CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

The development of sunflowers as a major Australian and World crop has been a protracted process. Archeological evidence (Smith,1965; Lawton, Wilke, DeDecker and Mason,1976) and more recent observations (Putt,1978) suggest that the common annual sunflower *(Helianthus annuus* L.) was utilized and even domesticated by some of the aboriginal cultures in North America *circa* 3000 B.C. as a source of food, oils for use in cosmetics, medicines and dyes. Sunflowers entered Europe from seed collected by the colonizers of the New World and was grown as an ornamental. It was not until the 18th century in Russia that the potential of sunflowers as a source of edible oil was recognized and exploited. Breeding programs aimed at developing improved cultivars for oil seed production followed, particularly in the U.S.S.R. Russian lines of *H.annuus* var. *macrocarpus* Ckll. have since been introduced into all sunflower growing areas of the world. The incorporation of high oil germplasm into other breeding programs has enabled sunflowers to become one of the top four edible oil producing crops in the world (FAO,1984).

In Australia, *H.annuus* was grown originally as ornamental plants (McAlpine,1906). At various times during the twentieth century attempts were made to promote sunflowers as a crop. Boyd(1915) stated that sunflowers "*will grow in almost any soil, and in any climate. It will withstand cold or heat, drought or rain. It is subject to no disease, and to no climatic disqualification.*" The plant could be utilized totally with the seed used as a source of edible oil or for poultry feed, the leaves as cattle silage and any trash converted to protein-rich ash (Boyd,1915). Other suggested uses for sunflower included its use as a source of fibre for cloth making and yellow dyes (Anon,1923), a source of pith for life preservers (Anon,1916a) and as a vermicide in horses (Keane,1915). Interest in the crop as an oilseed was heightened during the two World Wars when other edible oils could not be imported (Anon,1916b; Anon,1941). The absence of commercial oil mills (Keane,1920) prevented further development of the crop at that time.

The introduction of the wheat quota system in Australia in 1969 forced growers to look for alternative crops (Lovett and Lazenby,1979). This lead to the revival of interest in sunflower in Australia. In the period from 1960/1 to 1986/7 the area planted to sunflowers in Australia increased from 1,800ha to an estimated 273,900ha with the greatest planting of 350,500ha in the 1984-85 season (Table 1.1). Australia has few specialist sunflower growers and most of the area is planted opportunistically to take advantage of climatic and market fluctuations. There are wide fluctuations in production levels from year-to-year (Table 1.1) for a number of reasons. These include the use of unsuitable planting and harvest machinery;

Table 1.1 Historical sunflower production figures for three states of Australia over a 26 year period.

000' Hectares / 000' tonnes[1]

	Queensland		New South Wales		Victoria		Total		
	HA	Tonnes	HA	Tonnes	HA	Tonnes	HA	Tonnes	Tonnes /ha
1960/61	1.7	1.1	0.07	0.02	0.05	0.04	1.82	1.16	0.64
1961/62	3.8	2.5	0.00	0.04	0.08	0.1	3.88	2.64	0.68
1962/63	3.7	2.1	0.1	0.06	0.06	0.04	3.86	2.2	0.57
1963/64	3.1	1.9	0.02	0.01	0.02	0.03	3.14	1.94	0.62
1964/65	4.3	2.4	0.04	0.0	0.0	0.0	4.34	2.4	0.55
1965/66	5.1	3.4	0.06	0.0	0.0	0.0	5.16	3.4	0.66
1966/67	3.3	1.6	0.4	0.2	0.2	0.07	3.9	1.87	0.48
1967/68	5.7	3.0	0.3	0.2	0.06	0.01	6.06	3.21	0.53
1968/69	11.5	4.7	1.8	1.2	0.4	0.4	13.7	6.3	0.46
1969/70	15.6	6.3	9.1	6.0	1.2	0.9	25.9	13.2	0.51
1970/71	20.1	14.1	54.1	43.2	1.4	1.6	75.6	58.9	0.78
1971/72	53.7	24.0	238.9	121.0	2.1	2.4	294.7	147.4	0.50
1972/73	80.1	45.4	158.6	54.3	2.1	2.1	240.8	101.8	0.42
1973/74	67.5	43.2	79.0	37.8	3.3	2.5	149.8	83.5	0.56
1974/75	104.9	68.4	94.1	37.5	8.0	4.8	207.0	110.7	0.53
1975/76	62.4	38.2	62.6	32.6	7.8	5.7	132.8	76.5	0.58
1976/77	77.8	40.3	38.8	21.8	13.3	8.4	129.9	70.5	0.54
1977/78	133.6	101.6	62.8	35.4	14.0	11.3	210.4	148.3	0.70
1978/79	161.8	121.6	72.1	43.1	14.2	11.0	248.1	175.7	0.71
1979/80	158.7	96.7	43.0	26.6	9.4	7.3	211.1	130.6	0.62
1980/81	146.3	103.3	35.6	19.1	8.2	8.6	190.1	131.0	0.69
1981/82	128.3	71.2	31.0	27.4	12.0	10.1	171.3	108.7	0.63
1982/83	119.7	72.8	53.2	28.3	1.1	0.8	174.0	101.9	0.59
1983/84	141.3	99.6	75.1	54.7	14.1	12.5	230.5	166.8	0.72
1984/85	192.7	158.6	145.4	115.1	12.4	14.4	350.5	288.1	0.82
1985/86	148.0	98.0	112.9	98.9	13.0	15.9	273.9	212.8	0.78
1986/87E	79.0	41.0	82.0	75.0	10.0	14.6	171.0	130.6	0.76

1. Australian Bureau of Agricultural Economics

* E : BAE Estimate

lack of knowledge of the most satisfactory cultural practices in the diverse Australian environments; expansion of sunflower cultivation into marginal growing areas; occurrence of detrimental environmental conditions such as drought; and losses caused by the increased incidence of diseases and pests. These fluctuations have occurred despite the implementation of extensive public and private breeding programs. To consolidate the position of sunflower in Australian agriculture it is desirable that the effects of the constraints on production be understood and minimized where possible.

Sunflower is a broad-acre relatively 'low value' crop in which the cost and difficulty in implementing chemical measures to control diseases prohibit their use. The tendency of growers therefore has been to grow cultivars with recognized disease resistance when these are available and when a particular disease problem is anticipated. The alternative is to accept losses due to disease. Rust of sunflower caused by the fungus *Puccinia helianthi* Schw. has the potential to be a major constraint to the attainment of optimum yields (Middleton and Obst,1972; Brown, Kajornchaiyakul, Siddiqui and Allen,1974). Knowledge about this fungus is important so that control strategies can be formulated.

1.1 The Causal Organism

Puccinia helianthi was first described by Lewis von Schweinitz in 1822 from specimens collected on *Helianthus mollis* Salem in the eastern United States (Putt and Sackston,1957). The earliest record of the pathogen in Australia was from the Mudgee district of New South Wales in 1887 while the first record of its occurrence in Queensland was at Ipswich in 1888 (McAlpine,1906).

Puccinia helianthi is a macrocyclic, autoecious and heterothallic rust. The autoecious nature of the fungus was first reported by Woronin(1872) after he obtained pycnial infections on *Helianthus annuus* from basidiospores derived from teliospores of *Puccinia helianthi* taken from *H.annuus*. Arthur(1903) and Kellerman(1905) demonstrated that the fungus was autoecious and macrocylic. Carleton(1904) and Bailey(1923) reported that occas ionally the aecial stage did not occur. Craigie(1927a) showed that two separate pycnial infections of opposite mating types must coalesce for aecia to form. The fungus was therefore heterothallic. Pycniospore transfer in pycnial nectar between pycnia of opposite mating types also initiated aecial development in *P. helianthi* (Craigie,1927b).

The normal life cycle of *P.helianthi* consists of the following five distinct spore stages in a regular sequence (McAlpine,1906; Arthur and Cummins,1962; Laundon and Waterson,1965).

Stage 0 Pycnia (syn.spermogonia) bearing receptive hyphae and pycniospores (syn. spermatia): These are described as Type 4 in the Systems of Hiratsuka and Cummins(1963) and Hiratsuka and Hiratsuka(1980). The

pycnia are subepidermal, determinate and have a strongly concave hymenium bound by periphyses. They are amber to orange in colour and usually amphigenous but may also develop on petioles and young stems. The pycniospores (syn.spermatia) are small, oval and hyaline.

Stage I Aecia bearing aeciospores: Aecidioid aecia are normally hypophyllous in groups directly below pycnia. Each aecium is orange-red with white lacinate margin and 0.2 - 0.3mm in diameter. Length dependant on environment. Short (<2mm) if humid conditions allow rupture and aeciospore discharge. Aeciospores orange with yellow cytoplasm and ellipsoidal to polygonal (16-23um x 20-28um) with colourless, irregularly verrucose walls.

Stage II Uredinia bearing urediniospores: These are most commonly amphigenous, pulvenerate hypophyllous, dark cinnamon-brown round sori up to 1mm in diameter. Also found on petioles and stems. Urediniospores with cinnamon-brown finely echinulate walls. Shape is sub-globose or obovoid (19-26um x 23-34um) flattened laterally with 2 germ-pores.

Stage III Telia bearing basidiospores: These brownish-black oval sori are chiefly hypophyllous and are formed by transition of uredinia. Teliospores are cylindrical to clavate (20-30um x 36-58um) with two cells each with one germ-pore. Non-deciduous pedical is pale and longer than the spore. The teliospores are classified as Type IV in the ontogenic system of Hiratsuka(1988).

Stage IV Basidia bearing basidiospores: These have not been described.

Study of the life cycle of *P.helianthi* and the environmental factors that influence it has been limited primarily to the urediniophase. Much of this work was nec essarily conducted *in vivo* because *P.helianthi* like the Uredinales in general was considered to be an obligate parasite. Nozzolillo and Craigie(1960) successfully established dual cultures of *P.helianthi* in callous tissue grown from rust-infected stem and hypocotyl sections of sunflower seedlings. Maheshwari, Hildebrandt and Allen (1967) failed to obtain infection of tissue cultures of sunflower with urediniospores of *P.helianthi*, while Hennessey and Sackston(1970) cultured the complete life cycle on detached leaves of sunflower maintained on water agar. The cycle from basidiospores to teliospore production was completed in about 80 days. True axenic culture of the fungus has been achieved (Coffey and Allen,1973).

1.2 Distribution and Host Range

Puccinia helianthi is considered to have a worldwide distribution occurring on all continents except Antartica (Putt and Sackston,1975). It is most prevalent in temperate and sub-tropical regions and rare or absent in the tropics and the artic regions (Laundon and Waterston,1965). This distribution follows that of its hosts.

The principle hosts of *P.helianthi* are members of the genus *Helianthus*, all 74 species and sub-species of which are native to the Americas (Rogers, Thompson and Seiler,1982). Other hosts belong to the closely allied genera *Iva*, *Heliospsis* and *Viguira* (Laundon and Waterston,1965) and *Xanthium strumarium* (Tranzschel,1909 cited by Arthur and Cummins,1962). Arthur and Cummins(1962) listed 35 species of *Helianthus* as hosts. More recent taxonomic examinations of the genus *Helianthus* (Heiser, Smith, Clevenger and Martin,1969; Rogers *et al*,1982) reveal that some of the "species" recorded by Arthur and Cummins (1962) are actually synomyms (eg. *H.fascicularis* Greene = *H.nuttallii* T.& G.) or natural hybrids (eg. *H.laetiflorus* Pers. = *H.rigidus* (Cass) Def. x *H.tuberosus* L.). The recorded hosts and results of cross-inoculation experiments can be found in Arthur(1903), Kellerman(1903), Arthur(1904), Kellerman(1905), Arthur(1906), Bailey(1923), Putt and Sackston(1957), Arthur and Cummins(1962), Hennessey and Sackston(1972), Zimmer and Rehder(1976) and McCarter and Kays(1984).

1.3 Aetiology and Cytology

Classically, the life cycles of the Uredinales commence with the germination of the teliospores. In areas where seasonal conditions (usually cold) prevent growth of the host the teliospores act as overwintering spores. Teliospores of *Puccinia helianthi* are generally regarded as needing a resting period before germination (Maneval,1922; Bailey,1923; Hennessey and Sackston,1970) but germination and subsequent infection of plants from teliospores which had not undergone any resting period have been reported (Carleton,1903; Sackston,1957). Volunteer sunflower seedlings with pycnia have been observed by the author (*unpublished data*) in Southern Queensland during Autumn and aecia were observed in October in the Callide-Dawson area by J.K. Kochman(*pers.comm.*).

Several workers have attempted to develop methods to stimulate and optimize teliospore germination in the laboratory. Hennessey and Sackston(1970) used alternating cycles of washing and drying to induce germination while Maneval(1922) and Bailey(1923) favoured prolonged floating of the spores on water. Attempts by Bailey(1923) and Maneval(1927) to stimulate germination of teliospores of *P.helianthi* by exposing them to various chemicals were not successful. Maneval(1922) found that teliospore germination and basidiospore formation was greatest at pH 4.6-6.5. Exposure to alternate light and dark cycles during incubation resulted in greater germination than incubation in continuous darkness (Maneval,1927).

No reference has been found in the literature describing the behaviour of the dikaryotic haploid nuclei in the teliospore during germination. If consistent with other species of *Puccinia* then the two nuclei undergo karyogamy with the initiation of teliospore germination (Savile,1939; Berkson and Britton,1969). The teliospore is then technically a probasidium. With most rusts the diploid nucleus then moves into the developing metabasidium where it undergoes meiosis to produce four haploid nuclei. Septa then form which results in a four-celled metabasidium. A single basidiospore is formed upon the sterigma that develops from each cell of the metabasidium (Bailey,1923). Variation in nuclear behaviour in the metabasidium of *Puccinia* spp. has been reported by Kulkarni(1958) and Kakishima, Sato and Sato(1984). Anikster(1983) showed that basidiospores of *P. helianthi* were binucleate but the nuclear cytology leading to the production of the binucleate state was not reported.

Infection of *Berberis vulgaris* L. following germination of basidiospores of *Puccinia graminis* Pers. was shown to occur by direct penetration of the cuticle and epidermis (Waterhouse,1921). Intracellular hyphae that grew through host cells and haustoria that terminated in host cells then formed and gave rise to pycnia. The cells of the thallus of the pycnia of *P.helianthi* contain a single haploid nucleus. The pycniosporophores of the central hymenial layer and the pycniospores produced and exuded in a nectar are also monokaryotic (Craigie,1959). Transfer of pycnial nectar from pycnia of one mating strain to pycnia of a compatible mating strain was found to initiate creation of the hetero-dikaryotic diploid state(Craigie,1927b). Craigie (1933,1959) demonstrated that the transferred pycniospores fuse with flexuous or receptive hyphae that extend through the ostioles of the recipient pycnia. The pycniospore nucleus enters the flexuous hyphal strand and migrates via the hyphae to the protoaecial cells where rapid multiplication of the pycniospore nucleus provides dikaryotization of the protoaecia. Anastomosis of haploid mycelia of different mating strains within the leaf can also result in dikaryotization through somatogamy (Craigie,1927a; Brown,1935) as can the coalescing of haploid infections with dikaryotic uredinial infections (Brown,1932).

Aecia begin to develop once the hetero-dikaryotic state is established. The aeciospores are dikaryotic (Craigie,1959). The aeciospores are released under humid conditions probably with their forcible ejection from the aecium as found in other species of *Puccinia* (Buller,1924). If these spores contact a sunflower leaf under conditions of free moisture and suitable temperatures they germinate by producing a single germ-tube. Leaf penetration occurs via the stomata following formation of an appressorium over a stoma(Bailey,1923). The dikaryotic intercellular thallus that results develops into an uredinium. The optimum temperature for aeciospore-derived infections was recorded as 16°C by Persic(1957) and 19°C by Bailey(1923).

Uredinia are the most conspicuous stage of the life cycle. They may develop on the leaf lamina, petioles, stems and involucral bracts as round sori up to 1mm in diameter. Infections limited to the older leaves may indicate a previous period when conditions were conducive to infection but which subsequently changed allowing the new growth to escape infection (Sackston,1957). Infection all over the plant indicates recurring periods of conditions favourable for infection or a single large influx of urediniospores during periods of conditions conducive to infection. Severe uredinial infections may cover most of the leaf area but often before this can occur the leaf prematurely senesces.

Favourable conditions for germination of the wind-borne urediniospores and subsequent infection of the host have been found (Bailey,1923; Sood and Sackston,1970,1972; Brown, Allen, Kajornchaiyakul and Siddiqui,1982; Goulter,1983) to include temperatures of between 15-25°C, darkness and the presence of free moisture on the host surface. The single germ-tubes issued on germination of urediniospores are initially dikaryotic (Craigie,1959). If the germ-tube encounters a stoma it may produce an appressorium. Appressoria may also be produced over the junctions of epidermal cells. However infection only occurs through stomata (Goulter,1983). Penetration occurs when a penetration peg grows from the lower surface of the appressorium and enters the stomatal aperture to form an "H-shaped" vesicle composed of the cytoplasmic contents of the appressorium. Post-penetration development begins with the intercellular growth of infection hyphae from the sub-stomatal vesicle. When the intercellular infection hyphae come into contact with host cell walls the invading hyphae may delimit haustorial mother cells from which haustorial pegs penetrate the cell walls and enlarge into simple spherical haustoria. The intercellular hyphae is dikaryotic (Coffey, Palevitz and Allen,1972a).

In susceptible hosts the hyphae continue to grow through the leaf and radiate out. Sporogenous cushions that develop beneath the upper and lower epidermis produce a new generation of urediniospores which ruptures the epidermis and cuticle to become erumpent sori. Urediniospores within sori are inhibited from germinating by the presence of a volatile chemical identified by Macko, Staples and Renwick(1971,1972) as the cis-isomer of methyl 3,4-dimethoxy cinnamate.

The potential of the uredinial stage to increase rapidly and reinfect the host is associated with yield losses that are of concern in agro-ecosystems. Coffey *et al*(1972a,b) described the ultrastructural and biochemical changes that occurred during uredinial infection of the susceptible sunflower cultivar "Mammoth Russian". They found that the host cells closest to sori became totally disorganized with degenerate organelles whose structural changes were analogous to the changes that occur in normal senescing tissues. The further host cells were from a sorus or radiating hyphae the more normal they appeared to be. Physiological changes induced by

infection include an increase or decrease in photosynthesis depending on severity of infection, an increase in transpiration and a decrease in chlorophyll content (Patil and Kulkarni,1978). Dark transpiration increases due to non-stomatal transpiration resulting from damage to the epidermis caused by erumpent sori (Siddiqui,1980). The translocation of assimilates from infected leaves was found to be reduced by rust infection (Siddiqui,1980). The decreased translocation of assimilates was a product of the greater local demand caused by the rust colony, greater rate of respiration of diseased tissue and a reduction in amount of assimilate produced (Siddiqui,1980). Yarwood and Childs(1938) reported that the dry weight of infected leaves was higher than that of healthy leaves. Siddiqui and Brown(1977) found that repeated inoculations could reduce the stem dry weight by up to 62%.

Initial biochemical changes following infection by *P. helianthi* were characterized by the enhanced synthesis, in host cells, of proteins and RNA and alterations to a number of biosynthetic pathways including increased use of the pentose-phosphate shunt (Coffey *et al*,1972b). Photosynthates were transformed into lipids in the host cells and were moved to the mycelium and incorporated into the developing urediniospores. This "synthetic phase" of parasitism lasted until sporulation and was followed by senescence (Coffey *et al*,1972b).

Late in the season or as the host leaves senesce uredinial infections may commence producing teliospores. Under suitable conditions these teliospores germinate to produce the basidiospores which begin the new life cycle.

1.4 Effect on Yield

The physiological effects of uredinial infections on the functioning of host leaves may be ultimately expressed by a reduction in the yield of plants. Yield of oilseed sunflowers is normally assessed as grain yield and the oil content of that grain. Quantitative assessments of the effect of rust on sunflower yields has usually been achieved by comparing the yields of experimental plots in which the disease has been controlled with fungicides with those of unsprayed plots. Using this technique yield losses (kg oil/ha) in highly susceptible open-pollinated cultivars have been found to be as high as 70% (Middleton and Obst,1972; Brown *et al*,1974; Goulter,1983). In general, the severity of the disease in the field has been found to be lower in susceptible hybrids than the older open-pollinated cultivars (Allen, Brown and Kochman,1980). Nevertheless, in Argentina, Juncos and de Romano(1985) found that by decreasing the severity of disease in two hybrids from 34% to 24% and 18% to 10%(leaf area infected), they were able to increase oil yield by 21% and 6% respectively. Ivancovich, Bruniard, Ludaena and Oliva(1985) reported similar results.

Fick and Zimmer(1975) used near-isogenic resistant and susceptible

lines to evaluate the effect of rust infection on crop yields in the absence of any chemical sprays. Two resistant hybrids had only 1% leaf area infected while in the susceptible counterparts the severity reached 31% and 43%. This was associated with oil yield losses of 38% and 83% respectively in the susceptible lines.

1.5 Epidemiology

The initial inoculum for commencment of epidemics on new season crops must survive inter-crop periods. Teliospores are usually considered to be the resting or over-wintering stage in the life cycle of many Uredinales (Petersen,1974). This may occur with *Puccinia helianthi* in areas where cold winters kill the host. Where conditions are milder the fungus may survive as latent mycelial infections or even as active uredinial infections on surviving sunflowers (Zimmer and Hoes,1987). It is presumed that in Australia *P.helianthi* survives during the inter-crop period on volunteer plants and on populations of wild sunflowers, such as *H.annuus* and *H.debilis* Nuttall that have become established in the major sunflower growing areas. The role of teliospores in Australia has not been accurately assessed. Reid(1981) described sunflower rust as commencing each season from pycnidial(?) infections on volunteer seedlings and spreading to cultivated crops but no evidence was given to support that contention. Pycnia and aecia are rarely observed in the field (Kajornchaiyakul, 1974).

Epidemics may be initiated from influxes of inoculum from distant areas. Urediniospores can be found on the surface of sunflower seeds but there is no evidence of seed-borne transmission (Laundon and Waterston,1963). In South America, Sackston(1957) encountered heavily rusted sunflower crops in isolated areas of land cropped for the first time. He speculated that widespread dissemination of urediniospores by air-streams had introduced the initial inoculum into these areas. Laundon(1973) found rusted sunflower plants in New Zealand despite the implementation of strict quarantine procedures. It was speculated that the source of inoculum was urediniospores from rusted crops in Australia that had been blown across the Tasman Sea to New Zealand, a distance of about 2500km.

It can also be speculated that the rust epidemics that occurred in south east Queensland during the spring of 1985 (Kochman and Criddle,1986) may have developed from inoculum derived from the large plantings of winter-grown sunflowers in Central Queensland. These epidemics were atypical because sunflower rust is rarely a problem in spring crops. The 1957-1986 average surface wind analyses at 1500h for Emerald (Longitude 148 10 E) in Central Queensland show that in August around 25% of winds have a northerly orientation and consequently a southern destination (Bureau of Meteorology Data). This increased to 35% in September and 39% in October. In southern Queensland, winds at Dalby (Longitude 151 16 E) were 25, 36 and 43% from the north during August, September and October respectively (Bureau of

Meteorology Data). It would seem therefore, that there would have been ample opportunity for air-borne urediniospores to move from Central Queensland sunflower areas to the sunflower areas of southern Queensland.

Further epidemic development from initial infections is dependant on a conducive environment. In general, urediniospores are not forcibly discharged but are passively disseminated from uredinia by air-streams (Smith,1966; Ingold,1978; Littlefield,1981). Eremeyeva and Karakulin(1929) estimated that in light winds over 14000 urediniospores may settle onto a single sunflower leaf in three hours. Optimal conditions for infection of leaves by urediniospores include a temperature of between 18-22°C, darkness and free moisture on the leaf surface for at least 8 hours (Goulter,1983). Exposure to different light intensities and photoperiods can modify the susceptibility of sunflower hybrids (Goulter, Kochman and Brown,1984).

1.6 Control

1.6.1 Biological

Predators and parasites have been used in attempts to obtain biological control of rusts. The hyperparasitic Deuteromycete *Darluca filum* (Biv.-Bern. exFr) Cast. has been observed to parasitise teliospores of *P.helianthi* (Muntanola,1954). Muntanola(1954) also referred to 'spore-eating cecidomyid larvae' active on rusted leaves. In Australia, cecidomyid fly larvae predacious on urediniospores have been identified (R.H. Broadly, *pers.comm.*) as belonging to the genus *Nycodiplosis*. However, the importance or potential of parasites and predators in controlling *P.helianthi* in Australia has not been investigated.

1.6.2 Cultural

The areas suitable for growing sunflowers commercially are often also climatically suitable for development of *P.helianthi*. Disease escape through an unfavourable climate has been recorded in Chile by Sackston(1956) where sunflowers were grown under irrigation in areas of low rainfall and little dew. Spore germination and infection was therfore prevented by the absence of free water on leaf surfaces.

Putt and Sackston(1957) suggested that cultural practices that reduce the amount of initial inoculum in a crop include crop rotation, field sanitation and field isolation. A three year rotation strategy was recommended to reduce the number of viable teliospores in the soil (Putt and Sackston, 1957). Other control measures recommended in North America include destruction of crop debris either by deep incorporation into the soil or removal. Volunteer or wild sunflower plants in commercial sunflower areas should be destroyed as these are potential sites for the build-up of primary inoculum. Susceptible crops should be planted early in the season when inoculum levels are low. They should also be up-wind of areas previously

cropped to sunflowers to avoid influxes of inoculum.

1.6.3 Chemical

The application of protectant and systemic chemicals to control sunflower rust has been used under experimental conditions. Protectants are applied to the plant surface where they create a chemical barrier to prevent infection. Effective fungicides in this class include copper compounds and the bithiocarbamates mancozeb and zineb (Pawar and Patil,1976). Systemic fungicides are absorbed by and translocated through the host tissue. Most systemic fungicides act by inhibiting successful infections while some are also capable of killing established infections. The latter are termed therapeutants. Systemic fungicides that have been reported to be effective against *Puccinia helianthi* include triadimefon (Singh and Musymi,1979) and oxycarboxin (Pawar and Patil,1976).

The application of fungicides to sunflower crops is often considered to be impractical. Aerial application is required to spray the large areas grown. However, penetration of the dense canopy and effective coverage with protectant chemicals is difficult (Zimmer and Hoes,1978). This mechanical difficulty and the expense of spraying the generally low value crop make chemical control of rust an unattractive economic alternative to the utilization of genetic rust resistance in the host.

1.6.4 Rust Resistance

Arthur(1929) considered that selection of sunflower cultivars resistant to sunflower rust was the most promising method of controlling the disease. At that time in the United States sunflowers were used as a silage crop. Spragg and Down(1920) reported that in Michigan the South American cultivar 'Kaeurpher' was resistant to sunflower rust. Before 1955, no rust resistant oil-seed cultivars were available in any country (Putt and Sackston,1957). In 1955, the rust resistant open pollinated cultivar, Beacon, produced in Canada, was released and grown extensively in North America. This line possessed pathotype-specific resistance to the prevalent pathotype of rust,North American Race 1, but was susceptible to North American Race 2 of the fungus (Putt and Sackston,1963).

The germplasm for rust resistance in the early Canadian sunflower lines was derived from out-crosses of susceptible cultivars with wild *Helianthus annuus* growing around the winter breeding nurseries at Renner,Texas. From that material Putt and Sackston(1963) identified two genes for resistance to rust, termed R1 and R2, which were dominant, non-allelic and inherited on a monofactorial basis. These genes have been used extensively in breeding programs both in North America and elsewhere (Zimmer,1977).

The presence in sunflower lines and species of other genes for resistance to rust has been suggested but these have not been identified

(Sackston,1962; Jabbar Miah and Sackston,1970a; Zimmer,1977). The Canadian line CM 403-4 presumedly possesses another gene, R3, but there are no remaining seed stocks of the line to test this hypothesis. More recently, Senetiner, Antonelli and Ludaena(1985) reported the identification of two genes in crosses of the Argentinian sunflower lines L.C.74/75-20620, Pergamino 71/538, MP555 and MP557 which were designated Ph1 and Ph2. The latter of these may be part of an allelic system. The relationship of these genes and the R1 and R2 genes has not been established.

Miller, Rodrigues and Gulya(1988) reported the identification of two more genes for resistance to *Puccinia helianthi*. The R4 gene was present in the lines HA-R1, HA-R3, HA-R4 and HA-R5 while the R5 gene was present in the line HA-R2. The lines HA-R1 to HA-R5 were selected on the basis of resistance to the North American pathotype Race 4 and were derived from the Argentinian lines Pergamino 71/538, Impira INTA, Charata, Saenz Pena 4-1-2 and Guayacan INTA (Gulya,1985). The ability to impart resistance to Race 4 distinguished the R4 and R5 genes from the R1 and R2 genes. Yang, Dowler and Luciano(1989) reported the identification of a sixth gene, the Pu6 gene, for resistance to sunflower rust.

The sunflower rust differential set suggested by Yang, Antonelli, Luciano and Luciani(1986) contained 13 lines with resistance to rust but the genotypes of 11 of those were not known. The differential set suggested by Gulya and Masirevic(1987) introduced the two newly described genes R4 and R5 derived from Argentinian lines.

Russian plant breeders have used interspecific crosses to obtain resistance which seemed to be conditioned by the action of recessive genes or dominant genes for susceptibility (Pustovoit and Slyusar,1979). Hennessey and Sackston(1972) found the sunflower species *H.argophyllus* T.& G., *H.debilis* and *H.petiolaris* Nutt. possessed resessive genes for resistance to rust. Further resistance to rust among the wild sunflower species of North America (Zimmer and Rehder,1976; Quresh, Gulya and Jan, 1989) may be utilized in breeding programs in the future. Wild species of sunflower that have become adapted in some regions of Australia also possess resistance genes to rust that have been used in breeding programs by Pacific Seeds (*unpublished data*).

Pathotype non-specific resistance is expressed by reduced rates of disease progress or slow-rusting and has been identified in sunflower lines by Kochman and Goulter(1982) and Goulter(1983).

1.7 Experimental Aim

Puccinia helianthi was first recorded in Australia over a century ago. The uredinial stage of the life cycle is common and is the cause of serious yield losses. Pathogenic specialization on different commercial sunflower hybrids has been observed. The complete life cycle has only rarely been seen and has not been studied. The importance of the sexual cycle in the epidemiology of sunflower rust in Australia is therefore uncertain. The role

of the sexual cycle in the origin of new pathotypes of sunflower rust in Australia is also not known.

The purpose of the studies reported in this thesis were to investigate and describe aspects of the sexual cycle of *P. helianthi* in Australia. It was proposed to use this information to develop techniques to study the genetics of virulence of the pathogen. This knowledge should enable a better understanding of why new pathotypes of sunflower rust have been rapidly appearing in the rust population in Australia in recent years.

A second aspect of the study reported in this thesis was concerned with the effectiveness of manipulating host genes for rust resistance by creating intra-crop heterogeneity in controlling sunflower rust. The effect of this intra-crop heterogeneity on the yield of sunflower hybrids was also examined.

CHAPTER 2

FACTORS AFFECTING THE GERMINATION OF TELIOSPORES OF *PUCCINIA HELIANTHI* Schw.

2.1 INTRODUCTION

In the pleomorphic life cycles of the Uredinales, it is often the urediniophase that has attracted most research and study since it is this stage that is directly involved in the development of disease epidemics in crops. However the teliophase is also important because the teliospores represent the teleomorph of the fungus with its importance as the site of karyogamy and sexual reassortment of genes controlling virulence and other characteristics.

Teliospores are regarded as resting spores by which the obligately parasitic rust fungus can survive in the absence of its host and during adverse climatic conditions. Often this resting phase is associated with a constitutive dormancy that must be removed before teliospore germination can occur. The inability to reliably activate or induce teliospore germination has hindered studies of the genetics of pathogenicity in the rust fungi(Groth and Mogen,1978).

Many techniques for inducing teliospore germination have been reported. The following are methods that have been successfully used: (i) The natural weathering of telia-laden leaves over winter (Gold and Mengden,1983a; Lambert,1929), (ii) alternate cycles of freezing and thawing the teliospores under laboratory conditions (Lambert,1929), (iii) alternate cycles of wetting and drying the teliospores under laboratory conditions (Hooker and Yarwood,1966), (iv) a combination of freezing and thawing together with wetting and drying cycles (Statler and Zimmer,1976; Lumbrosa, Anikster, Moseman and Wahl,1977), (v) presoaking and storing wet telia-bearing material at 4°C in darkness (Anikster,1986), (vi) treating teliospores with volatile host extracts (Binder, Klisiewicz and Waiss,1977; Gold and Mengden,1983b; French, Turner, Sonnett, Pfeffer and Piotrows,1988), citric acid (Thiel and Weiss,1920) or buffers (Maneval,1927), (vii) prolonged incubation of teliospores on water or water agar (Maneval,1922; Arthur,1929; Groth and Mogen,1978) and (viii) exposure of teliospores to a heat treatment (Gold and Mengden,1983a).

Although some teliospores in the telia of *Puccinia helianthi* Schw. will germinate without a resting period most spores exhibit a dormancy (Carleton,1903; Maneval,1922; Bailey,1923). Mengden(1983) termed this phenomenon as intermediate (or nonuniform) dormancy which has also been found in the cereal rusts *Puccinia sorghi* Schw.(Hooker and Yarwood,1966) and *P.graminis* f.sp. *avenae* (Hingorani,1952).

Many of the techniques listed above have been used to induce

germination of teliospores of sunflower rust. Maneval(1922) studied the germinability of teliospores after prolonged floatation on water. The effect of the hydrogen ion concentration (pH) of the incubation media on germination was also studied. Maneval(1927) expanded these studies to examine germination of teliospores of *P.helianthi* in solutions of a range of organic and inorganic compounds. He found that weak solutions of KH_2PO_4 and $KMnO_4$ had some stimulatory effect in some experiments but the results were inconsistent. Citric acid solutions as used by Thiel and Weiss(1920) to stimulate germination of teliospores of *Puccinia graminis* f.sp. *tritici* were found to be ineffective with *P.helianthi* (Bailey,1923; Maneval,1927). Jabbar Miah and Sackston(1970) and Bailey(1923) concluded that the length of time that teliospores were stored was the critical factor in breaking dormancy. Storing telial material outdoors over winter or dry in the laboratory were equally effective in breaking dormancy. The use of wet-dry cycles by Hennessey and Sackston (1970) was successful but gave inconsistent results.

The environment in which the teliospores are produced can influence their subsequent germinability. Jabbar Miah and Sackston(1970) speculated that due to seasonal effects only teliospores produced at certain times of the year germinated. Maneval(1922) had previously concluded that teliospores produced as the season approached late winter/early spring germinated after a much shorter incubation period than those produced in the early autumn. For example, it took about 103 days for teliospores produced in October (Northern Autumn) to germinate but only 1 day for those produced in April (Northern Spring) to germinate. Flor(1942) observed that germination of teliospores of *Melampsora lini* (Ehrenb.)Desm. was best when they were produced in April (Northern Spring). Ericksson(1898) believed that only teliospores of *P. graminis* that matured in late autumn would germinate in the following spring.

Conflicting observations have been made on the role that the temperature regime under which teliospores are produced has on their germinability (Mengden,1984). Teliospores of *Puccinia hordei* Otth. germinated best when produced below 18°C (Joshi,1965). Hennessey and Sackston(1970) found that teliospores of *P.helianthi* produced on sunflower leaf discs at a constant 10°C could be induced to germinate while those produced at 20-22°C could not. In contrast, Jabbar Miah and Sackston(1970) found that teliospores of sunflower rust produced on plants grown at 27-18°C (day/night) were less dormant (as assessed by the number of wet-dry cycles needed to induce germination) than those produced at 20-15°C. Those produced at 10-7°C were more dormant.

Other factors that have been shown to influence dormancy in teliospores of *Puccinia helanthi* are the maturity of the spores and the physiologic race of the fungus. Jabbar Miah and Sackston(1970) and Hennessey and Sackston(1970) observed that the longer spores were allowed to mature on the plant the more easily dormancy could be broken. Jabbar Miah and

Sackston(1970) working with whole plants and Hennessey and Sackston(1970) with detached leaves and leaf discs found that the teliospores of Race 2 germinated most freely, Race 1 most poorly and Race 3 and 4 were intermediate.

Teliospore germination in the genera *Puccinia* and *Uromyces* is most often compared to that of *Puccinia graminis* (Petersen,1974). Under conducive conditions teliospores of *P.graminis* germinate when an unbranched metabasidium develops from one of the cells of the spore (probasidium). As the contents of the cell move into the metabasidium, septa are formed and four cells become delimited. Each cell contains a single haploid nucleus that has resulted from meiosis of the diploid nucleus of the teliospore. From each cell a peg-like sterigma is formed from which a single basidiospore forms asymmetrically at the tip. The asymmetry is thought to aid the basidiospores be forcibly discharged in the manner of ballistospores (Ingold,1971). The basidiospores then infect a suitable host to initiate the haploid thalli which produce the pycnia. This classical pattern has been shown to occur in *Puccinia malvacearum* Mont. (Allen,1933), *Puccinia lobata* Berk. and Cust. (Berkson and Britton,1969), *Uromyces fabae* (Kapooria,1971) and other urediniales. Exceptions to this system occur in species such as *Puccinia vitata* Hennen and Hodges (Gardner,1988), *P.penniseti* (Kulkarni,1958) and *P.japonica* (Kakishima, Sato and Sato, 1984) where basidiospore formation is replaced by the development of direct infection hyphae.

Teliospore germination is less sensitive to environmental conditions than the development of the metabasidium which requires more specific conditions. Arthur(1929,p292) reviewed the general abnormalities seen in teliospore germination as a result of suboptimal conditions.In general, teliospores require moisture to germinate but should not be submerged since the metabasidium will grow indefinately and does not differentiate into individual cells until in contact with air (Blackman,1903; Arthur,1929). Teliospores of *P.helianthi* germinate on media over the pH range 3.5-8.4 while the optimum pH for basidiospore formation was 4.6-6.5 (Maneval,1922). At optimal temperatures teliospore germination and basidiospore formation are maximized. Supraoptimal temperatures are associated with reduced production of sterigmata and basidiospores (Maneval,1922). For *P.helianthi* the cardinal temperatures for teliospore germination have been reported to be 20, 24 and 28°C by Fraizer(1920) and (not given), 20 and 32°C by Maneval(1922). At the higher temperatures abnormal metabasidia which were not described and fewer basidiospores formed (Maneval,1922). Bailey(1923) reported that teliospores of *P.helianthi* germinated well at temperatures between 6 to 21°C with the optimum being about 18°C. Other workers such as Gold and Mendgen(1983a) have studied the effect that incubation temperature had on the mortality of teliospores.

Teliospore germination is influenced by light especially its diurnal periodicity (Carter and Banyer,1964; Pady and Kramer,1971). The

influence of light on teliospore germination seems to be species specific (Anikster,1986). Lambert(1929) found that germination of teliospores of *Puccinia graminis* was not affected by storage in darkness or light. In contrast Sato, Katsuya and Sato(1980) found that teliospores of *Uromyces aloes* would not germinate in continuous light while Maneval(1927) reported that teliospores of *Puccinia xanthii* Schw. germinated very poorly in continuous darkness. Teliospores of *Uromyces appendiculatus* only germinated normally in an environment of alternating light and dark with the transition to darkness initiating the germination process (Gold and Mengden,1983b). Maneval(1927) found that basidiospore formation as well as germination was greatest when teliospores of *P.xanthi* and *P.helianthi* were exposed to periods of alternating light and darkness.

The pycnial and aecial stages in the macrocyclic life cycle of *Puccinia helianthi* are rarely found in the Australian environment (McAlpine,1906; Kajornchaiyakul,1974). Consequently, the importance of the sexual process in the development of new pathotypes of sunflower rust in Australia cannot be estimated. There is a paucity of thorough information dealing with the gametophytic or sexual stage in the life cycle of *P.helianthi*. The work reported in this and succeeding chapters is believed to be the first attempt to study the biology of the gametophytic stage of sunflower rust in Australia. It is contended a thorough understanding of the gametophytic stage of *Puccinia helianthi* is essential for future studies of the genetics of virulence of the pathogen.

The material contained in this chapter deals with:

i) the characterisation of normal germination of teliospores of *P.helianthi*

ii) an examination of a technique to activate or induce teliospore germination

iii) an examination of the effects of incubation media on teliospore germination

iv) an examination of the effect of light regimes on teliospore germination and the differentiation of metabasidia and

v) a study of the effects of incubation temperature on the germination of teliospores and the differentiation of metabasidia.

2.2 EXPERIMENTAL

2.2.1. Examination of germination characteristics of teliospores of *Puccinia helianthi* using light and scanning electron microscopy.

On 25 May 1987 volunteer sunflower seedlings in a mature crop of the hybrid Cargill Dynamite growing 5 km west of the township of Clifton in southeastern Queensland were found bearing pycnia of *Puccinia helianthi*. Many of the mature plants bore dried leaves which had up to 12% of their area occupied by telia of *P.helianthi*. Harvest of the mature crop had been delayed

by uneven plant maturation and by wet weather. It was suspected that the pycnial infections were derived from basidiospores originating from the germination of the teliospores in the crop.

Materials and Methods.

Leaf material bearing telia (designated accession Pht-047) was collected from the field and taken to the laboratory. At 1630h on the same day of collection three 9cm Petri dishes containing solidified 0.5% distilled water agar (WA) were each inoculated with 2ml of a teliospore suspension of Pht-047. These dishes were incubated at 18°C which was reported by Bailey(1923) to be the optimum temperature for teliospore germination. At noon on the following day the dishes were collected. They were sprayed with 0.1% lactophenol trypan blue to fix and stain the germination structures and were examined microscopically. A Badger brand air-brush connected to a small air-compressor was used to apply an atomized spray of stain.

For Scanning Electron Microscopy (SEM) leaf material bearing telia of collection Pht-047 was placed on wet filter paper in a Petri dish and incubated at 18±2°C. Periodic examination under a dissecting microscope revealed those telia containing germinating teliospores. These telia were removed and prepared for SEM examination using the method given in Appendix I.

Results.

Germination had commenced and proceeded to various stages of metabasidial differentiation in 19.9% (n=1276) of cells of teliospores incubated at 18°C. Germination was observed to have commenced when the metabasidial germ-tube first emerged from either or both of the apical germpores of either cell of the teliospore (Figure 2.1a,b). Once the cytoplasmic contents of the teliospore cell had moved into the elongating germtube a septum formed to bisect the cytoplasm into roughly equal volumes. Following this a second septum formed to delimit the cytoplasm from the lumen of the germ-tube and cell (Figure 2.1c). Each subdivided cytoplasm was then bisected by another septum so that the metabasidium consisted of four cells (Figure 2.1d). From each cell a peg-like sterigma extended (Figure 2.1e). A swelling at the tip of each sterigma became the basidiospore which contained the cytoplasmic contents of the metabasidial cell (Figure 2.1f,g). Once mature, the basidiospores were liberated leaving an empty metabasidium (Figure 2.1h). Basidiospores did not form and mature in a set sequence. Examination of 105 metabasidia where it could be determined with certainty which basidiospores were developing first revealed that the first basidiospore developed on the third cell in 60% of cases, the apical cell in 27.5% of cases, the second cell in 8.6% of cases and the basal cell in 3.8% of cases (Figure 2.2).

Metabasidia produced on 0.5% water agar varied greatly in length

Figure 2.1 Stages in the development of the metabasidium by teliospores of *Puccinia helianthi* (x400)

a) Germination of apical cell of teliospore
b) Germination of basal cell of teliospore
c) Cytoplasm moved to end of metabasidium and 2 septa (s) formed
d) Metabasidium divided into four cells

(Bar = 10um)

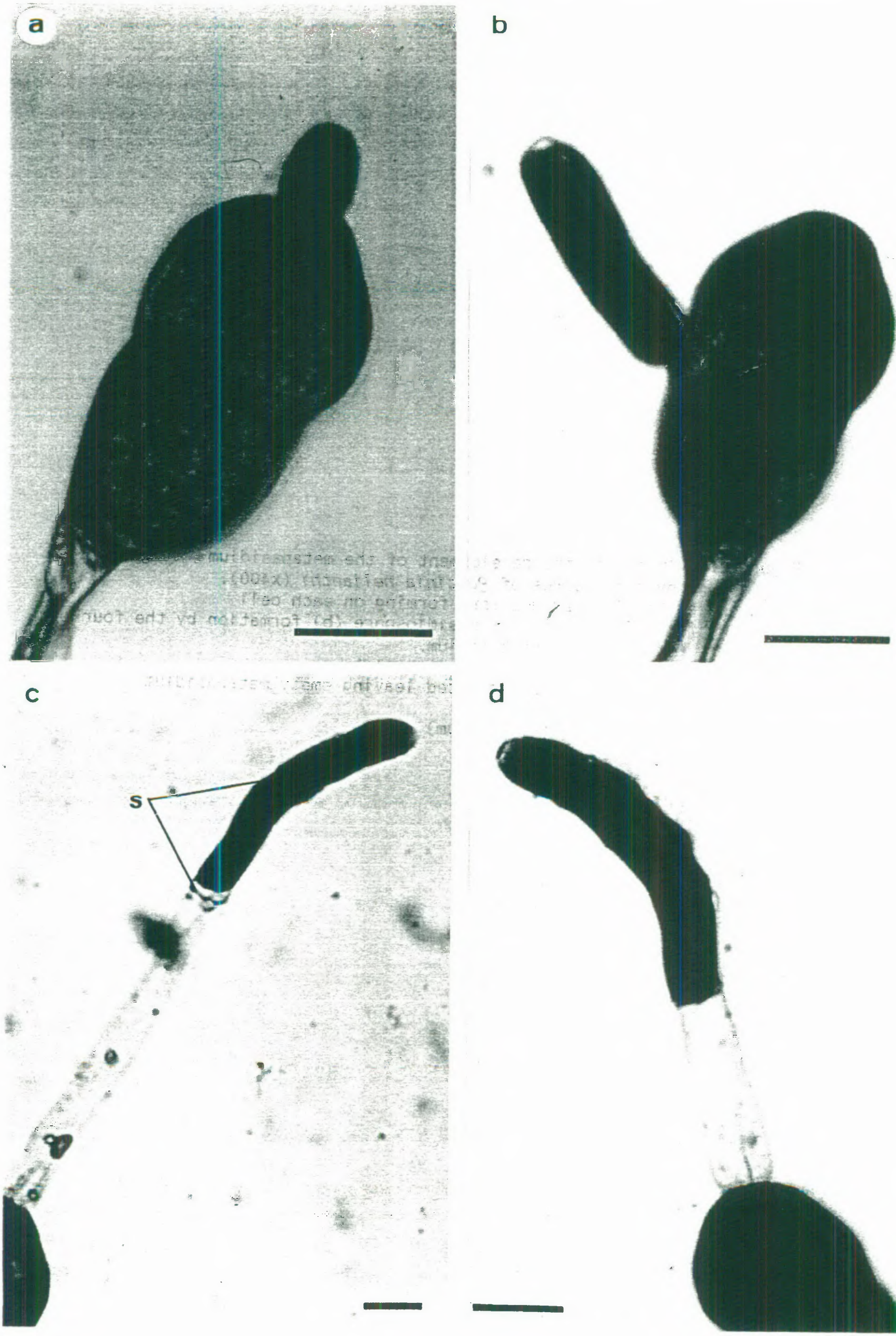
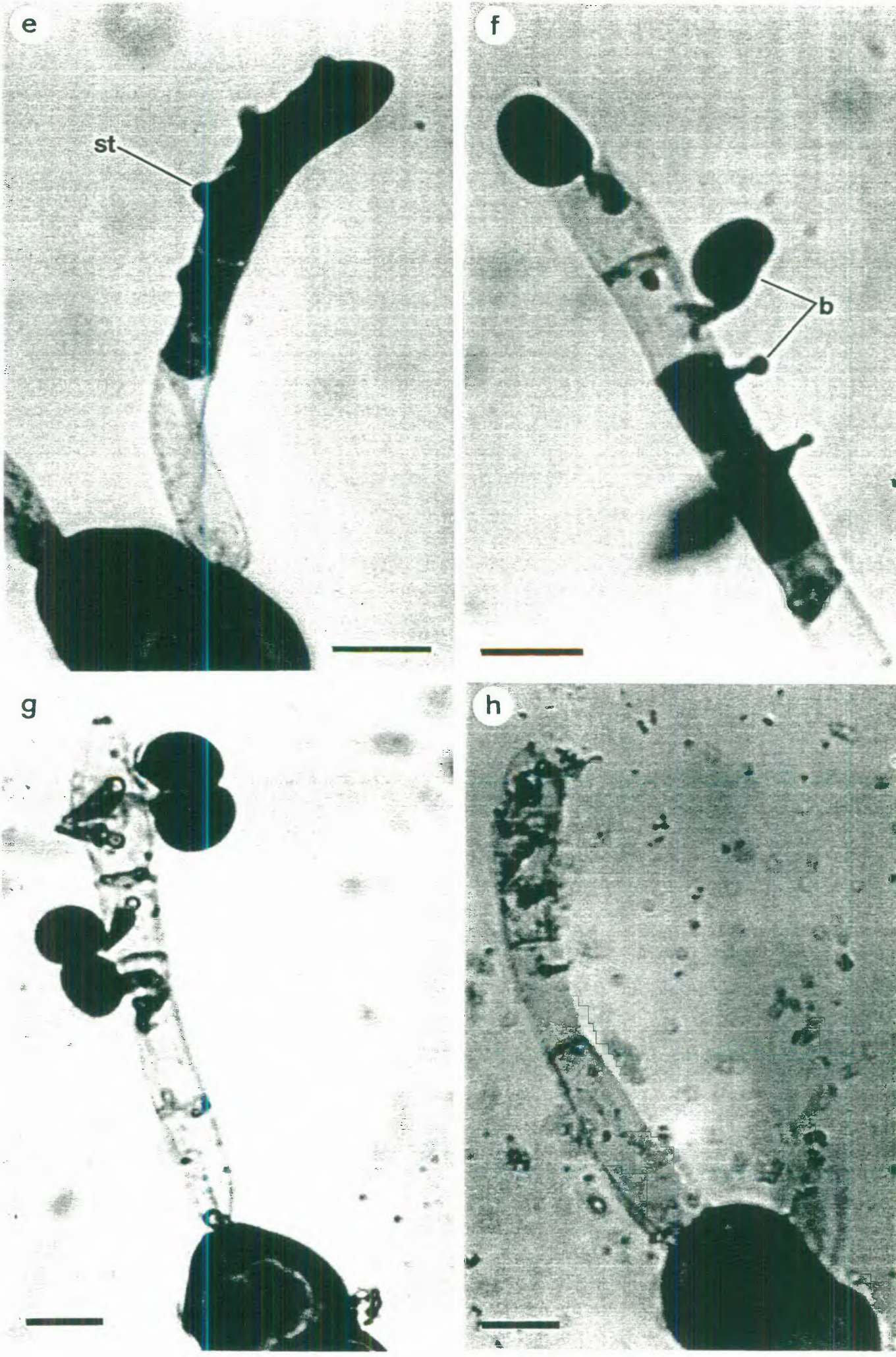

a
b
c
s
d

Figure 2.1. Stages in the development of the metabasidium by teliospores of *Puccinia helianthi* (x400).

e) Single sterigma (st) forming on each cell
f) Various stages of basidiospore (b) formation by the four cells of a metabasidium.
g) Basidiospore formation complete
h) Basidiospores liberated leaving empty metabasidium

(Bar = 10um)


e
st
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but were of a consistent diameter in the range 5.0-(7.0)-9.5 um. The length of sterigmata also varied with the longest still bearing a basidiospore being 8um long. Mature basidiospores were 10.2-(12.4)-15.3um x 8.2-(8.8)-10.2um in dimension, and with a distinct apiculus. Basidiospore walls were too thin to accurately measure using light microscopy.

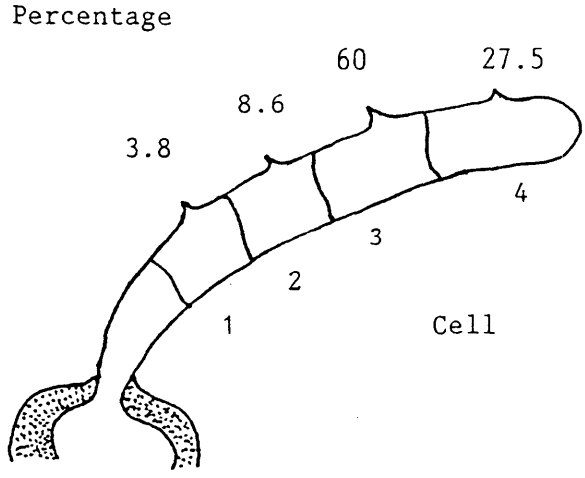


Figure 2.2 Relative frequency of first basidiospore formation for each of the four cells of the metabasidium.

Figures 2.3a-h show various stages of teliospore germination as seen by scanning electron microscopy. Figure 2.3b shows that not all teliospores in a telium neccessarily germinated synchronously. Figure 2.3f shows the swelling at the tip of the sterigma that will become the basidiospore and Figure 2.3g shows the asymmetric placement of the maturing basidiospore on the sterigma.

2.2.2 Effect of storage time and pre-germination conditioning on the germination of teliospores of *Puccinia helianthi*.

Collection Pht-047 of *P. helianthi* was exceptional in that a high percentage of teliospores germinated without the application of any artificial stimulation. Other field collections studied failed to germinate as readily. Preliminary experiments where freeze-thaw and wet-dry cycling were used to break dormancy gave inconsistent results. A modification of the pre-germination (preconditioning) procedure used by Anikster(1983,1986) for other species of the Uredinales was found to provide more consistent results and was used in this study. The preconditioning technique consisted of presoaking and storing wet telia-bearing material at 3-5^{0}C in darkness. This treatment will be referred to as the cold soak preconditioning treatment in this thesis. This experiment was designed to study the effect of storage time and preconditioning treatment on teliospore germination.

Figure 2.3 Scanning Electron Micrographs of metabasidial formation by teliospores of *Puccinia helianthi.*

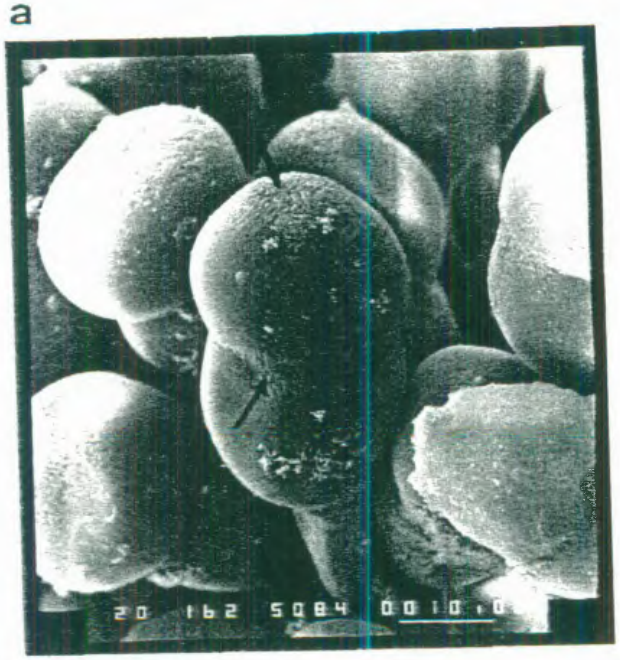
a) Teliospores showing germ-pore regions (arrowed)
b) Telium with germinating teliospores (Note: not all germinating)
c) Telium with germinating teliospores (Note: majority germinating)
d) Metabasidia emerged and curving

Micrograph Key:

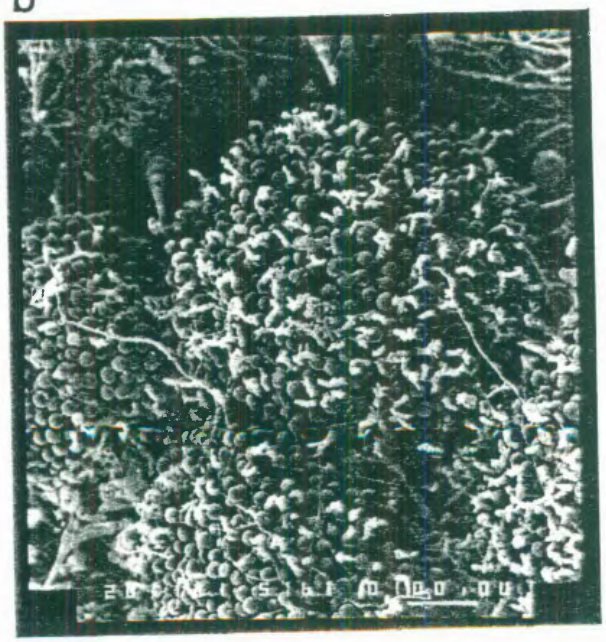
Example 2.3a 20 162 5084 0010.ou

20 - accelerating voltage
162 - magnification i.e. $16 \times 10^2 = 1600$
5084 - photograph number
0010.ou - length of bar=10 um

a



b

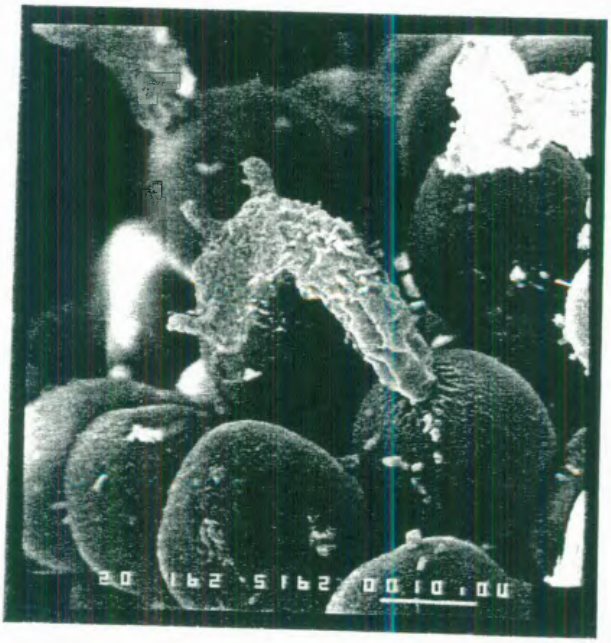


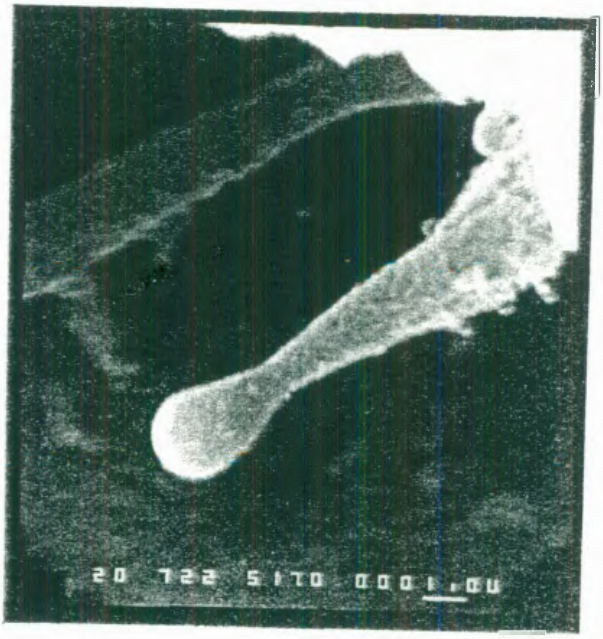
c

d

Figure 2.3 Scanning Electron Micrographs of metabasidial formation by teliospores of *Puccinia helianthi*

e) Sterigmata forming (arrowed)
f) Swelling of tip of sterigma which will become a basidiospore
g) Asymmetric placement of basidiospore on sterigma
h) Basidiospores





g

Materials and Methods.

Leaves containing telia that were collected during the period March 1982 to Febuary 1988 were airdried for 48h after collection and then stored in brown paper bags in a domestic refrigerator at 3-5°C. On 12 March 1988 twenty dry telia were taken from fifteen individual collections. The telia from each collection were squashed and teased on a watchglass to release and mix individual teliospores. The teliospores from each collection were then divided into two parts. One part was placed in a dry empty 6cm diameter Petri dish and placed in the refrigerator at 3-5°C. An aqueous suspension using distilled water at room temperature was prepared with the second part. The suspension was used to inoculate 0.5% water agar contained in 6cm diameter Petri dishes. After excess moisture had evaporated the Petri dishes were sealed with Parafilm™ sealing film to prevent drying of the agar and placed in the same refrigerator as the dry teliospores.

On the 26 March 1988 (14 days storage) aqueous suspensions were prepared with the teliospores that had been stored dry in Petri dishes in the refrigerator. These suspensions were then used to inoculate 0.5% water agar in 6cm diameter Petri dishes. The dishes of inoculated water agar which had been stored for 14d were removed from the refrigerator. All dishes were then incubated at 18°C and exposed to 12h photoperiod at 1500lx.

The number of teliospore cells that had germinated in a sample of at least 200 teliospores ie 400 cells was assessed after 48h incubation at 18°C. Those collections which did not attain 25% germination of cells of preconditioned teliospores after 48h were re-examined after 96h incubation.

Results.

From Table 2.1 it can be seen that 5 general responses to the preconditioning treatment and extended incubation could be distinguished among the different isolates of *P. helianthi*. These included; (1) no germination after extended incubation and preconditioning, considered dead e.g. Pht-034, (2) a marked response to preconditioning which was expressed as increased germination with either a) no great response to extended incubation eg. Pht-14, Pht-36, Pht-37 or b) a further response to extended incubation to a level attained by reconditioning eg. Pht-27, (3) no response to preconditioning eg. Pht-21.

There was a varied response to duration of storage. Generally teliospores showed high levels of longevity. High germination percentages were recorded for Pht-3 (6 years storage), Pht-12 (4 years), Pht-13 (3.75 years), Pht-14 (3 years), Pht-15 (3 years) and Pht-18 (3 years). Collections Pht-36 and Pht-37 which had only been in storage for 1 month showed relatively low germinations (Table 2.1).

Table 2.1 Effect of preconditioning teliospores on water agar at 4°C for 14 days on germination of teliospores held in storage.

Teliospore Collection Accession	Length of Storage (months)	Teliospore Cell Germination (%) Control[1] Incubation Time 48h	Control[1] Incubation Time 96h	Preconditioned Incubation Time 48h	Preconditioned Incubation Time 96h
Pht-3	72	4.2	7.7	53.6	___
Pht-4	70	0.5	3.1	25.7	___
Pht-8	58	18.1	18.0	23.3	27.5
Pht-10	58	0.6	4.4	12.4	19.6
Pht-12	46	18.8	___	53.0	___
Pht-34[2]	41	0.0	0.0	0.0	0.0
Pht-13	39	9.3	___	53.4	___
Pht-14	37	5.4	6.4	63.1	___
Pht-15	36	21.8	___	46.2	___
Pht-18	34	20.6	___	43.4	___
Pht-21	15	37.8	___	37.6	___
Pht-24	12	24.2	___	37.8	___
Pht-27	12	13.9	20.6	21.6	22.0
Pht-36	1	1.0	1.8	9.4	14.4
Pht-37	1	3.9	4.3	21.6	29.9

1. Control-teliospores stored dry in darkness at 3-5°C until incubation.
2. Pht-34 glasshouse derived culture collected October,1984 stored at room temperature until June,1987 then introduced to cool storage. Considered to be 100% mortality of teliospores.

2.2.3 Comparison of storage methods and length of storage during a preconditioning period on subsequent germination of teliospores.

The work reported in the preceding section showed that the germination of teliospores was enhanced when the spores were placed on water agar and stored at 3-5°C for 14d before being incubated at 18°C. The experiments reported in this section were made to compare the germination of teliospores that were stored *in situ* in telia on wet leaf material with those that had been stored after having been inoculated onto water agar. The ability of teliospores to germinate after having been taken from leaves that had been left in the field was also examined.

Materials and Methods.

Leaf material bearing telia was collected on 5 January, 1989 from the sunflower hybrid Cargill Dynamite growing at Toowoomba. The plants were approximately 4 weeks post-anthesis (Growth Stage R9, Schneiter and Miller, 1981). One leaf was taken at random from the upper third of each of ten plants. These leaves had not yet begun to show visible signs of senescence.

The teliospores to be stored on water agar were collected by using a scalpel blade to scrape teliospores from more than 20 telia on each leaf. An aqueous suspension which was made with these spores was filtered through a 250um aperature mesh to remove leaf trash and clumps of teliospores and then placed on 0.5% water agar contained in 6cm diameter Petri dishes. Three replicate batches of the inoculated dishes were stored in an inverted position in a sealed plastic container in a refrigerator kept at about 4°C for periods of 0 to 89 days. To maintain humidity and prevent desiccation of the water agar the container was lined with wet paper towelling. Germination tests were conducted on samples of three dishes removed at irregular intervals. The dishes were incubated at 18°C and exposed to a 12h photoperiod at 1500lx for 24h or 48h. The number of germinated teliospore cells was assessed in a sample of 100 teliospores in each dish.

The teliospores to be stored on leaf material were prepared by cutting squares (5cm sides) from five of the collected leaves. These squares were then washed briefly in tap water, and while still wet, placed in 9cm diameter Petri dishes which were lined with wet filter paper discs. A sample of teliospores from the leaves was taken so that an initial germination test could be performed. The dishes were sealed with Parafilm™ sealing film and then placed in the same refrigerator as the teliospores that had been inoculated onto the water agar plates. Germination tests were conducted after irregular intervals of storage. To test the germinability of the teliospores an aqueous suspension was prepared by mixing teliospores from ten telia taken from each of the 5 dishes. The suspension was used to inoculate 0.5% water agar in five 6cm Petri dishes. These dishes were then incubated at 18°C and exposed to a 12h photoperiod at 1500lx for 24h or 48h. The number of germinated cells was assessed in a sample of 100 teliospores in each dish.

Teliospores exposed to field conditions were collected from dried leaves bearing telia on 1 Febuary 1989. These leaves came from the upper third of the sunflower plants remaining in the field. This was about 8 weeks post-anthesis and the plants had reached harvest maturity. These leaves were stored in a brown paper packet in the same refrigerator as the other material. Aqueous suspensions were made at irregular intervals from 50 telia taken from the dry leaf material. The suspensions were used to inoculate 0.5% water agar in five 6cm Petri dishes. The dishes were then incubated at 18°C and exposed to a 12h photoperiod at 1500lx for 24h or 48h. The number of germinated cells was assessed in a sample of 100 teliospores in each dish.

Results.

From Table 2.2 it can be seen the germinability of teliospores stored on water agar increased with length of preconditioning storage. The relationship between storage time on water agar at 3°C and germination had a strong positive linear correlation ($r^2 = 0.98^{**}$) (Figure 2.4).

Table 2.2. Effect of preconditioning storage techniques on germination of teliospores of *Puccinia helianthi*

			Teliospore Cells Germinated (%)					
Sample	Date	Days in Storage	Storage on Water Agar		Storage on Wet Leaf		Field Collection	
			Incubation		Incubation		Incubation	
			24h	48h	24h	48h	24h	48h
0	5 Jan,89	0	0.33	___	0.5	___		
1	9 Jan,89	4	1.00	___	___	___	Field	
2	13 Jan,89	8	3.00	___	___	___	Exposure	
3	17 Jan,89	12	3.67	___	___	___		
4	21 Jan,89	16	5.00	___	2.6	___		
5	1 Feb,89	26	7.33	___	6.0	20.3	20.2	___
6	8 Feb,89	33	12.33	___	___	___	___	___
7	22 Feb,89	47	15.33	22.0	15.7	35.7	20.3	23.0
8	8 Mar,89	61	25.0	44.0	___	___	31.8	37.0
9	22 Mar,89	75	29.7	41.3	21.7	59.7	26.3	41.3
10	5 Apr,89	89	38.3	___	54.3	___	___	___

Analytic comparison between the storage techniques was not applied since ~~the experiment involved~~ the samples of teliospores used in each technique could not be directly compared. The germinability of teliospores left *in situ* on leaf material or removed and stored on water agar were similar (Table 2.2). Initially the teliospores that were exposed on dry leaf material in the field had a higher germination than spores that were preconditioned on water agar. For example, teliospores of Sample 5 which had been stored for 26 days on water agar had a germination of 7.3% while the teliospores exposed in the field had a germination of 20.2%. However, germination of teliospores preconditioned on water agar for 61 days (Sample 8) was greater than for the field matured spores which by then had been in cold storage in the refrigerator for 35 days.

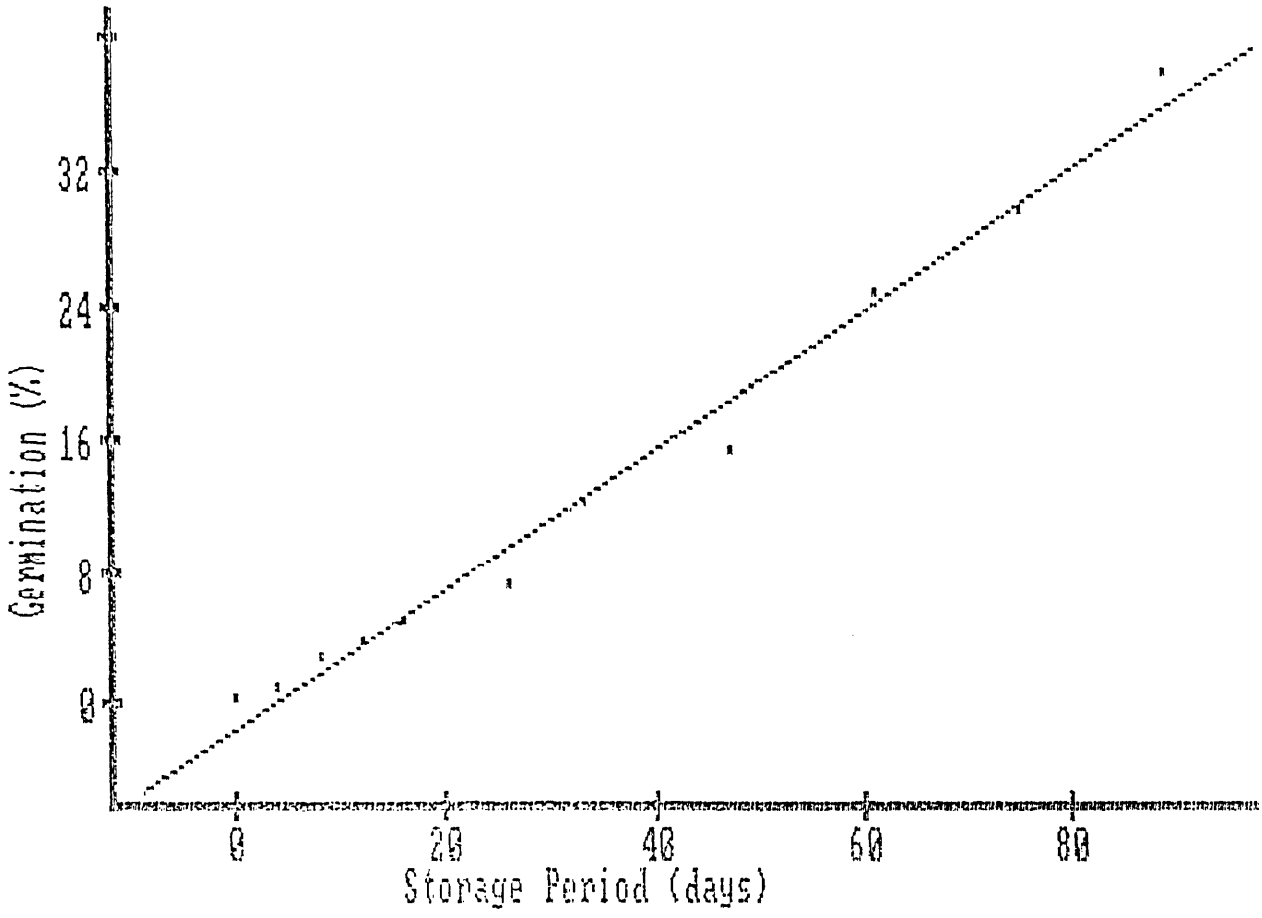


Figure 2.4 Effect of length of storage on 0.5% water agar at 3-5°C on the subsequent germination of teliospore cells incubated at 18°C. (Regression line: Y= -1.33 + 0.406X)

2.2.4 Effect of agar concentration in the medium on germination of teliospores of *Puccinia helianthi*.

Early workers (Maneval,1922; Lambert,1929) generally floated teliospores on water to test their germinability. More recent researchers have favoured the use of 0.2% water agar (Gold and Mengden,1983; Anikster,1988) or 3.5% water agar (Anikster,1983) to study teliospore germination. Dilute agar (0.5% water agar) was used in the preliminary work reported in this chapter since this concentration was found to be optimal for germination of urediniospores of *Puccinia helianthi* (author, unpublished data). In the experiments reported in this and later chapters the use of solid media was desirable. A solid media allowed ease of handling especially when teliospores were to be suspended over plants for basidiospore infection experiments. The experiments reported in this section were designed to determine the optimum agar concentration in water agar for germination of teliospores of *Puccinia helianthi*.

Materials and Methods.

Five concentrations of water agar (Difco Bacto Agar) were initially prepared; 0.25, 0.5, 1.0, 2.0 and 4.0% (weight/volume). All were in the pH range of 5.0-5.5. Ten millilitre aliquots of molten agar were dispensed into 6cm plastic Petri dishes. When the agar had set the dishes were sprayed with an aqueous suspension of teliospores (45,000/ml) of Pht-047 by use of an air-brush. The dishes were then exposed to the air-stream in a laminar flow hood until excess moisture had evaporated from the agar surface. Four dishes of each concentration were then inverted and placed in each of three chambers of a multiple temperature incubator (11.5,16.5 and 22.0°C) which also contained open containers of water for maintaining a saturated atmosphere. The lowest concentration used (0.25%) was discarded because it could not be easily handled during microscopy. The results for this treatment are therefore excluded. The experiment was conducted twice. This gave a multifactorial experiment with 4 agar concentrations x 3 temperatures x 4 replicates x 2 repetitions

The dishes were incubated for 24h (12h light at 1000lx / 12h dark) before being removed from the incubator and sprayed with 0.1% lactophenol trypan blue to fix and stain the germination structures for light microscopy. The germination percentage of teliospore cells and stage of development of the metabasidia were determined for the cells of 200 teliospores in each dish.

Results.

Analysis of the data for total germination revealed no significant differences (P>0.05) between the agar concentrations each time the experiment was conducted (Table 2.3). Temperature did have a significant influence on

Table 2.3 Effect of agar concentration and temperature on germination of teliospore cells of *Puccinia helianthi*.[1]

	Agar Concentration (% w/v)				
Temperature(C)	0.5[2]	1.0	2.0	4.0	Means
11.5	16.5*a*	17.0*a*	15.5*a*	22.0*a*	17.8
16.5	54.5*a*	48.8*a*	58.8*a*	49.3*a*	52.8
22.0	59.5*a*	55.5*a*	56.3*a*	49.5*a*	55.2
MEANS	43.5	40.5	43.5	40.3	

1. Percentage of total cells assessed (n=1600) i.e. two times the number of spores.
2. Means in each row were not significantly different (P>0.05)

germination ($P<0.05$) but a temperature x agar concentration interaction was absent since the effect of temperature was consistent at each agar concentration (Table 2.3).

Microscopic examination revealed the presence of many abnormal metabasidia. The following aberrant forms of metabasidia were observed:
i) indeterminate growth of the germ-tube (Figure 2.5a) where the germ-tube failed to differentiate into a metabasidium and continued to elongate and became vacuolate. These were most frequent at the low agar concentrations.
ii) branching of the metabasidium (Figure 2.5b).
iii) branching without septation (Figure 2.5c).
iv) normal septation with terminal cell producing an apical germ-tube (Figure 2.5d).
v) normal septation with elongate (Figure 2.5e) or malformed sterigmata (Figure 2.5f).
vi) normal septation, branched sterigmata with or without basidiospores (Figure 2.5g).
vii) separation of metabasidium at septa (Figure 2.5h).

Aberrations (ii-vii) were most common at 22.0°C and increased in frequency at the higher agar concentrations. Since the formation of aberrant sterigmata occurred at low frequency on 0.5% water agar this concentration was selected for subsequent experiments reported in this thesis.

In subsequent experiments examining the effect of environmental factors on teliospore germination and metabasidial differentation the following classes were used:
1) Non-germinated - no evidence of teliospore germination
2) Germination - primary germ-tube emerged and clearly distinguishable
3) Indeterminate germination - the germ-tube failed to differentiate into a metabasidium
4) Septation - at least one discernable septum formed but no sterigmata evident
5) Sterigmata - at least one discernable sterigma had formed
6) Aberrant sterigmata - sterigmata elongated or branched without forming basidiospores
7) Basidiospores - at least one distinct swelling at tip of sterigmata indicating formation of a basidiospore
8) Complete - all basidiospores formed and liberated

2.2.5. Effect of light on the germination of teliospores and differentiation of metabasidia of *Puccinia helianthi*.

Once teliospores have been conditioned to germinate the initiation and completion of the germination processes are influenced by environmental

Figure 2.5. Aberrant forms of metabasidia of *Puccinia helianthi*.
a) Indeterminate elongation of metabasidium (x100)
b) Branching of metabasidium (x400)
c) Branching without septation
d) Apical germ-tube from terminal cell

(Bar = 10um)

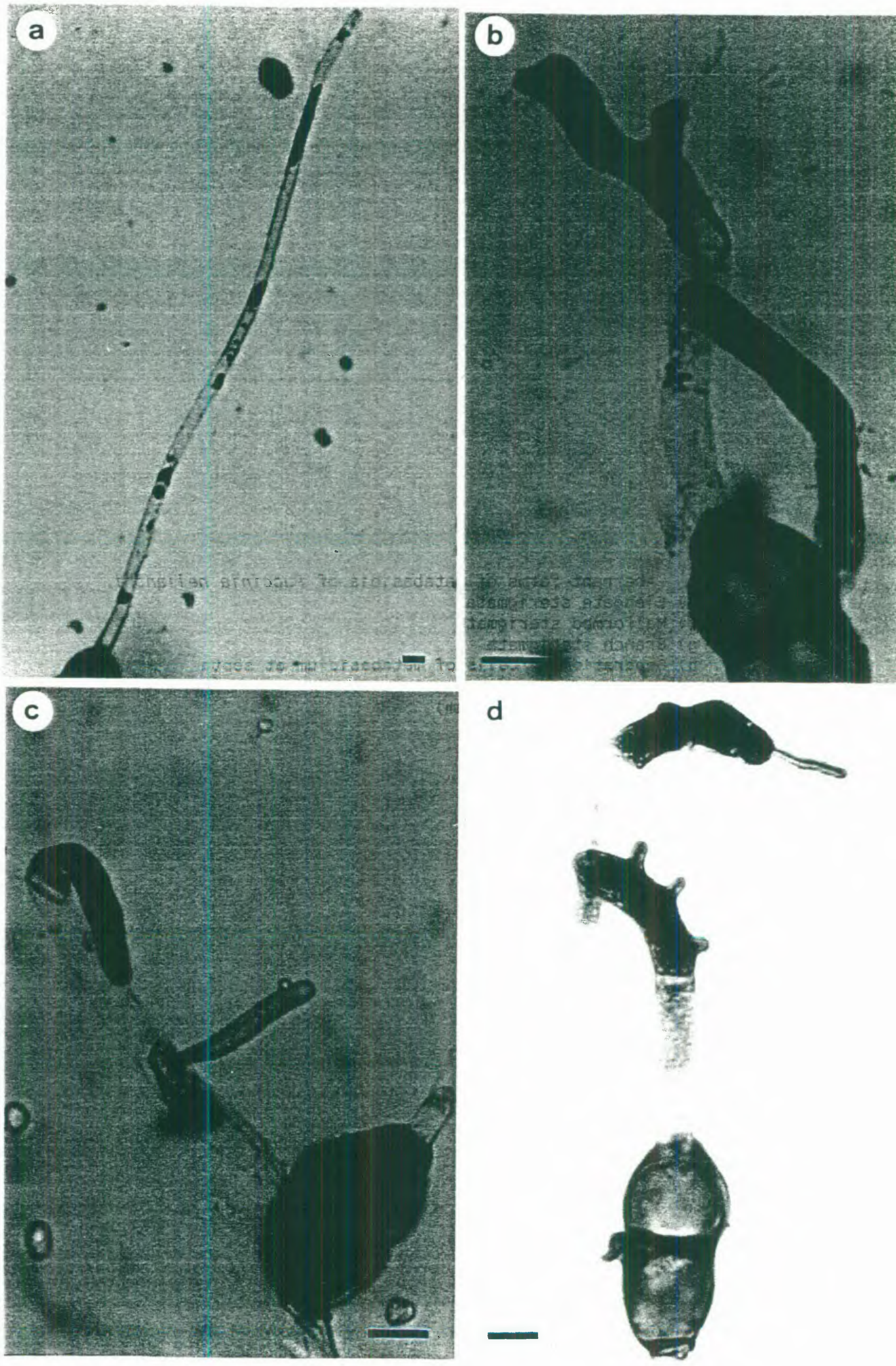
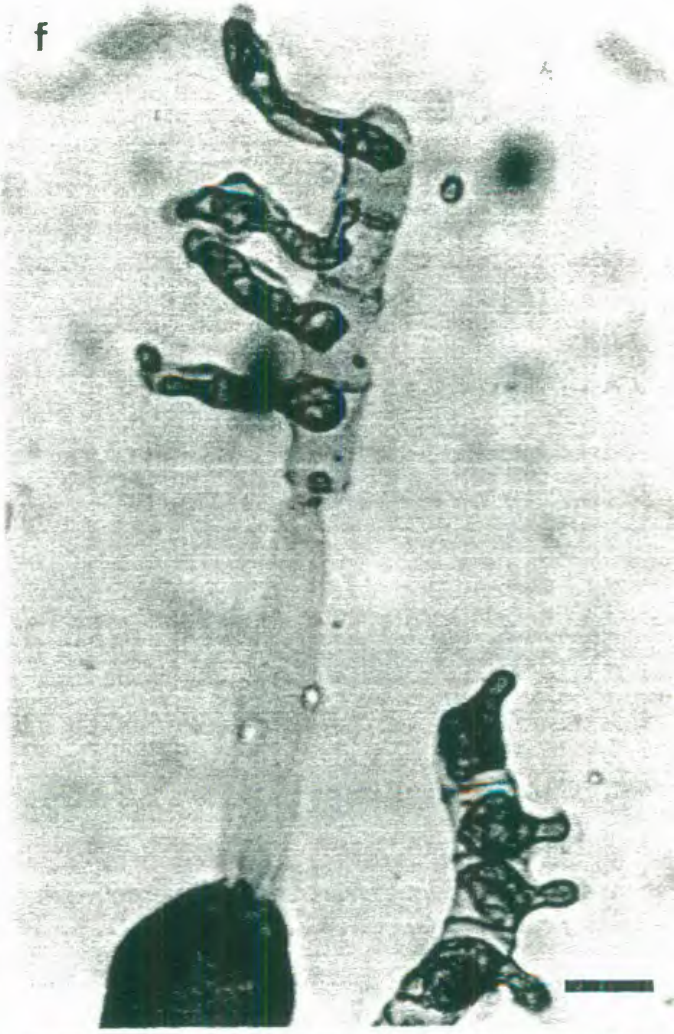

a
b
c
d

Figure 2.5. Aberrant forms of metabasidia of *Puccinia helianthi.*
e) Elongate sterigmata
f) Malformed sterigmata
g) Branch sterigmata
h) Separation of cells of metabasidium at septa

(Bar = 10um)


e


f


g


h

factors such as moisture, light and temperature. Maneval(1927) stated that exposure to light increased the proportion of teliospores of *P.helianthi* that successfully produced basidiospores. However, he gave no quantitative data to support this observation. The experiment reported in this section was made to examine the effect of an initial photoperiod on teliospore germination. It was assumed that any response of germination was only conditioned once the teliospores were rehydrated.

Materials and Methods.

The experiment was repeated twice at weekly intervals during May 1988 using teliospores that had been stored dry on leaf material for 12 months at 4°C. A suspension of teliospores was prepared by mixing telia taken from leaf material in Pegasol 3440 Special Light mineral oil (Mobil Oil Company). The teliospore suspension was sprayed onto 0.5% water agar contained in one hundred and sixty-two 6cm diameter Petri dishes under the light of a red-orange photographic safelight (<5 lx in the vicinity of the dishes). Immediately after inoculation of the dishes ~~was completed~~ the safelight was extinguished so the mineral oil could evaporate and allow the teliospores to commence hydration in darkness.

After ten minutes the dishes were closed and a random sample of 27 were wrapped individually with two layers of aluminium foil to exclude all light. These provided the total darkness treatment. All 162 dishes were inverted and randomly arranged 60cm below a bank of 4x36 W cool white fluorescent tubes which gave a light intensity of 1100 lx at the agar surface (teliospore level). The temperature was 17.5 $\pm$ 0.5°C for the duration of the experiment.

Six photoperiod treatments were used. Total darkness, continuous light and exposure to 2, 4, 8 or 12h light before imposition of darkness. At the completion of each photoperiod treatment (2,4,8 and 12h duration) the dishes for that treatment were wrapped in aluminium foil. Three replicate dishes from each treatment were taken at 2,4,6,8,12,14,16 and 24h after the commencement of incubation at 17.5°C and were sprayed with 0.1% lactophenol trypan blue to fix and stain the germination structures. The percentage germination of teliospore cells was assessed for 150 teliospores in each replicate dish at each sample time.

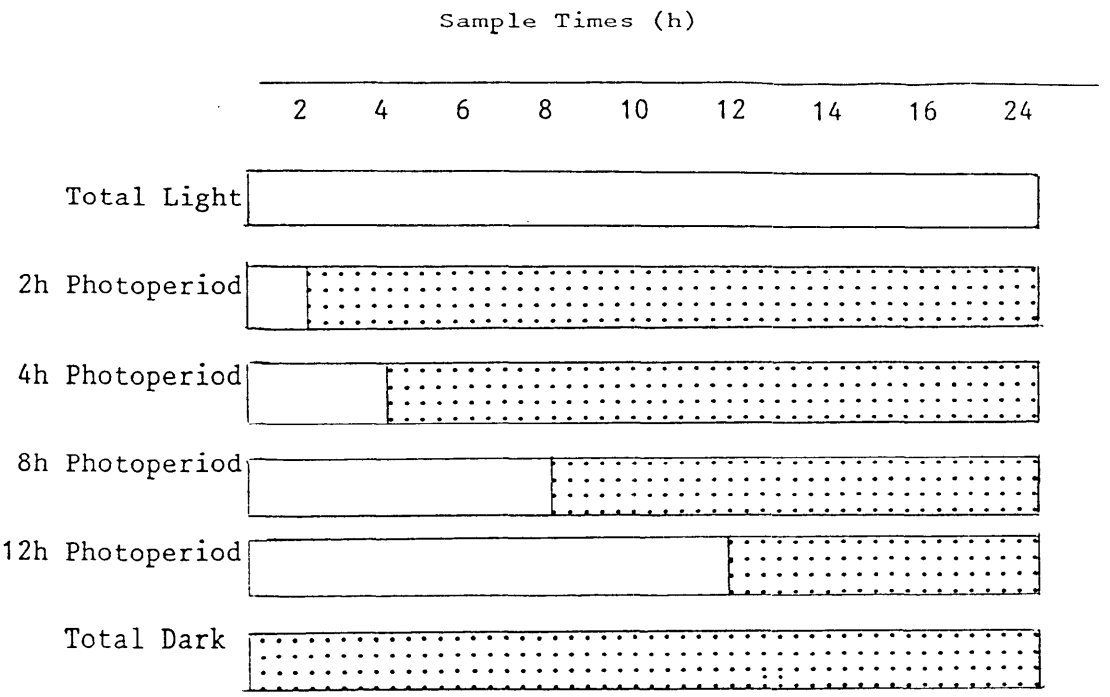


Figure 2.6 Diagram of photoperiod treatments subjected to teliospores. Open area represents period of light (1100 lux) and shaded area period of total darkness

Results.

The results are shown in Table 2.4. After 24h incubation it was found that teliospores exposed to the short photoperiods (0,2h, and 4h) showed significantly more ($P<0.5$) indeterminate germ-tubes than those subjected to other light treatments. Abnormal sterigmata were not influenced by the photoperiod treatments tested (Table 2.4). Exposure to longer photoperiods (4h and longer) resulted in more teliospores forming metabasidia and completing basidiospore formation. Germination of teliospores was first evident after 4h incubation and continued to some extent throughout the 24h period of the experiment in all treatments.

The results of total teliospore cell germination after 24h incubation under each photoperiod treatment showed that greatest germination occurred after exposure to the 8h and 12h photoperiods. Exposure to total darkness was least favourable for germination while the 2h, 4h and total light photoperiod treatments gave intermediate results. Some stimulation of germination occurred and peaked around 8h following the switch from a photoperiod of sufficient duration to darkness. For example, germination in the 4h photoperiod treatment was the highest of all treatments at the 12h sample. However, in later samples it was no greater than the longer photoperiod treatments. In the 8h photoperiod treatment germination was significantly greatest ($P<0.05$) at the 16h sample but was not significantly better ($P>0.05$) than the 12h photoperiod treatment after 24h incubation.

Table 2.4 The effect of light on germination of teliospores and differentiation of metabasidia of *P. helianthi*

Time After Inoculation (h)	Photoperiod Treatment (h)	Germination Deter-minate	Germination Indeter-minate	Septation	Sterigma Normal	Sterigma Aberrant	Basidiospore Formation	Germination Complete	Total
4	0	0.1[1]	—	—	—	—	—	—	0.1
	2	0.1	—	—	—	—	—	—	0.1
	4	0.1	—	—	—	—	—	—	0.1
	8	0.0	—	—	—	—	—	—	0.0
	12	0.1	—	—	—	—	—	—	0.1
	24	0.0	—	—	—	—	—	—	0.0
6	0	1.5	0.15	0.15	—	—	—	—	1.8 *b*
	2	2.8	0.0	0.5	—	—	—	—	3.3*a*
	4	2.4	0.0	0.5	0.1	—	—	—	3.1*a*
	8	2.7	0.0	0.3	—	—	—	—	3.0*ab*
	12	2.6	0.0	0.5	—	—	—	—	3.1*ab*
	24	2.3	0.0	0.7	—	—	—	—	3.0*ab*
8	0	5.7	1.2*a*[2]	0.65	0.15	—	—	—	7.7*a*
	2	6.0	0.6*ab*	1.4	—	—	—	—	8.0*a*
	4	6.8	0.5*ab*	2.3	—	—	0.7	—	10.2*a*
	8	5.8	0.7*ab*	1.3	0.3	—	0.2	0.2	8.5*a*
	12	4.5	0.2 *b*	3.1	0.3	—	0.1	0.1	8.3*a*
	24	8.5	0.1 *b*	2.3	0.3	0.2	0.9	0.0	12.3*a*
10	0	9.0	2.15*a*	2.0	0.5	0.0	0.65	1.0*a*	15.3*a*
	2	11.0	2.2*a*	3.7	0.5	0.1	1.3	0.5*a*	19.3*a*
	4	13.7	0.1*a*	5.3	1.3	0.0	1.3	1.1*a*	22.8*a*
	8	8.3	1.6*a*	6.0	0.7	0.0	2.7	1.5*a*	20.8*a*
	12	11.8	0.7*a*	6.1	0.8	0.1	1.3	0.5*a*	21.3*a*
	24	11.5	0.7*a*	4.0	0.6	0.0	0.6	0.4*a*	17.8*a*
12	0	12.4	5.6*a*	5.0	0.8	0.1*a*	1.5	2.3 *bc*	27.7 *b*
	2	12.0	3.5*ab*	5.5	1.8	0.1*a*	1.8	1.8 *c*	26.5 *b*
	4	14.0	4.5*a*	8.5	1.5	0.1*a*	4.4	5.7*a*	38.7*a*
	8	8.9	3.1*ab*	7.4	1.6	0.3*a*	5.9	4.0*ab*	31.2*ab*
	12	9.0	2.1 *b*	6.7	1.6	0.2*a*	5.3	3.1 *bc*	28.0 *b*
	24	7.6	1.6 *b*	6.0	1.6	0.5*a*	5.1	3.3 *bc*	26.7 *b*
14	0	6.1	6.5*a*	5.1	0.7	0.5*a*	3.4	3.5 *c*	25.8 *b*
	2	5.6	6.0*a*	5.3	1.7	0.7*ab*	4.0	5.0 *bc*	28.3 *b*
	4	9.1	6.2*a*	7.5	1.8	0.1 *b*	7.0	9.1*ab*	40.8*a*
	8	7.5	3.5 *b*	7.5	3.0	1.3*a*	11.0	8.2*ab*	42.0*a*
	12	8.9	2.4 *bc*	7.1	4.1	0.7*ab*	9.3	8.8*ab*	41.3*a*
	24	9.0	2.0 *c*	7.0	2.8	1.0*ab*	9.2	11.5*a*	42.5*a*
16	0	8.0	8.6*a*	5.5	1.2	0.7*a*	5.5	5.8 *b*	35.3 *b*
	2	9.1	7.8*a*	5.9	1.1	1.0*a*	8.5	7.9*ab*	41.3 *b*
	4	5.8	7.4*ab*	6.5	2.8	0.7*a*	6.2	11.1*ab*	40.5 *b*
	8	6.0	3.8 *bc*	9.3	3.7	1.5*a*	11.5	13.9*a*	49.7*a*
	12	5.7	2.8 *c*	6.8	2.8	1.3*a*	10.7	12.5*a*	42.3 *b*
	24	5.6	2.4 *c*	6.0	2.8	0.8*a*	12.1	12.1*a*	41.8 *b*
24	0	3.3	8.8*a*	3.5	0.6	2.5*a*	6.3	21.7 *c*	46.7 *c*
	2	2.6	7.6*a*	4.9	1.0	4.0*a*	8.2	28.5 *bc*	56.8 *b*
	4	1.5	7.3*a*	2.4	0.5	2.3*a*	8.5	31.1*abc*	53.6 *b*
	8	2.8	2.9 *b*	2.8	1.0	2.2*a*	8.9	42.7*a*	63.3*a*
	12	2.1	2.9 *b*	3.1	0.8	2.4*a*	10.6	39.8*a*	61.7*a*
	24	1.8	1.5 *b*	3.8	1.3	2.7*a*	9.5	34.4*ab*	55.0 *b*

1). Results are percentage of metabasidia in each class as measured by assessing 900 teliospores.
2). Results in each column for each sample time that are followed by at least one lower case letter in common do not differ significantly ($P<0.5$).

2.2.6 Effect of temperature on germination of teliospores and differentiation of metabasidia of *Puccinia helianthi*.

The optimum temperature for germination of teliospores of *P. helianthi* has been reported to be around 18°C (Bailey,1923), 20°C (Maneval,1922) or 24°C (Fraizer,1920). At temperatures above the optimum for germination abnormal metabasidia and fewer basidiospores formed (Maneval, 1922). The experiment reported in this section was to determine the optimum temperature for the germination of Australian collections of teliospores.

Materials and Methods.

This experiment was conducted five times using teliospores from the following collections; Pht-047 (2x), Pht-015, Pht-017 and Pht-022. Teliospore suspensions were prepared by mixing dry spores of Pht-047 or hydrated preconditioned spores of the other cultures in distilled water. Suspensions of Pht-047 were then stored for 2h at 4°C to allow the spores to rehydrate. Teliospore suspensions were then sprayed using an air-brush onto 0.5% distilled water agar that had been pre-cooled to 4°C. A relatively even distribution of 25 teliospores.mm^{-2} was achieved.

The inoculated dishes were inverted and placed in the chambers of a thermogradient plate-type incubator. Temperatures were recorded in each chamber using mini-thermistor sensors connected to a Grant Instruments Squirell Multi-Channel Data Logger. Data retrieved included minimum temperature, maximum temperature, mean temperature and standard deviation. The inoculated dishes were exposed to light at 1000lx for 4h and then darkness for the remainder of the incubation period. An red-orange photographic safelight was used to provide sufficient light to enable sample dishes to be taken during dark periods. This was done to prevent exposing the remaining dishes to high intensity light that might affect germination. The sample dishes were then sprayed with 0.1% lactophenol trypan blue and examined microscopically.

The temperatures under which the inoculated dishes were incubated were 4.4 ± 0.4, 7.6 ± 0.4, 10.4 ± 0.8, 14.4 ± 0.4, 17.6 ± 0.8, 20 ± 0.8, 22.8 ± 1.2, 25.2 ± 0.8 and 27.6 ± 0.4°C (variation is standard deviation). Germination percentage and stages of development of the metabasidia were assessed at 0,1,2,3,4,5,6,8,10,12 and 16h after exposure to the various temperatures. Each sample consisted of four dishes. Two hundred teliospores were examined in each dish. To avoid sampling errors care was taken not to assess teliospores that were grouped in clumps.

Results.

The results presented are for Pht-015 only since these results were typical and germination of this culture was greatest.After 16h no

germination was observed in teliospores that had been incubated at 4.4°C. This was the case in each of the experiments. Germination occurred at low levels at 7.6°C and increased in frequency as temperature increased to 17.6°C and then decreased at higher temperatures (Table 2.5). At 27.6°C teliospores germinated to produce stunted germ-tubes which did not differentiate into metabasidia. This was considered to be non-functional germination.

Figures 2.7 and 2.8 show the effect of temperature on the total germination of teliospore cells and on the proportion of metabasidia that successfully produced basidiospores. Temperature had a significant effect on teliospore germination ($P<0.05$). The optimum temperatures for total germination at both the 8 and 16h sample times were 17.6 and 20.0°C. New germinations were still occurring at the completion of the experiment especially at 17.6°C where 6.5% of cells had produced pre-septate metabasidia (Table 2.5).

The first basidiospores were observed after 4h incubation at 17.6°C, 5h at 14.4 and 20°C, 6h at 10.4 and 22.8°C and 16h at 7.6°C (Table 2.5). Basidiospores were not formed at 25.2°C. Successful production and liberation of all four basidiospores by the metabasidia only occurred at 10.4, 14.4, 17.6 and 20.0°C. Completion of metabasidial formation was most rapid and reached the greatest level at 17.6°C (Figure 2.8). At 22.8°C basidiospores formed on some sterigmata of some metabasidia but in all cases these metabasidia were otherwise aberrant with elongated or branched sterigmata. Aberration of metabasidial formation increased with temperature. At 25.2°C, 76% of metabasidia formed were indeterminate while 11% formed aberrant sterigmata.

2.3 DISCUSSION

Teliospores were defined by Hiratsuka(1973) as the basidia-producing spores of the rust fungi. The teliospore functions as the site of karyogamy, and serves as the probasidium. It germinates to produce the metabasidium in which meiosis occurs. The haploid basidiospores are produced from the cytoplasmic and nuclear contents of the metabasidium (Petersen,1974). The germination and differentiation of metabasidia by teliospores of *Puccinia helianthi* conform with the generalized descriptions of the structural morphology of the external metabasidia of other members of the Pucciniaceae (Buller,1924; Petersen,1974; Littlefield,1981; Mengden,1984).

In many species the teliospore also functions as a perennating structure that enables the rust fungi to survive unfavourable periods. Hence the terms 'resting','winter' and 'over-wintering' spores have been used to describe teliospores (Cummins and Hiratsuka,1983). Teliospores possess a degree of longevity and an intrinsic dormancy to prevent germination immediately on maturation. This enables them to survive drought periods or

Table 2.5. Effect of incubation temperatures on germination of teliospores and differentiation of metabasidia of *Puccinia helianthi*

a) 7.6°C

Time after Inoculation (h)	Germination Determinate	Indeterminate	Septation	Sterigmata Normal	Sterigmata Aberrant	Basidiospore Formation	Germination Complete	Total
0	—	—	—	—	—	—	—	—
1	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—
3	1.3(100)[1]	—	—	—	—	—	—	1.3
4	1.5(100)	—	—	—	—	—	—	1.5
5	2.0(100)	—	—	—	—	—	—	2.0
6	1.5 (60)	—	1.0(40)	—	—	—	—	2.5
8	2.5 (71)	—	1.0(29)	—	—	—	—	3.5
10	2.5 (45)	—	1.5(27)	1.0(18)	—	—	—	5.5
12	0.5 (8)	—	4.5(70)	1.4(22)	—	—	—	6.4
16	2.0 (25)	—	3.5(44)	2.0(25)	—	0.5 (6)	—	8.0

b) 10.4°C

Time after Inoculation (h)	Germination Determinate	Indeterminate	Septation	Sterigmata Normal	Sterigmata Aberrant	Basidiospore Formation	Germination Complete	Total
0	—	—	—	—	—	—	—	—
1	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—
3	1.5(100)	—	—	—	—	—	—	1.5
4	3.5(100)	—	—	—	—	—	—	3.5
5	6.0 (92)	—	0.5 (8)	—	—	—	—	6.5
6	9.0 (69)	—	3.0(23)	0.5 (4)	—	0.5 (4)	—	13.0
8	8.5 (55)	0.5 (3)	5.5(36)	0.5 (3)	—	0.5 (3)	—	15.5
10	15.0 (48)	2.5 (8)	7.0(22)	2.5 (8)	—	3.0 (9)	1.5 (5)	31.5
12	14.0 (43)	4.0(12)	7.5(23)	0.5 (2)	—	3.0 (9)	3.5(11)	32.5
16	0.5 (1)	4.5(13)	2.5 (7)	2.0 (5)	—	11.5(33)	13.5(39)	34.5

c) 14.4°C

Time after Inoculation (h)	Germination Determinate	Indeterminate	Septation	Sterigmata Normal	Sterigmata Aberrant	Basidiospore Formation	Germination Complete	Total
0	—	—	—	—	—	—	—	—
1	—	—	—	—	—	—	—	—
2	1.5(100)	—	—	—	—	—	—	1.5
3	5.0(100)	—	—	—	—	—	—	5.0
4	8.5 (85)	—	1.5(15)	—	—	—	—	10.0
5	10.0 (69)	1.5(11)	1.5(11)	0.5(3)	—	0.5 (3)	0.5 (3)	14.5
6	13.5 (69)	1.0 (5)	3.0(15)	1.0(5)	—	0.5 (3)	0.5 (3)	19.5
8	13.5 (44)	2.0 (6)	5.5(18)	1.5(5)	—	3.5(11)	5.0(16)	31.0
10	6.5 (17)	5.5(14)	7.5(20)	2.0(5)	—	4.5(12)	12.0(32)	38.0
12	5.5 (15)	3.5 (9)	4.5(12)	2.5(6)	—	5.5(15)	16.0(43)	37.5
16	3.0 (7)	3.5 (8)	2.5 (5)	1.5(3)	0.5(1)	5.0(11)	29.0(65)	45.0

d) 17.6°C

Time after Inoculation (h)	Germination Determinate	Indeterminate	Septation	Sterigmata Normal	Sterigmata Aberrant	Basidiospore Formation	Germination Complete	Total
0	—	—	—	—	—	—	—	—
1	—	—	—	—	—	—	—	—
2	3.5(100)	—	—	—	—	—	—	3.5
3	8.5 (94)	—	0.5 (6)	—	—	—	—	9.0
4	15.0 (81)	1.0 (5)	1.0 (5)	0.5(3)	—	0.5 (3)	0.5 (3)	18.5
5	17.0 (67)	4.0(16)	2.5(10)	0.5(2)	—	1.0 (3)	0.5 (2)	25.5
6	18.0 (56)	4.0(13)	3.0 (9)	2.0(6)	—	3.5(11)	1.5 (5)	32.0
8	13.5 (30)	5.0(11)	6.5(14)	1.5(3)	—	11.5(25)	7.5(17)	45.5
10	8.5 (16)	5.5(11)	3.5 (8)	4.0(8)	0.5(1)	12.0(25)	14.5(30)	48.5
12	4.5 (9)	7.0(14)	4.0 (8)	4.0(8)	0.5(1)	6.5(14)	22.5(46)	49.0
16	6.5 (10)	8.0(13)	2.5 (4)	1.0(2)	1.0(2)	5.5 (9)	37.0(60)	61.5

Continued:
e) 20.0°C

0	—	—	—	—	—	—	—	—
1	—	—	—	—	—	—	—	—
2	1.5 (75)	—	0.5(25)	—	—	—	—	2.0
3	8.0 (94)	—	0.5 (6)	—	—	—	—	8.5
4	15.5 (82)	1.5 (8)	2.0(10)	—	—	—	—	19.0
5	16.5 (60)	5.5(20)	4.5(16)	0.5(2)	—	0.5 (2)	—	27.5
6	21.0 (54)	8.0(20)	4.5(12)	1.5(4)	—	3.5 (9)	0.5 (1)	39.0
8	12.0 (26)	7.5(16)	8.5(18)	3.5(7)	1.5 (3)	12.0(26)	2.0 (4)	47.0
10	6.5 (12)	12.5(24)	4.5 (9)	3.0(6)	6.5(13)	10.0(19)	9.0(17)	52.0
12	4.5 (8)	13.5(25)	6.0(11)	0.5(1)	8.5(16)	10.5(20)	10.0(19)	53.5
16	3.5 (6)	11.5(20)	3.0 (5)	1.0(2)	4.5 (8)	5.5 (9)	29.5(50)	58.5

f) 22.8°C

0	—	—	—	—	—	—	—	—
1	—	—	—	—	—	—	—	—
2	1.0(100)	—	—	—	—	—	—	1.0
3	5.5 (92)	0.5 (8)	—	—	—	—	—	6.0
4	14.0 (90)	1.0 (6)	0.5 (4)	—	—	—	—	15.5
5	16.5 (69)	4.0(17)	2.0 (8)	0.5(3)	1.0 (4)	—	—	24.0
6	17.5 (56)	9.0(29)	3.0 (9)	0.5(2)	0.5 (2)	0.5 (2)	—	31.0
8	10.5 (26)	15.0(37)	6.5(16)	1.0(2)	6.5(16)	1.0 (2)	—	40.5
10	8.5 (19)	16.5(38)	6.5(15)	0.5(1)	11.5(16)	0.5 (1)	—	44.0
12	6.0 (12)	19.5(40)	5.5(11)	0.5(1)	16.5(35)	0.5 (1)	—	48.5
16	2.5 (5)	19.0(38)	5.0(10)	0.5(1)	23.0(46)	—	—	50.0

g) 25.2°C

0	—	—	—	—	—	—	—	—
1	—	—	—	—	—	—	—	—
2	0.5(100)	—	—	—	—	—	—	0.5
3	3.0(100)	—	—	—	—	—	—	3.0
4	8.0 (94)	—	0.5 (6)	—	—	—	—	8.5
5	14.5 (76)	3.5(18)	1.0 (6)	—	—	—	—	19.0
6	17.0 (79)	3.5(16)	1.0 (5)	—	—	—	—	21.5
8	19.0 (75)	5.0(19)	1.5 (6)	—	—	—	—	25.5
10	7.5 (24)	17.5(56)	3.5(11)	—	2.5 (8)	—	—	31.0
12	10.5 (30)	19.5(56)	1.0 (3)	—	4.0(11)	—	—	35.0
16	3.0 (8)	30.5(76)	2.0 (5)	—	4.5(11)	—	—	40.0

1. Numbers within parentheses are percentage of germinated teliospore cells in each class for each sample time.

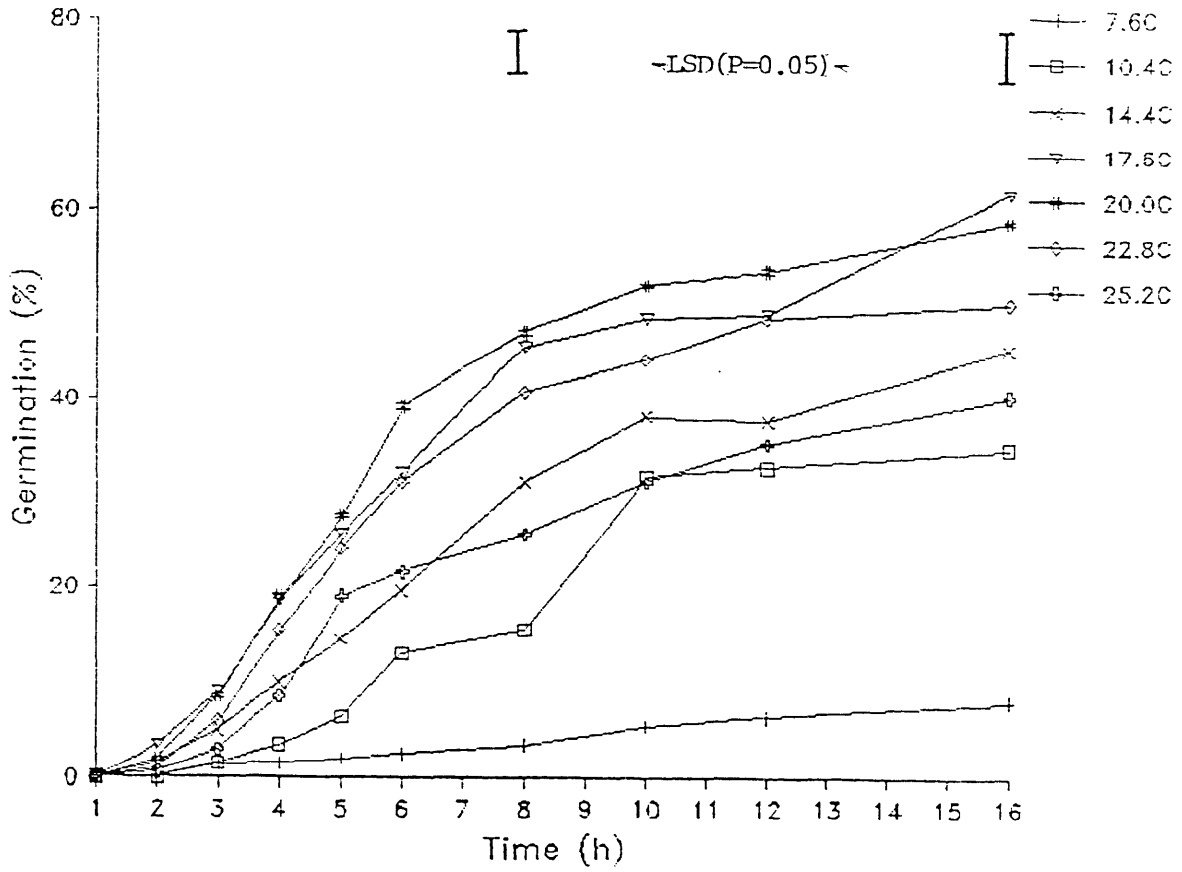


Figure 2.7 Effect of temperature on total germination of teliospores of *P.helianthi*.

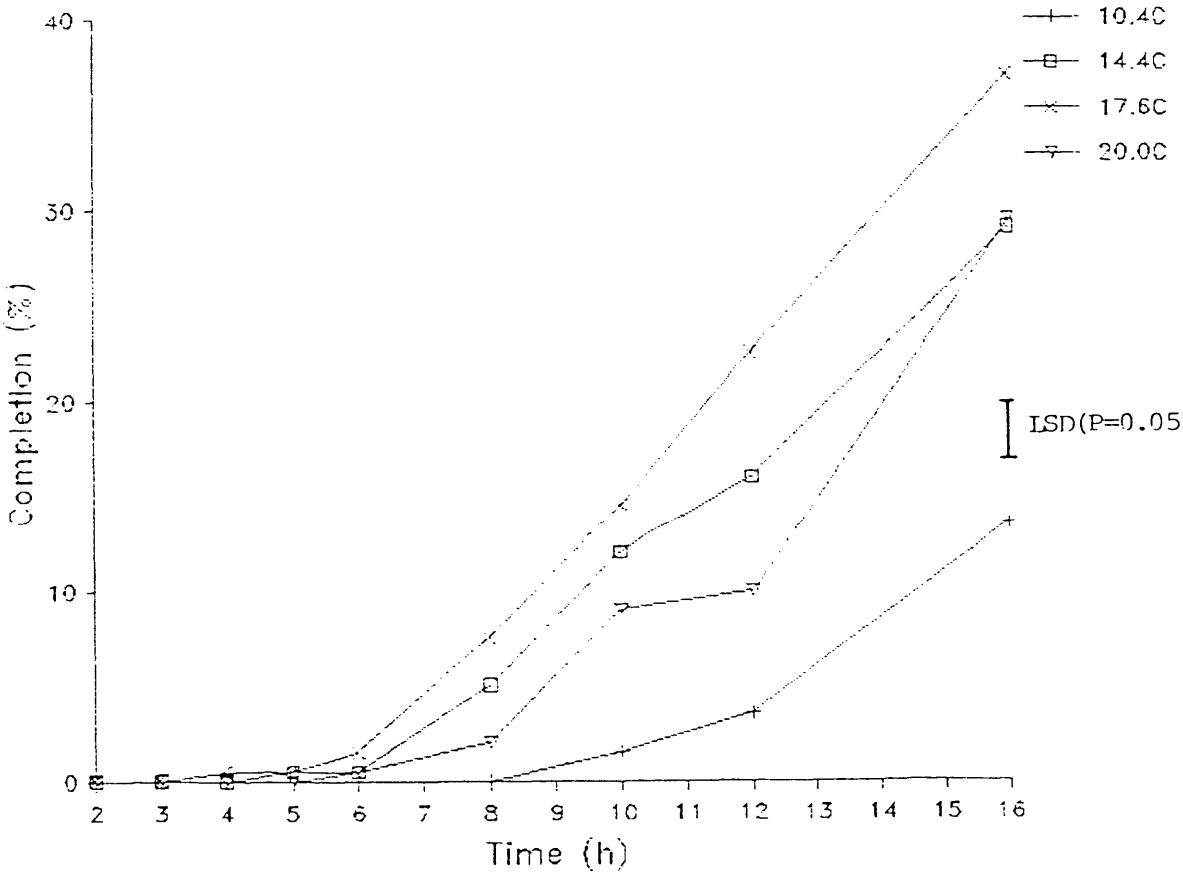


Figure 2.8 Effect of temperature on proportion of teliospores that completed liberation of basidiospores

cold winters when the hosts are absent or when conditions are unsuitable for infection by basidiospores. The thick and pigmented walls of teliospores protect them from desiccation and damage by ultraviolet radiation (Savile,1976).

Puccinia helianthi probably co-evolved with *Helianthus* spp. in North America. In the northern states of the United States and in Canada teliospore dormancy would be advantageous as a means of surviving during the cold winter environments. Teliospores of Australian isolates of *Puccinia helianthi* also possess a dormancy and are capable of surviving relatively well under controlled conditions. Teliospores of some collections that had been stored dry in paper bags at 4-5°C for 6 years showed between 50 and 60% germination. Several other collections were still viable after being stored for 2 to 5 years. This contrasts with the longevity of teliospores reported for other species of rusts. Anikster(1986) found that teliospores of *P.hordei Otth., P.recondita Rob.& Desm., P.lagenophorae*(Cooke) and *Uromyces scillarum* (Grev.) Wint. that had been stored in a similar way were incapable of germination after 3 years.

There did not seem to be an obligate necessity for teliospores of Australian isolates of *P.helianthi* to be exposed to "winter" conditions before being capable of germination. The isolate Pht-047 after being exposed to an unknown number of weeks of field conditions germinated well in May (late southern autumn) while teliospores produced in the middle of Summer and also exposed to field conditions on the host plants showed 20% germination when incubated in the laboratory in February(late summer) . Field exposure of teliospores on senescing or dead host tissue may be analogous to the wet-dry cycling procedure used in the laboratory to induce germination.

It is possible that the time of year when teliospores were produced may have influenced their subsequent dormancy (Eriksson,1898; Bailey,1923; Maneval,1927; Flor,1942). Maneval(1922) found that teliospores of *P.helianthi* that had been produced in summer and autumn and floated on water did not germinate until the following spring. In contrast to this, teliospores of Pht-063 were capable of germinating in summer. This phenomenon, if consistent for other Australian isolates of the fungus, together with the observation that telia form most frequently in late summer and autumn (94%,n=64 collections) may have implications for the biology of the fungus in Australia. In the North American winter the teliospores are prevented from germinating by the cold weather. However, in the milder Australian autumn and winters the teliospores may be continually induced to germinate. This may explain the presence of pycnia and aecia on plants of sunflower crops in autumn in Australia. The importance of these autumn aecial infections in completing the life cycle of *P.helianthi* in Australia is uncertain. It is possible that the crop may mature and die or that volunteer plants may be destroyed by cultivation before the aecia mature.

Many techniques have been used to break the dormancy in teliospores

of rust fungi (Maneval,1922; Lambert,1929; Hooker and Yarwood,1966; Statler and Zimmer,1979; Gold and Mengden,1983a). The use of wet-dry cycling to induce germination of teliospores of *P.helianthi* gave inconsistent results (Jabbar Miah and Sackston,1970; Hennessey and Sackston,1970). The technique of pre-soaking and storing wet telia-bearing material at about 4°C in darkness (Anikster,1983; Anikster,1986) has been found to work successfully with teliospores of *P.helianthi* and has the advantage of requiring little maintenance.

Induction of germination was observed not to occur equally in all teliospores of a population. The germination of teliospores preconditioned on 0.5% water agar kept at about 4°C showed a positive response to duration of preconditioning. In the cold wet preconditioning environment the teliospores became hydrated and were induced to germinate but did not because of the subminimal temperatures. The number of spores capable of germinating in the population therefore accumulates as more are induced to germinate.

Prolonged presoaking of teliospores was not recommended by Anikster(1986) since it resulted in mortality of activated spores. Observations with *P.helianthi* suggest that mortality due to prolonged presoaking is related to the degree to which the teliospores are activated to germinate (author, *unpublished data*). Populations of teliospores with a low initial frequency of germination due to dormancy could be presoaked for several months without reducing their capacity to germinate. The presoaking of populations in which many of the teliospores had already been activated for more than 2-3 weeks resulted in reduced germinability.

Teliospores that are activated to germinate may not do so immediately they are placed under optimal conditions but may exhibit a lag period before germinating. Gold and Mengden(1983a) found that teliospores of *Uromyces appendiculatus* activated to germinate by several techniques needed to be incubated for 3-5 days before germination commenced. Other published references to lag periods include 2-4 days for *Gymnosporangium juniperi-virginianae* (Pady and Cramer,1971), *Cronartium fusiforme* (Powers and Roncadori,1966) and *Uromyces viciae-fabae* (Freytag, Bruscaglioni, Gold and Mendgen,1988). Teliospores that had been activated by presoaking have a reduced lag period (Anikster,1983). A definite lag period was not observed in the teliospores of *P.helianthi* that were activated to germinate by presoaking at 3-5°C in the experiments reported in this thesis.

Following activation the degree of germination was influenced by fungal isolate used and the prevailing environmental conditions. The time course studies reported in this thesis showed that teliospores of *P.helianthi* germinated most rapidly and frequently in the temperature range 17 - 23°C. At these temperatures the first germ-tubes were discernable after 2h incubation and at 17.6°C the first basidiospores were seen after 4h incubation. However, the rate and frequency of basidiospore formation fell at temperatures above and below 17.6°C. Basidiospores were not produced at

temperatures above 22.8°C but were replaced by aberrant metabasidia and sterigmata. Below 17.6°C the rate of germination of teliospores decreased and aberrations were exceptions. The effect of temperature on abnormalities of metabasidia were reported but not quantified by Maneval(1922). Sato *et al*(1970) observed increased septation in *U.aloes* as the temperature approached the maximum for teliospore germination. Temperature optima are normally given for teliospore germination and not for completion of basidiospore production and liberation. For example, 18°C was given as the optimum temperature for germination of teliospores of *Uromyces appendiculatus* (Gold and Mengden,1983a), 17°C for *U.aloes* (Sato *et al*,1980), 18°C for *P.graminis* (Lambert,1929) and 25°C for *P.cacabata* Arth. and Hollw. For *P.helianthi* the optima for both teliospore germination and production and liberation of basidiospores were found to be around 17-18°C. This is in agreement with the findings of Bailey(1923) but lower than the 20°C reported by Maneval(1927) and 24°C reported by Fraizer(1920).

The formation of aberrant metabasidia was also observed to be influenced by agar concentration. As the agar concentration of the incubation media increased the frequency of metabasidial abnormalities also increased. Recently Anikster(1988) showed that a reduction in the water potential of an agar medium (achieved by increasing the concentration of mannitol in the medium) resulted in the metabasidia of *P.recondita* and *U.scillarum* producing undischarged basidiospores, aberrations of sterigmata and elongation of metabasidia without basidiospore formation.

The effect of light on teliospore germination varies. Gold and Mengden(1983b) classified the reported responses as i) equal germination in continuous light or dark, ii) normal germination in continuous dark with inhibition by light, iii) normal germination in light-dark cycles but not in continuous dark or reduced by continuous light.

Germination of teliospores of *U.appendiculatus* was completely inhibited in continuous darkness but occurred in darkness following a light to dark transition (Gold and Mengden,1983b). Initial exposure to light at an intensity of 15lx was sufficient to stimulate germination. Neuhaus(1969) reported that exposure to light at an intensity of 1000lx for 1min was sufficient to stimulate germination of teliospores of *P.sorghi*. The release of basidiospores by *U.appendiculatus* reached its maximum at approximately 7h after the light-dark phase change (Gold and Mengden,1983b; Freytag *et al*, 1988). Concentration of basidiospores of *P.malvacearum* in the air spora peaked at 2300h and was essentially zero during daylight hours (Carter and Banyer,1964). The experiments reported in this chapter showed that the germination of teliospores of *Puccinia helianthi* occurred in continuous dark, continuous light and in darkness following a light-dark transition. Exposure to photoperiods of 8h and 12h at a light intensity of 1100lx resulted in significantly greater germination ($P<0.05$) than photoperiods of 2h,4h, continuous darkness or continuous light. The asynchronous germination

of teliospores observed in these experiments made it difficult to identify with certainty whether the change from light to darkness had an influence on subsequent germination. There seemed to be a peak in germination approximately 8h after the phase change. Incubation in the longer periods of darkness (0,2 and 4 photoperiods) resulted in formation of significantly more ($P<0.05$) indeterminate metabasidia but did not affect production of aberrant metabasidia. Maneval(1927) reported that incubation in darkness resulted in production of fewer basidiospores of *P.helianthi*.

In Australia teliospores of *Puccinia helianthi* are not only produced in response to approaching winter but also as a response to host maturation. Periods of exposure to extreme cold are not neccessary to induce germination of teliospores of Australian isolates of *P.helianthi*. Teliospores produced in summer at Toowoomba were also capable of germinating in a matter of weeks. Whether or not these teliospores produced basidiospores would have been a function of temperature and moisture availability.