B. *PUCCINIA*

The genus *Puccinia* belongs to the family Pucciniaceae, order Uredinales, sub-class Protobasidiomycetes and the class Basidiomycetes. Alexopoulos (1962) placed this genus under the family Pucciniaccae, order Uredinales, sub-class Heterobasidiomycetidae and the class Basidiomycetes.

The genus *Puccinia* with about 1800 cosmopolitan species are parasitic on flowering plants including many cereals such as wheat, barley, oats etc. and cause great losses to the cultivated crops. *Puccinia* and other genera under the order Uredinales are obligate parasites and are commonly known as 'rust fungi', because they produce *rust-like spores* in the form of rusty spots or streaks on stems, leaf-sheaths, leaves etc. of the host plants.

Common species of Indian wheat rusts and the name of the diseases caused by them are mentioned in the next page :—

Name of *Puccinia* sp.	Name of host plants	Name of diseases	Characteristic features
1. *Puccinia graminis*	Wheat (*Triticum aestivum*) and Barberry (*Berberis vulgaris*)	Black rust or Black stem rust of wheat	Attacks firstly the stems, next leaf sheaths, leaves and ears. Uredosori mature early, they are elongated, brown in colour, coalescing ; these are exposed by the rupture of host epidermis. Teleutosori like uredosori but black in colour, appear almost on all green parts.

Life history of Puccinia graminis—Refer para one, article 4.3 B.

Puccinia graminis[1] Pers., commonly called "black stem rust" is the most well known among rust fungi. It is a heteroecious[2], macrocyclic[3] and polymorphic[4] rust. The life history can be divided into five stages, such as :—

1. UREDINIAL OR RED RUST STAGE—In the late spring (February), vertically elongated reddish-brown pustules i.e. sori appear on the stems and leaves of wheat plant. Each pustule is a sorus called *uredosorus* or

uredinium. Uredosori are formed just beneath the host epidermis. At maturity each uredosorus contains numerous reddish or orange-red spores called *uredospores*. Each uredospore, borne on a long stalk, is unicellular, binucleate (n+n), oval or globose in structure with a fairly thick wall which

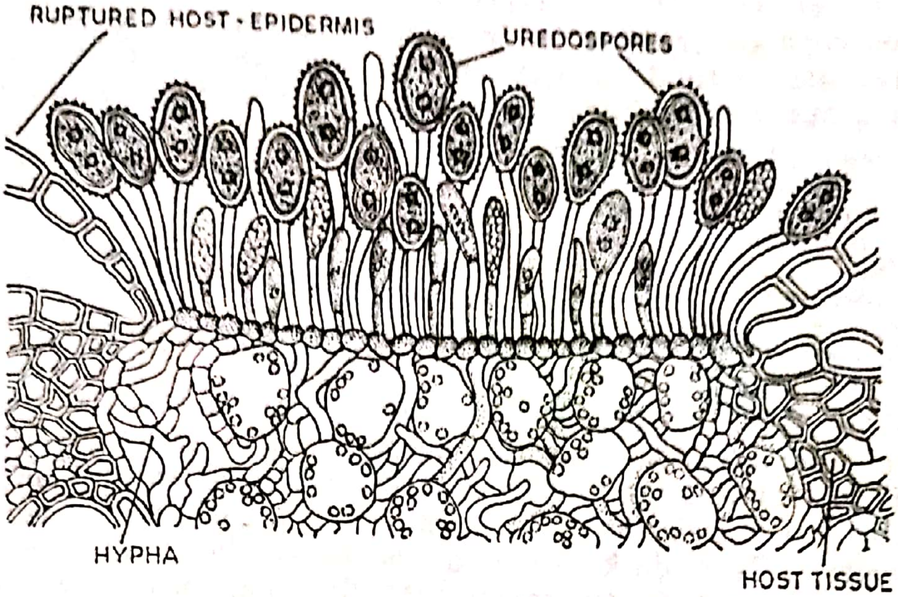


Fig. 4.6—*Puccinia graminis*. Section through uredosorous showing uredospores.

is differentiated into outer spiny exospore and inner smooth endospore. Each uredospore is provided with a number (4 to 5) of germ-pores. When the spores become fully mature, these are freely exposed due to rupturing of the host (wheat plant) epidermis, underneath of which the sori are located. These spores are easily detached and carried to other hosts i.e. new wheat plants by wind where they germinate within a few hours by putting forth germ-tubes in presence of moisture. One of the germ tubes grows faster over the host epidermis than the rest and on reaching a stoma enters inside forming an elongated *appresorium*. From this a narrow branch also enters inside

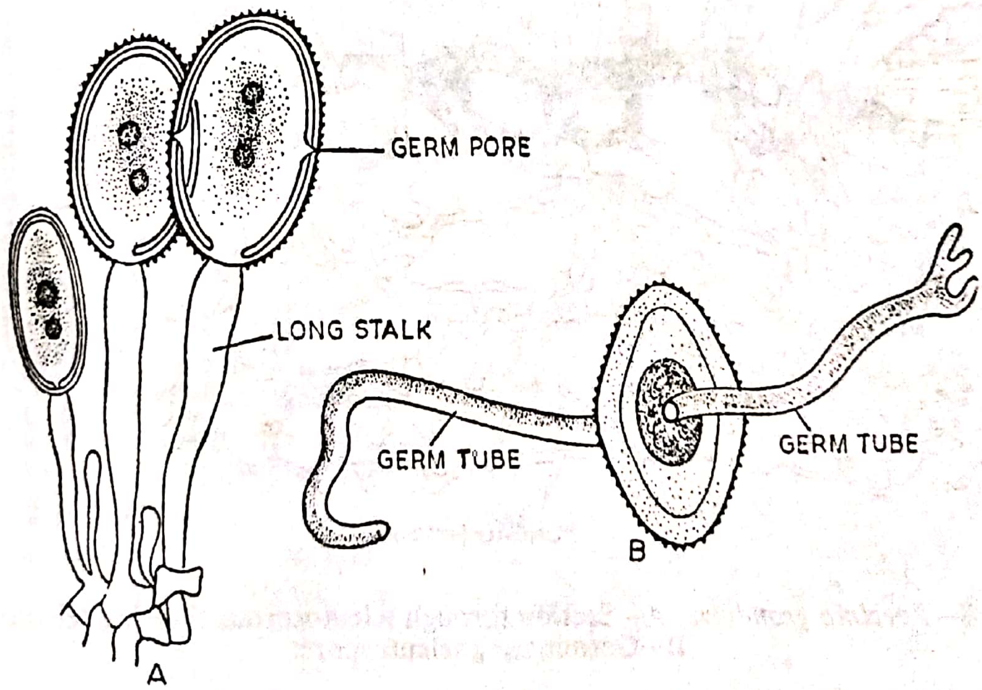


Fig. 4.7—*Puccinia graminis*. A—A few uredospores (enlarged). B—Germination of a uredospore.

and swells up to form a substomatal vesicle. This substomatal vesicle eventually gives rise to a much branched dikaryotic (n+n) i.e. secondary mycelium which spreads near about between the cells and often sends out haustoria. Infection is now complete and the secondary mycelium (n+n) continues its growth within the host. Within 10 days, the mycelium absorbs sufficient food from the host to form a new crop of uredospores—this uredospores again become mature and ready for dissemination by wind in another 10 days time. If the weather remains cloudy, it is possible to produce successive crops of uredospores in a single season—it is for this reason, uredospores are often designated as *repeating spores*. Consequently the disease from one or a few infected plants may spread over a large area.

Development of Uredospores—While the dikaryotic mycelium is developing inside the stem, leaf etc. of the host, some of its hyphae begin to collect in the form of compact masses of cells at certain points beneath the epidermis. From such a mass, a layer of binucleate parallel cells called *basal cells* arises pressing against the epidermis. Each basal cell divides transversely into a lower *foot cell* and an *upper cell*. The foot cell remains sterile while the upper cell divides again into two cells. Its upper daughter cell constitutes the main body of uredospore and the lower one develops into a stalk of the spore. The epidermis of the host is raised up with the maturity and elongation of uredospores. Finally, the epidermis is ruptured in a slit-like manner exposing uredospores.

2. Telial or Black rust stage—Towards the middle of the summer season, the same mycelium which has been producing uredospores or mycelia originating from germination of uredospores, begin to form black pustules or black sori called *teleutosori* or telia consisting of dark coloured (black) masses of spores. These dark coloured spores produced in teleutosori are known as *teleutospores* or *teliospores*. At first i.e. during the transitional

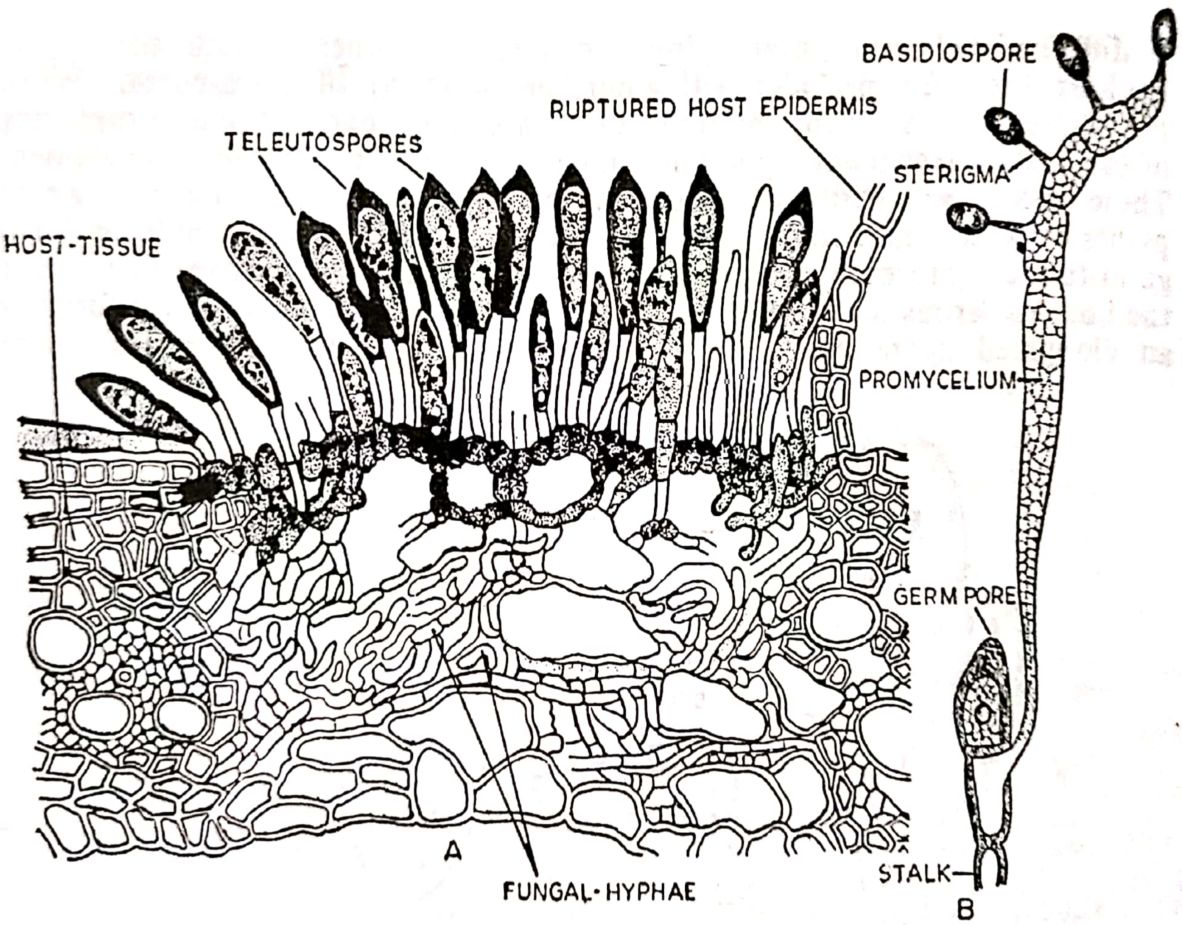


Fig. 4.8—*Puccinia graminis*. A—Section through teleutosorous showing teleutospores. B—Germinating teleutospore.

period between the development of uredosori and teleutosori, both uredospores and teleutospores may be formed in the same sorus, such a sorus is often called *mixed sorus*. Later on, new sori called *teleutosori* containing teleutospores only begin to develop on the wheat plant. Telial stage is more destructive than the uredinal stage. The change from uredospore production to teleutospore production by the same mycelium is supposed to be governed by the photosynthetic activity of the host plant. Each teleutospore is stalked, dark-brown to black in colour, binucleate, two-celled, spindle-shaped and thick-walled structure; the apex of the upper cell is rounded or sometimes pointed. Each cell of a teleutospore contains two nuclei i.e. dikaryotic (n+n) in young condition which fuse together into a single diploid nucleus (2n) as the spore matures. Each cell has a germ-pore—in the upper cell it is apical while in the lower cell it is lateral.

The teleutospores are dormant spores and unable to germinate immediately. Therefore the teleutospores are resting spores which carry the fungus over to the following season. Unlike uredospores, they do not have any specific host to stick on. They remain dormant on dead host tissues or on the soil, then they germinate freely. But their survival in the plains of India is very difficult since the temperature during March to June is considerably high.

Development—Development of teleutospores takes place in the same manner like that of uredospoeres except that the upper cell cut off from the parallel basal cell divides further into a row of 3 cells. The upper two cells of the row become enlarged to form the main body of the 2-celled teleutospore and the lower one forms the stalk.

3. Basidial stage—Under favourable conditions of temperature and moisture, each cell of the teleutospore germinates independently sending forth an erect tubular outgrowth from each cell. Such tubular outgrowth is known as *epibasidium* or *promycelium*. The fused diploid nucleus moves to the promycelium and there undergoes reduction division to form four haploid daughter nuclei, then transverse septa are formed separating the nuclei from one another into four cells (uninucleate)—this is the *basidium* proper. A short lateral outgrowth, known as sterigma, is developed from each cell of the basidium. Each sterigma cuts off at its tip a single, colourless, minute and uninucleate basidiospore (haploid). At maturity the basidiospores are forcibly ejected by the water droplet method and are carried away by wind (Buller, 1924). As *Puccinia graminis* is heterothallic, the four basidiospores that are produced in a basidium are of two different strains or sexes (+ and −), *i.e.* two are of (+) strain and the other two are of (−) strain. These basidiospores are incapable of infecting the wheat plants, but basidiospores can germinate only when they come in contact with the alternate *i.e.* secondary host, *e.g.* the barberry plant (*Berberis vulgaris*).

4. Pycnial or Spermogonial stage—Typical infection takes place when basidiospores of two opposite sexes i.e. (+) and (−) strains are carried away by wind and reach barberry plant. A basidiospore (+ or −) on falling upon a leaf or young twig of a barberry plant, germinates by putting forth a germ tube which directly penetrates the epidermis. It elongates inside, becomes divided into uninucleate cells and ultimately branches forming a branched uninucleate *primary i.e. monokaryotic mycelium* within the intercellular spaces of the host tissue, and obtains nourishment from the host cells through haustoria. The nuclei of this monokaryotic

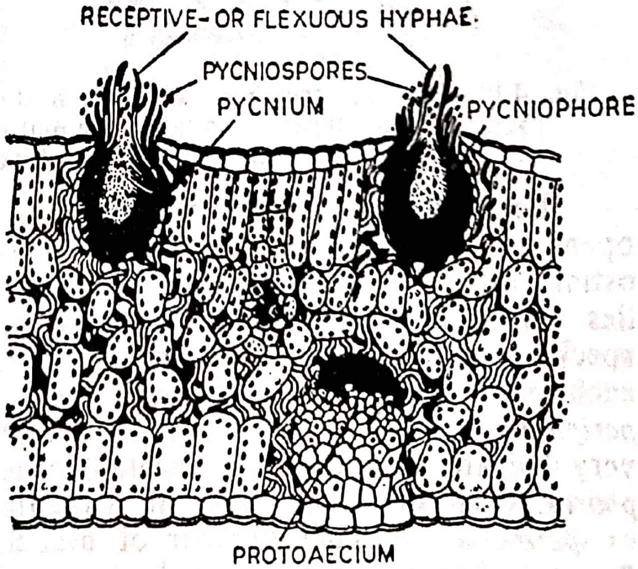


Fig. 4·9—*Puccinia graminis.* Section through barberry leaf showing two pycnia at the upper epidermis and an aecial primordium (protoaecium) near the lower epidermis.

mycelium carry either (+) or (−) factor which the parent basidiospore happens to carry. Within a few days after infection, isolated dense hyphal mats begin to appear both beneath the upper and lower epidermis of the leaf. Later on the hyphal mats near the upper epidermis represent the *primordia of spermogonia* and that near the lower epidermis represent the *primordia of aecidia* (also called *protoacidia* or *aecidial initials*). Within a few days (7 to 10) the upper primordia i.e. primordia of spermogonia matures into clusters of *spermogonia* or *pycnia* (singular : *spermogonium* or *pycnium* respectively).

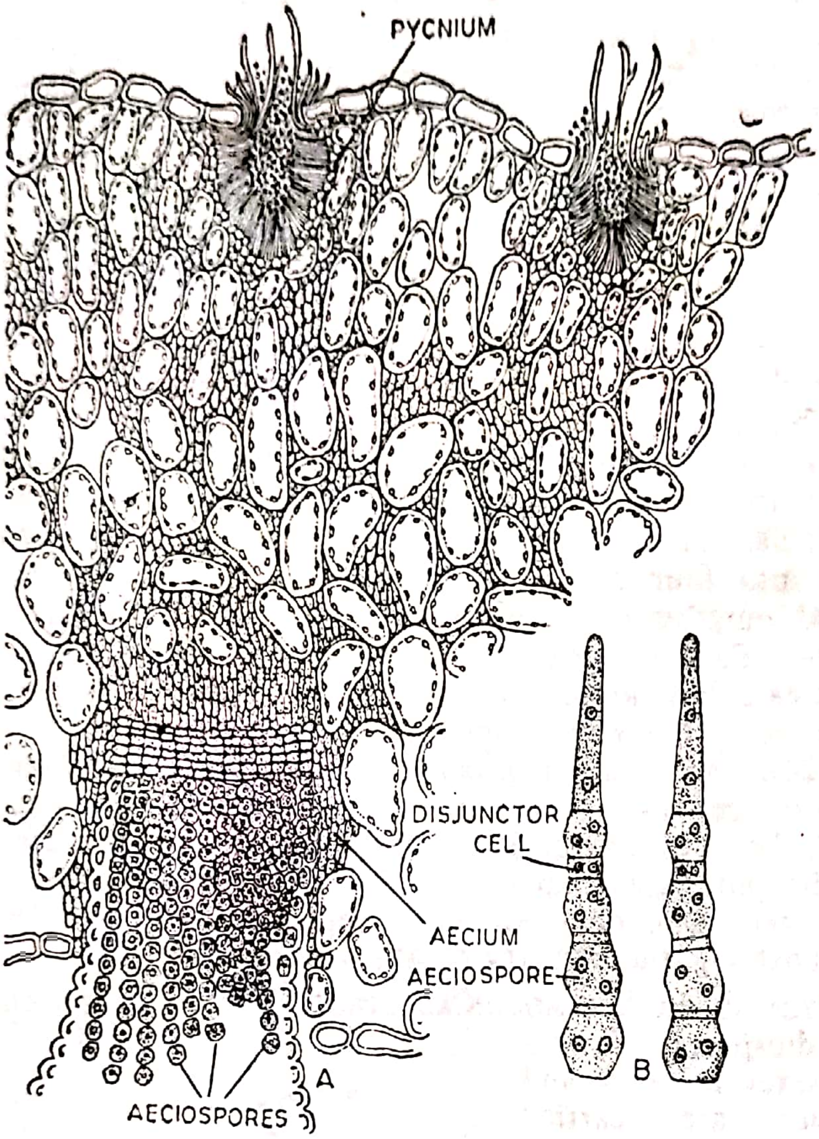


Fig. 4.10—*Puccinia graminis.* A—Section through the barberry leaf showing two pycnia at the upper epidermis and a mature aecium containing aeciospores at the lower epidermis. B—Two aeciospore chains.

Each spermogonium or pycnium is a oval or flask-shaped body which opens to the upper surface of the leaf by a minute pore called *ostiole*. The ostiole only projects above the surface of the leaf, the remaining portion lies embedded within the leaf. Each pore i.e. ostiole appears as a minute speck in surface view. The transverse section of a pycnium shows that each pore i.e. ostiole is guarded by a number of sterile hyphae called *periphyses*. The cavity of each pycnium is lined inwardly with numerous, very delicate, elongated uninucleate hyphae, called *spermatiophores* or *pycniophores*, each of which cuts off at the tip a succession of small *pycniospores* or *spermatia*. A small number of branched or simple hyphae, known as *flexuous hyphae* or *receptive hyphae* are also seen amidst periphyses but project out further beyond them. The spermatia or pycniospores are expelled through the ostiole and collect in mass on a droplet of liquid (nectar) exuding from the ostioles. The nectar also covers the periphyses and flexuous hyphae. The pycnia containing pycniospores, flexuous hyphae, periphyses etc. are all therefore haploid structures and depending upon the

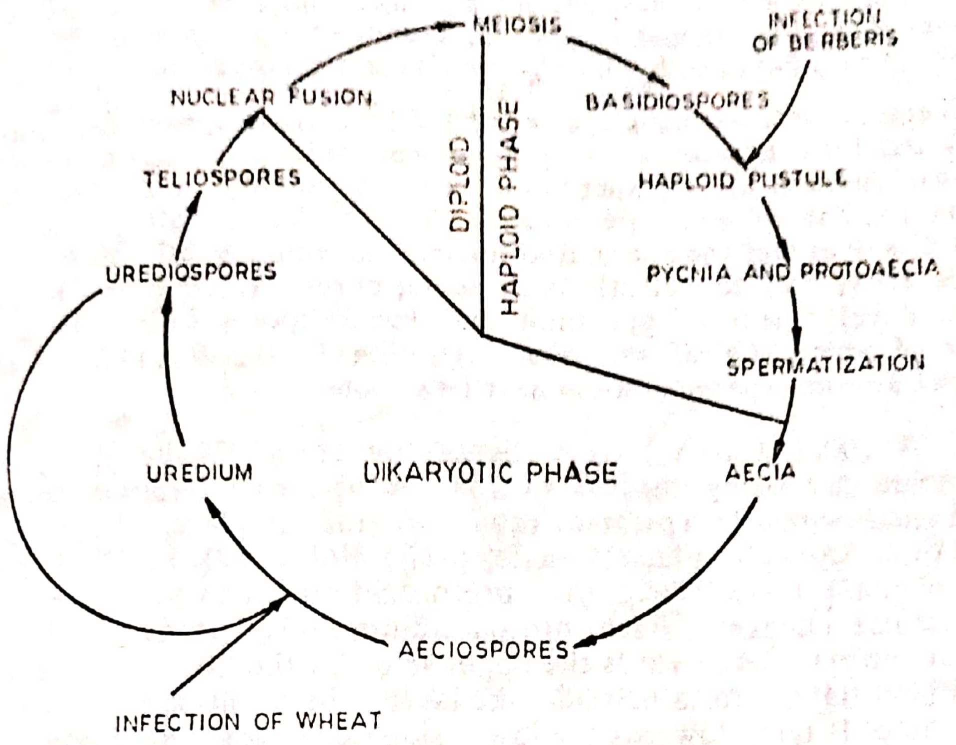
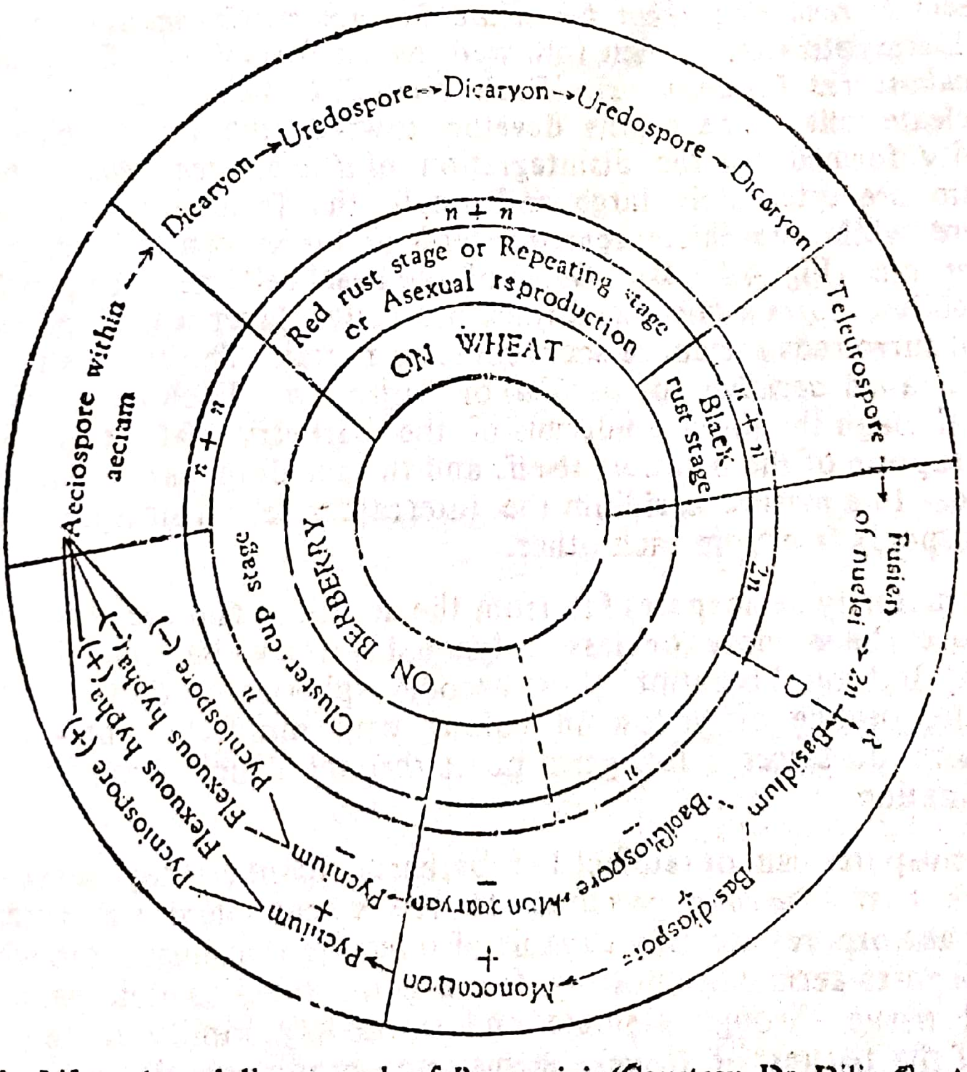


Fig. 4.11—Word diagram of the life cycle of *Puccinia graminis* (*Modified after Webster, 1970*)

nature of basidiospores from which they develop, they may be either of (+) or of (−) strain. Spermatization now takes place through the agency of



4.12—Life-cycle and disease-cycle of *P. graminis* (Courtesy, Dr. Dilip Gupta).

insects. Insects are attracted by the fragrance of the spermogonial mass and the sweet taste of the nectar. While insects visit pycnia, the spermatia or pycniospores of a pycnium of one sex are carried to the pycnium of opposite strain—as a result spermatization i.e. fusion of a pycniospore of one strain with the tip of a flexuous hypha of opposite strain takes place.

Therefore spermatization is effected if (+) pycniosposes are transferred to (−) flexuous hyphae or (−) pycniospores to (+) flexuous hyphae. At the time of spermatization, spermatia fuse on coming in contact with the flexuous hyphae of opposite strains. The common wall at the point of contact dissolves and the spermatial nucleus on entering the flexuous hypha migrates from cell to cell through septal pores. It has been observed that there is development of aecidium and aecidiospores only when there is transfer of spermatia of one spermogonium to the flexuous i.e. receptive hyphae of another spermogonium near its ostiole.

5. Aecial (Aecidial) or Cluster-cup stage—While the pycnia i.e. spermogonia are being formed twards the upper epidermis of the barberry leaf and simultaneously spermatizations are taking place, the development of *aecia* or *aecidia* takes place (Craigie, 1927 ; Buller, 1950). While formation of spermogonia takes place, the protoaecidium also undergoes certain developmental changes. Each protoaecidium looks at first a globose mass of hyphae but its cells towards the upper side (of the lower epidermis) very soon differentiate into a palisade-like layer—these cells are called *basal cells.* Rest of the cells lying towards the lower side i.e. facing the lower epidermis are called *displacement cells.* During spermatization, the nuclei of pycniospores (i.e. spermatial nuclei) which pass from the spermatia (i.e. pycniospores) into the flexuous hyphae, travel down the hyphae, move through the septal perforations of the mycelium and reach the basal cells of the protoaecidia, *rendering them binucleate i.e. dikaryotic condition* (dikaryotization). Dikaryotization is then followed by the formation of *aecidia* (*aecia*) and *aecidiospores* (*aeciospores*). Each binucleate basal cell cuts off a chain of binucleate cells. The chains develop towards the lower epidermis into the cavity formed by the disintegration of *displacement cells.* The cells of the chain are alternately large and small, the former matures into an *aeciospore* while the latter remains sterile and is known as *intercalary cell* or *disjunctor cell* (Fig. 4.10, B). Some of the basal cells near the periphery of a protoaecidium form a one-celled thick protective layer called *peridium*—this peridium surrounds the entire spore mass all-round. The entire structure thus formed is called *aecidium* (pl. *aecidia*) or *cluster cup.* Each aecidium elongates pushing through the lower epidermis of the barberry leaf ; this is followed by the rupture of the peridium itself, and the aecidium assumes a bell-shaped structure. In a mature aecidium the intercalary cells disintegrate separating the aeciospores free from each other.

At maturity aeciospores fall from the aecidium and are carried by wind. Aeciospores have more or less polygonal form while remaining within the aecidium, but on liberation they become spherical ; they are unicelluar, binucleate, orange or yellow in colour with minutely warty thick walls. Each spore possesses a few germ-pores through which germ tubes come out on germination.

Aeciospores cannot reinfect the barberry plant ; they serve to return the black rust (*Puccinia graminis*) to the wheat plant i.e. primary host, because aeciospores are only capable of infecting that host. On wheat plant, the aeciospores germinate putting forth germ tubes which gain access to the host tissue through stomata and ultimately ramify in the intercellular spaces of the tissues of leaves, stems etc. producing dikaryotic mycelium (n+n). This dikaryotic mycelium within 1 to 2 weeks again produces uredospores in uredosori. In this way the cycle is continued.

Thus the life cycle of *P. graminis* is a completed one, consisting of five distinct stages. Teleutospore constitute the perfect stage in the life cycle, since it is the structure in which karyogamy and meiosis take place. In this rust, the life cycle is long-cycled *i.e.* macrocyclic type and consists of five distinct stages produced in a regular sequence, these are as follows :—

Stage O—Pycnia (spermogonia) bearing pycniospores (i.e. spermatia) and flexuous i.e. receptive hyphae

Stage I—Aecia bearing aeciospores

Stage II—Uredia bearing uredospores

Stage III—Telia bearing teleutospores

Stage IV—Basidia bearing basidiospores

All the spore-forms, with the exception of basidiospores, are produced in definite sori.

Nuclear cycle—In *Puccinia graminis*, the dikaryotic condition (n+n) is more well represented and persists through greater part of the life cycle than the monokaryotic condition (n). Dikaryotic condition is established by spermatization between a spermatium and receptive hypha of opposite strains (+and−); as a result of this, the two nuclei of opposite strains (i.e. one of + strain and the other of − strain) become ultimately associated as a dikaryon in the basal cells of aecial primordia or protoaecidia. The dikaryotic protoaecidia ultimately mature into aecidia composed of dikaryotic cells. Each aecidium also produces binucleate i.e. dikaryotic aeciospores (n+n). This dikaryotic condition persists from this (aecidial) stage upto the formation of young teleutospores. Young teleutospores are dikaryotic i.e. each contains two nuclei (n+n); at maturity i.e. in the developing teleutospores karyogamy (nuclear fusion) takes place and consequently they become diplophasic (2n). Meiosis of this diploid nucleus (2n) takes place during the germination of teleutospore—as a result monokaryotic condition (n) is established. This monokaryotic condition persists in the subsequent stage i.e. in basidiospores or sporidia (n), spermogonia (n), spermatia (n), receptive hyphae (n) and also in the protoaecidia (specially before spermatization)—all these are therefore haploid and either of (+) strain or of (−) strain. Of the five different stages in the life cycle (O to IV), only O and IV are monokaryotic (n) while the stages I to III are dikaryotic (n+n) with a very short diplophase (2n) [in the later stage of III]. Thus in the life cycle of *P. graminis*, there is a monokaryotic condition of short duration which is followed by a dikaryotic condition of longer duration.

Physiologic i.e. Biological specialization—Refer Chapter I, introduction, page 524.

Salient features of Puccinia graminis :—

(1) Obligate parasite on host plants.

(2) It is heteroecious, macrocyclic and polymorphic rust.

(3) Mycelium is generally intercellular which obtains nourishment by haustoria.

(4) Clamp connections, by dikaryotic mycelium, are rare.

(5) Teleutospores are formed from terminal cells of the dikaryotic mycelium.

(6) Basidiospores are definite in number and are borne on sterigmata.

(7) Teleutospore constitutes the perfect stage, in this structure karyogamy and meiosis takes place.

(8) Basidiocarps are lacking.

(9) Life cycle is long-cycled i.e. macrocyclic type consisting of five distinct stages,