craigie's (1927) subsequent discovery of the potential of pycniospores as dikaryotizing agents reopened the question. Allen (1934a) summarized several methods of dikaryotization. Moreover, it was later discovered that the dikaryon mycelium could dikaryotize monokaryon mycelia, essentially performing what became known in holobasidiomycete genetics as a "di-mon" cross. The question of the origin of the dikaryon in individual species (perhaps in individual infections) is still open. Unless the genetic constitution of the vegetative hyphae is known, the exact nuclear condition of the telial cells (and therefore teliospores) remains in question, as does nuclear behavior.

In several patterns of nuclear behavior in the teliospore and basidium no nuclear fusion or meiosis apparently takes place. If this is so, either some other provision for genetic recombination must be made (i.e., parasexuality and somatic recombination, see above), or the gene pool of the organism will be rendered static except for mutations, presumably an obvious disadvantage in competition with other such organisms, especially in the formation of "physiologic races."

Binucleate basidiospores have been found in several rusts (see Allen, 1933). For the most part, such spores receive a single meiotic daughter nucleus, with a mitotic division occurring in the basidiospore (i.e., *Puccinia malvacearum*). In others, however, a binucleate basidiospore may result from a binucleate basidial cell, with the nuclei either homokaryotic or heterokaryotic. If the basidiospores are functionally heterokaryotic, a normal dikaryon mycelium should result from basidiospore germination, imitating homothallic production of aecia and aeciospores. Olive (1947, 1949b) has described such spores, and Dodge (1929) mentioned multinucleate promycelia of *Caeoma (Gymnoconia) nitens*, although not describing multinucleate basidiospores.

BASIDIOSPORES

Germination of basidiospores may apparently take at least two forms. The production of a germ tube (termed "direct germination" by Bega, 1960) appears typical of germination on a suitable substrate for penetration, although also reported on inert or resistant substrates (Krebill, 1969, 1972). Krebill (1972) distinguished between long, slender vs. short, stout germ tubes in *Cronartium comandrae* Peck. Bega (1960) showed that pH 3.0-4.0 was optimum for direct germination, and linked time as an influential factor with pH and temperature in *C. ribicola*.

Under conditions not yet completely defined, a basidiospore may germinate by production of a short, delicate, tapering protuberance which acts as a sterigma, inflating apically and asymmetrically into a "secondary sporidium." DeBary (1887), true to form, was the first to describe the phenomenon in the rusts, but few accurate reports have followed. Observations of this type of germination (termed "indirect germination" by Bega, 1960, but herein called "germination by repetition" following the same terminology for the homologous event in other Heterobasidiomycetes) have been made in several genera (Spaulding & Rathbun-Gravatt, 1926; Bega, 1960), indicating that this form of germination is general throughout the order.

Kais (1963) reported up to five generations of repetitive sporidia, but observed the spores as passive, attached by isthmuses. Miller & Roncadori (1966) observed and illustrated violent discharge of secondary sporidia in *Cronartium fusiforme* Hedge. & Hunt. on water agar. Krebill (1972, 1969) plotted basidiospore germination types and percentages of *C. comandrae* on various substrates, noting long germ tubes on suitable substrates for penetration and short germ tubes (possibly abortive sterigmata according to Krebill) on inert or impenetrable substrates, and germination by repetition less common (maximum 13% on aspen leaves). Roncadori (1968) found relatively consistent pathogenicity of basidiospores, secondary and tertiary sporidia of *C. fusiforme* on two pine hosts. Bega (1960) reported up to six generations of sporidia in *C. ribicola*, also investigating the physiology of basidiospore germination (see above).

Germination by repetition may be used as a unifying character to link the rusts to other fungi usually placed in the subclass Heterobasidiomycetidae. In fact, recently this character has become more diagnostic of the subclass than the traditional presence of the septate basidium. Now included in the subclass by some authors is the order Ceratobasidiales, which exhibits a more or less typical holobasidium, but basidiospore germination by repetition. Whether the Uredinales, Tremellales and Ceratobasidiales can be linked by this single character, presumably easily the production of parallel evolution, is surely questionable.

SUPRAGENERIC CLASSIFICATION

Although Persoon was the first to include the rust fungi as a discrete and recognizable group, further refinement of taxonomy awaited the opportunity for microscopic examination. When the basidium, or promycelium, of the rust fungi was found to be transversely septate, in the same fashion as that of *Auricularia* and other well-known non-parasitic fungi, these, together with certain other organisms whose basidia were longitudinally (or "cruciately") septate were placed together (Patouillard, 1884) under the name "Hétéromycètes." The definition of the group was as follows: "Usually gelatinous fungi, rarely cartilaginous or corky, with *pluricellular* basidia, the spores mostly curved and *germinating with a*

promycelium producing secondary spores . . ." (cf. Donk, 1972a). In Patouillard's sense, the term promycelium denoted not the tube emanating from the teliospore of the rusts, but the small sterigma-like projection from the primary basidiospore, on which a secondary basidiospore was produced in the process of "germination by repetition." Later (Patouillard, 1887, 1900) the character of pluricellular basidium was slowly deemphasized, and the character of repetitive germination of basidiospores elevated. In fact, certain groups, now placed in the Tulasnellales and Dacrymycetales, were allowed entrance into the Hétéromycètes even though they exhibited unicellular basidia.

Most recent revisions of this basic system give the rust and smut fungi a more discrete position within the fungi. Talbot (1968), for example, proposed a class Teliomycetes to include the order Uredinales and Ustilaginales, while the other groups accepted by Patouillard were placed in classes Phragmobasidiomycetes and Holobasidiomycetes. Lowy (1968) proposed to segregate certain anomalous groups with either aseptate or partially septate basidia into subclass (under Basidiomycetes) Metabasidiomycetidae, as opposed to subclass Heterobasidiomycetidae, the latter including Auriculariales (inclusive of *Tremella*), Septobasidiales, Uredinales and Ustilaginales. Finally, Donk (1972a) emended Patouillard's basic system once more, but without proposing subclass or class rank changes. Instead, under Class Basidiomycetes (and restricted to taxa accepted by Patouillard as heteromycetous) Donk included the following orders: Uredinales, Septobasidiales, Auriculariales. No subordinate taxa were included under the two former orders, but the Auriculariales included two suborders: Auriculariineae and Tremellineae, the latter to include Tremellaceae, and Tulasnellaceae. Unlike most other emendations, Donk more or less flatly refused to include the Ustilaginales in this larger group until more evidence of its relationship was forthcoming.

Traditionally, the rust fungi have been included in two families, the Melampsoraceae and Pucciniaceae (or Uredinaceae). The classification schemes devised by early authors were reviewed by Arthur et al. (1929), but it was Dietel (1897, 1928) who employed the largest number of subfamilial divisions (presumably subfamilies although never designated as such). The treatment of the rust fungi in North American Flora (Vol. 7, 1906-1940) included three families under the order Uredinales, the Coleosporiaceae with internal basidia, the Uredinaceae with external basidia and crustose or powdery telia, and the Aecidiaceae with external basidia and solid or erumpent telia. Under the latter were listed eight subfamilial groups, paralleling several of Dietel's (1928) series. Arthur et al. (1929) stated that their treatment in North American Flora had been based on that by Dietel (1897), but gave an abbreviated scheme which further modified his former classification. In it, Arthur returned to the two-family concept, using Melampsoraceae and Pucciniaceae.

Arthur et al. (1929) concisely stated the four diagnostic principles used in their taxonomic scheme. First, "internal germination" or internal basidia should be deemphasized, hence the family Coleosporiaceae was eliminated, with the genera redistributed in the remaining two families. Second, life-cycle types were not used rigidly to relate genera within families. In some cases, microcyclic forms were separated from their macrocyclic counterparts. Third, it was to be recognized that genera of each family had obvious morphological similarities to genera of the other. In other words, the diagnostic character of pedicellate versus non-pedicillate teliospores was utilized somewhat arbitrarily. Fourth, the principle of priority of names adopted under the American Code of Botanical Nomenclature Anonymous, 1904) was abandoned in favor of well-known and commonly used names, with the hope that they could be conserved at a later date.

The two-family system was retained by Arthur (1934) throughout his career. In his final major work, the Melampsoraceae was divided into two subfamilies and the Pucciniaceae into four, with form-genera segregated independently. Later monographic and floristic treatments have largely followed this scheme closely. Wilson & Henderson (1966) resurrected the family Coleosporiaceae for those forms whose teliospores germinated internally, but no subfamilial groupings were offered. Kuprevich & Transhel (1957) dealt only with the Melampsoraceae, using five subfamilial groups.

Methods of teliospore production have been used by Hughes (1970) to suggest relationships with the Uredinales. Three methods were identified, namely, (*a*) melampsoraceous, a simple budding process by sporogenous cells producing teliospores within the host epidermis, (*b*) cronartiaceous, meristem arthospore production of teliospores in chains, and (*c*) pucciniaceous, sympodioconidial production of pedicellate teliospores. In the two-family system of classification, the first two teliospore production methods would be placed within the Melampsoraceae, and the third within the Pucciniaceae, but ideally the three methods would be separated.

PHYLOGENETIC RELATIONSHIPS

Perhaps the most difficult obstacle to be overcome in a discussion of phyletic relationships within the rust fungi is the reticulate nature of taxonomic characters within the group. An example of reticulation of taxonomic characters may be seen in the relationship of *Puccinia*, *Uropyxis* and *Cumminsiella* (Baxter, 1957). *Puccinia* and *Cumminsiella* share subepidermal spermogonia and aecidioid aecia, but *Puccinia* species exhibit a single germ pore per teliospore cell, while *Cumminsiella* species produce two. Conversely, teliospores of *Cumminsiella* and *Uropyxis* are very similar, but *Uropyxis* species produce subcuticular spermogonia and uredioid aecia. Hence relationships must be surmised based on a philosophy of the primacy of

spermogonial and aecial characters, versus teliospore characters. Such multidimensional character fields exist throughout the rust fungi, and make conjecture on evolutionary pathways difficult.

Such a reticulation has been made less confusing by highly informed accounts by a small number of authors. Hiratsuka & Cummins (1963) for example, by dealing specifically with the development and structure of the spermogonium, were able to conclude that perhaps three major lines of evolution had occurred within the Uredinales, in contrast to the long-held assumption that only two major lines had developed (sessile vs. pedicellate teliospores).

Rejecting teliospore characters as perhaps taxonomically valuable at the species level but not at the suprageneric, Leppik (1953) turned to the morphology of aecia on which to base a phylogeny of the rust fungi on coniferous hosts. From a prototype near *Uredinopsis* evolved the more sophisticated roestelioid, peridermioid and caeomoid aecial forms. The thickness of the peridial tissue was used as an indicator of climatic factors existent at the evolutionary onset of the form. As such, the site of origin of conifer rusts must have been the temperate northern hemisphere, with strong seasonality, warm dry springs and summers, and cold winters. Later (Leppik, 1973) these assumptions were tested against the theories of continental drift, in which the Laurasian subcontinent has been postulated as the site of origin of the Abietaceae, after the split of Pangeaea. Moreover, the warm, wet tropic equatorial belt has prevented the penetration of both members of the Abietaceae and their rust fungus parasites to the southern hemisphere, except in high mountains.

Leppik (1953) drew sharp contrast between phylogeny, governed intrinsically by the genetic constituency of the organism, and hologeny, extrinsically controlled by ecological or environmental factors exerting selective pressure against the organism. For parasites such as the rust fungi, hologenic forces include the host plant, which, when extrapolated to larger groups, allows a complex analysis of progression of rust fungus parasitism on evolving plant groups. Based on this analysis and "Tranzschel's law", Leppik surmised that in *Melampsora*, for example, from a basically teliosporous population on ferns ("proto-*Melampsora*") the next epochal generation was aeciosporous on conifers ("pino-*Melampsora*"). Next, a teliosporous era was born on Salicales ("salico-*Melampsora*") culminating in an aeciosporous generation on more modern angiosperms ("neo-*Melampsora*" on Saxifragaceae, Liliaceae, Euphorbiaceae, etc.).

A similar analysis of *Gymnosporangium* (Leppik, 1956) led to the conclusion that "proto-*Gymnosporangium*" occurred on "proto-Cupressaceae" and Cupressaceae as a teliosporous generation. Aeciospores were then formed on Pomaceae in a later era, and some of these have become restricted to such hosts, and were segregated into the genus *Coleopuccinia* Pat.

Leppik (1956) was led to the conclusion that melampsoraceous, gymnosporangiaceous and pucciniaceous lines were derived from a common ancestral pool called "histosorous." This general analytical process was expanded (Leppik, 1961, 1967) to all rust fungi, and these data correlated with continental drift theories (Leppik, 1953-1967) in a most fascinating series of publications.

Because of their more sophisticated life cycle, more complex morphology and less flexible habit, Leppik (1961) concluded that the Uredinales were evolved from, rather than ancestral to the Auriculariales.

In a most incisive discussion of the evidence for and against presumed phyletic relationships in the heterobasidiomycetous fungi, Donk (1972b) drew attention to similarities between genera "placed among the Auriculariaceae when their fruitbodies (teleutosori) develop extramatrically (*Herpobasidium* J. Lind., *Kriegeria* Bres., *Kweilingia* Teng.) and among the Uredinales when they are erumpent (*Goplana*)." In his opinion, all were referable to the monocyclic rusts without spermogonia. In *Kweilingia* (Thurumalachar & Narasimhan, 1951) probasidia (teliospores) are formed in compact, laterally coalescing chains or columns similar to those of *Dasturella* Mund. & Khes. and *Pucciniostele* Tranzsch. & Kom. Such observations appear to smudge the lines between the saprophytic, hymenium-producing auriculariaceous fungi, and the parasitic, erumpent fungi traditionally placed in the Uredinales.

Likewise, it is commonly known that smut fungi often produce yeast-like colonies when cultured on artificial media. Yet, in some "yeasts" a more or less well-defined septal pore cap is found, namely, in species of *Trichosporon* Behr., *Leucosporidium* Fell et al. and *Filobasidium* Olive (Kreger-van Rij & Veenhuis, 1971: 89). In one case (*Leucosporidium*), Donk (1973c) suggested similarities between the peculiar pore cap and vesicles observed capping the simple septal pore in some Uredinales (Ehrlich et al., 1968; Littlefield & Bracker, 1971). In those Uredinales thus far cultured on artificial media, mycelial colonies are produced, not yeast-like, further smudging the lines between the Uredinales and the smut fungi, especially if such "yeasts" are thought to have been derived from some common ancestral pool.

By far the most thought-provoking treatment of phylogeny within and of the rust fungi was that by Savile (1955). First stating numerous principles to which evolution was found to conform, Savile drew the rust fungi from a *Taphrina*-like ancestral form. Such an ancient fungal group was envisioned as self-sterile, of simple fruit body, parasitic (especially upon ancient green plant groups), and with plastic life cycle. Similarities were drawn between the asci of *Taphrina* species, which occasionally become septate or have been reported to produce exogenous spores, and the simple teliospores of Uredinopsis and related taxa, which produce basidiospores *in situ*. The *Taphrina*-like ancestral form concept was also supported from a genetic or interfertility basis by Raper & Flexer (1971).

Savile also discussed at length evolutionary trends within the rust fungi. The telium and teliospores were, or course, considered the most primitive spore and sorus form. The uredium was derived from the telium before great sophistication of the telium had occurred. Uredospores, as repeating, asexual units, were merely extensions of the teliospore principle, and on the same host plant. Aecia, considered clearly analogous to uredia, were derived from uredia, although the actual series of events leading to such a derivation were unclear. For Savile, certain uredial forms such as those found in *Chrysomyxa* Unger and *Coleosporium* (the so-called "aecidioid" or "peridermoid" uredia) were derived from aecia, with the uredospores remaining in chains separated by intercalary cells. Finally, the pycnium was morphologically unrelated to any other spore or sorus form within the rust fungi, and was considered seemingly an evolutionary advantage in and of itself.

Savile's derivation of the rust fungi from ascomycetous ancestors seems borne out by Hughes' (1970) repeatedly drawn homologies of spore production and spore forms with those of Ascomycetes (phialospores, arthrospores, etc.), and the uniqueness of some of these, especially the phialospore produced in the pycnium. Although not specifically stated, Hughes circumstantially had concluded that so many spore production methods mimicked those of ascomycetous fungi that the two groups must be closely related — perhaps more closely related than had been formerly postulated.

Other trends which Savile identified within the rust fungi included the following: progression from ancient hosts to more recent hosts (following a principle that a fossil record was of secondary importance to the living record of host plants and their known history and dates of origin). Presumably the most primitive rust fungus was to have been found on a Marattiaceous host, for the rust fungi now found on ferns and fern allies were among the most primitive of the living stock. Secondly, from the primitive intraepidermal telium, the development of the subepidermal telium of taxa such as *Pucciniastrum*, and then to the more sophisticated telia of the Pucciniaceae was traced. Thirdly, phylogenetic movement away from a peridiate uredium to an unprotected uredial sorus, was identified perhaps because the need for protection and the need for a pressure-increasing device against the host epidermis and cuticle had been reduced. This was the main stream, not withstanding the evolutionary diversion of the differentiated uredial peridia of *Melampsoridium* and allied genera. Finally, progression was from a non-pycnial form to the present differentiated pycnia, often with bounding tissues. The occurrence of the flask-shaped pycnium of the Pucciniaceae was envisioned as a convergent evolutionary structure rather than a clue to relationships with pycnidial forms of imperfect fungi as had previously been postulated.

Going further, Savile (1955) saw the rust fungi as very primitive in the context of the rest of the basidiomycetous fungi. In opposition to other workers, Savile thought the clamp not to be analogous to the crozier of the Ascomycetes, and the lack of clamp connections to generally be primitive rather than an advanced, reduced, character. Thus the rusts were primitive because they lacked clamps, and not derived because of the same character. Conversely, those groups within the Heterobasidiomycetes which possessed clamps were considered to be somewhat advanced over the rust fungi (i.e., the smut fungi, the Auriculariales, etc.). The Homobasidiomycetes were seen as derived from the heterobasidiomycetous stock, perhaps by the sophistication of the hypobasidium into the homobasidium, and the reduction (and eventual demise) of the phragmobasidium characteristic of the Heterobasidiomycetes.

A point raised by Savile, and much later discussed at length by Donk (1972a, 1973a, b) was the probable distance of the relationship of the smut fungi to the rust fungi. Although usually placed together in text books, and generally thought of as closely related because of similarities in gross morphology of the brand spore and the teliospore, further proof was needed of the relationship for both workers, for the smut fungi were culturable on artificial media (keeping in mind the dates of their publications), often bore clamp connections, produced "basidia" of little understood function and behavior, on which the "basidiospores" often acted more like conidia than like basidiospores.

Laundon (1973) accepted this line of phylogenetic reasoning, and in a more succinct manner presented further evidence for it. Among these points were the following: (*a*) the movement from ancient hosts to more modern hosts has been accompanied by a movement away from heteroecism toward autoecism, and from macrocyclic life cycles toward microcyclic; (*b*) more primitive rust fungi produce sessile teliospores, the more advanced produce pedicellate (Saville had also emphasized this point); (*c*) with advancement, the telial sorus became more and more complex.

Only a single "universal" character had been recognized in the classification of the rust fungi since the time of Dietel, namely, the pedicellate versus sessile teliospore, with the sessile always assumed the more primitive (cf. above, under suprageneric classification, for a discussion of this character and its nomenclatural ramifications).

In sharp contrast to the conclusions reached by Savile (1955), Jackson (1944) had previously drawn a number of parallels between the life cycle and morphology of certain floridean Rhodophycean organisms and ascomycetous fungi, especially those considered well advanced (i.e., perithecial and apothecial forms). Although such an idea lapsed into some dormancy, it was picked up and given new vitality by Bessey (1950) and Denison & Carroll (1966), who drew attention to more recent findings pointing in the same direction. Of

these, Bessey stated the case for phylogeny of rust fungi from such stock most carefully. Moreover, for Bessey, the Uredinales and Ustilaginales were enough closely related to have been derived from a common stock. Thus, for Bessey, the primitive characters could be stated as follows. "If, then, the two orders Ustilaginales and Uredinales are considered to be related we must conclude that they have arisen probably (*1*) from a group of fungi in which clamp connections occurred or their homologue, the croziers, characteristic of ascus formation; (*2*) from fungi in which spermatia were produced in well-organized spermogonia; (*3*) from fungi in which sexual reproduction was initiated by the spermatization of receptive hyphae; and (*4*) from fungi in which the monocaryotic and dicaryotic mycelial phases were both present." Of these principles, Savile (1955) would have agreed with only the fourth, and was vehemently opposed to all the others.

Bessey also referred to the discussion by Linder (1940), who drew phyletic parallels between the teliospore-probasidium combination of the Uredinales and the "germinated" bitunicate ascus of certain Ascomycetes, presumably the Dothideales and their relatives. These thick-walled asci often exhibited a thin spot at the apex, perhaps analogous to the germ spore of the teliospore, and germination was accomplished by the extrusion of the inner, thin-walled ascus. All that remained was the septation of the promycelium and the further extrusion of sterigmata and the production of exogenous spores. Clamp connections, assumed primitive by Linder, might have been lost with the strictly parasitic habit of the rust fungi.

Bessey took issue with Linder, but for the most part, Bessey's arguments appear to center on terminology rather than morphology or development, and Bessey's conclusions were a combination of those of Jackson (1944) and Linder (1940), drawing the basic Basidiomycetous stock from highly advanced fungi (i.e., the discomycetes), which, in turn, were most closely related to floridean algae.

Yet another set of theories were proposed be Rogers (1934), in which certain heterobasidiomycetous fungi usually placed loosely within the Tremellales, namely *Tulasnella* and close relatives, were assumed primitive basidiomycetes. The basidium represented the ascus initial, with septation occurring between the basidial body (the hypobasidium or probasidium) and the first protuberances at its apex (the epibasidium). These first protuberances were analogous to ascus pockets, within extensions of which (within sterigmata) were produced basidiospores, closely analogous to ascospores within the ascus walls. Thus basidiospores were not really exogenous, but endogenous spores. From this stock were drawn the rest of the Tremellales, with longitudinally (or "cruciately") septate basidia, and the homobasidiomycetes, without septation in the basidial body. From the tremellaceous forms were derived the phragmobasidial forms by

movement of the septa within the basidium from apical or longitudinal, to transverse. Another schema was adopted by Olive (1957), who used the variation in septation of the basidium of *Galzinia* as a possible *modus operandi* for the derivation of the "urnigera series" from phragmobasidial forms. No explanation was offered as to the origin of the phragmobasidial and holobasidial forms.

At the risk of introducing yet another unsubstantiated set of theories, it might be pointed out that all theorists have overlooked one additional possibility. In all briefs filed thus far, either the phragmobasidial forms have been derived from the holobasidial forms, or *vice versa*, but the basidiomycetous fungi have invariably been seen as a more or less monophyletic line. Savile came closest to departure from this by indicating that the holobasidial forms may have diverged from the phragmobasidial forms very early, drawing his conclusions largely from the common occurrence of clamp connections in the holobasidial forms (but overlooking their only slightly less common occurrence in the phragmobasidial forms).

Savile, I believe, enunciated many very valid evolutionary points, one of which is open to considerable additional consideration. If a fungus assuming very new developmental habits or invading a very new ecological niche is often not well adapted for these adventures, and may well be rather unspecialized in its exploitation of their possibilities, then there stands some reason to believe that the "*Taphrina*-like" ancestral form, producing "exogenous" ascospores for the first time, might not have done so in a well-defined, well-ordered manner. Must one assume that *all* asci produced *four* lateral "sterigmata," or could some of these asci have produced more or less than four, often in various configurations in addition to the lateral placement? Must every ascus have become *regularly transversely septate*, or could some of these asci have remained undivided? Could the condition of the host (dead or alive, well rotted and giving little resistence to the growth of the "sterigmata" or alive and with intact epidermis and/or cuticle) have permitted the survival of various clumsy attempts at producing these "exogenous" ascospores, at least until some regularity had been established for such procedures? If so, could both the phragmobasidial forms, with stout, forceful sterigmata able to grow indefinitely through a water film or through stomatal openings, and holobasidial forms, perhaps with equally stout sterigmata in primitive forms (*Tulasnella*, *Ceratobasidium*, etc.), but quickly with shorter, less forceful sterigmata, have been derived from a single ascomycetous stock, virtually at the same time? If so, then many of the seeming incongruities inherent in a monophyletic scheme might be relieved. For example, presence or absence of clamp connections in both holobasidial and phragmobasidial forms becomes a problem of rather low magnitude, as does the demand for movement of septa within the basidial body. At the same time, the rust fungi may still be considered relatively primitive within the

phragmobasidial forms, but the complexity of spore and sorus forms are not necessarily transferred to the holobasidial forms, nor is it necessary to account for their appearance in the Uredinales but not in the homobasidiomycetous fungi.

The following propositions concerning the phylogeny of the Uredinales and basidiomycetous fungi in general may be summarized: (*a*) Uredinales as primitive, holobasidial forms derived from them, either early or late; Uredinales derived from a "Taphrina"-like ancestor (Savile, 1955); (*b*) primitive basidiomycete derived from discomycetes, in turn arising from floridean algae; Uredinales well advanced within the phragmobasidial forms (Jackson, 1944; Bessey, 1950); (*c*) Uredinales derived from tremellaceous ancestral forms probably derived from ascomycetes (Rogers, 1934; Olive, 1957), and (*d*) holobasidial forms and phragmobasidial forms derived from a pool of ascomycetous fungi producing "exogenous" ascospores in a variety of methods, with two major lines persisting (Petersen, here).

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