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THE CULTURE OF CEREAL LEAF RUSTS FOR PHYSIOLOGICAL AND TAXONOMICAL STUDIES

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ABSTRACT. The results of studies concerned with the culture of cereal rust fungi are presented. Detached leaf culture of the uredinial spore generations of *Puccinia coronata, P. hordei, P. recondita, P. striiformis* and *P. triticina* (= *P. recondita* f.sp. *tritici*) on water agar containing 80ppm benzimidazole in controlled environment cabinets set at 14°C in a 16h light regime was found to be most useful and convenient. However the different rusts were found to have different temperature optima. The methods developed were successfully employed in physiological and taxonomical experiments.

INTRODUCTION

When studying rust fungi and other obligate parasites special difficulties arise because of the complicated relationship between the pathogen, the host and the disease as well as the influence of environmental conditions on all three. Fig. 1 shows the model of this relationship described by Loegering (1984).

One approach towards an understanding of this relationship is to investigate the physiological requirements and reactions to environmental changes of both host and pathogen in isolation. The growth of the host partner in controlled, aseptic conditions is relatively easy and has been described many times (Metzler, 1981; Chares *et al.*, 1983). However, the growth of rust fungi in pure culture has been difficult, and although early experiments with the culture of spermatia (Plowright, 1889) were successful, it was only recently that they could be reproduced (Deml *et al.*, 1982a,b). Until the early 1950s, experiments with other stages in the life cycle of the rusts, such as those of Fuchs & Gaertner (1958), were always abandoned in failure, although Colley (1918) and Wright (1977) reported the continuing growth of promycelium from germinating teliospores of *Cronartium ribicola* and *Puccinia striiformis* respectively. Hotson & Cutter (1951) reported the growth of *Gymnosporangium juniperi-virginianae* in tissue cultures of rust galls from *Juniperus,* and the subsequent axenic growth of the pathogen apart from the host tissue. Although this early success in axenic culture was not confirmed in a later paper (Hotson, 1953), and considerable controversy arose about its validity (Scott & Maclean, 1969), Cutter (1959) confirmed the earlier findings and extended them to other rusts. Meanwhile, other workers maintained dual tissue cultures of infected hosts (Bauch & Simon, 1957; Turel & Ledingham, 1957), where the rust was always dependent on living host tissue.

A major breakthrough in the axenic culture of rusts was achieved when Williams *et al.* (1967) succeeded in establishing growth with densely sown urediniospores of *P. graminis tritici* on relatively simple media containing yeast extract and peptone. Prior to this, several researchers had concentrated on this spore form as a basis for axenic culture, but mostly single spores or low spore densities had been used, in order to facilitate the observation of the process of transition from the germ tube stage to saprophytic growth (Arthur, 1928; Stock, 1931; Fuchs & Gaertner, 1958; Gaertner & Fuchs, 1962): no continuous

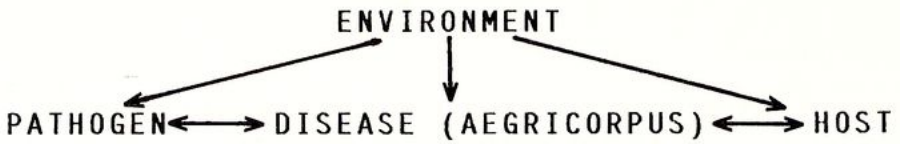

FIG. 1. Relationships in a host/pathogen ecosystem (after Loegering, 1984)

culture of rusts had been obtained. Since 1967 many rust isolates, including isolates of most cereal rusts (Helfer, 1986), have been taken in axenic culture starting from urediniospores. The literature of this work has been reviewed by Scott & Maclean (1969), Scott (1976), Maclean (1982) and more recently by Williams (1984).

Despite the successes, enthusiasm over the axenic culture of rusts has recently waned as the application of the results of physiological studies *in vitro* to host parasite interactions *in vivo* could be made only with great reservations. Furthermore, axenically grown rusts developed only very slowly and often atypically (Williams, 1984). For practical purposes it was, therefore, necessary to approach the questions concerning host-parasite relationships from a different angle.

As indicated in Fig. 1 the environment plays an important role in disease development, influencing all three 'organisms' (*sensu* Loegering, 1984) in the relationship, i.e. host, parasite and aegricorpus. Hence it is important to provide environmental conditions as uniform as possible when the effects of host and parasite on the development of disease are being studied. This can be accomplished by raising host plants in controlled environment cabinets where temperature, humidity, and light cycle and intensity can be controlled. Unfortunately this requires large amounts of space and equipment, especially if different rust isolates are being tested simultaneously and have to be isolated from each other to avoid cross contamination. A more economical and convenient means of obtaining controlled environmental conditions is to carry out the experiments with the rusts on the detached leaves of their respective hosts, the leaves being laid out in petri dishes on a suitable medium to prevent wilting and senescence. The first attempts to grow rusts in this way were made by Clinton & McCormick in 1918 with *Cronartium ribicola* on *Ribes nigrum* (Clinton & McCormick, 1924); they simply used water to keep the leaves fresh. Yarwood (1946) gives a good account of the methods used and the results obtained by earlier researchers. Subsequent, more advanced methods have employed various concentrations of benzimidazole to delay senescence (Person *et al.*, 1957; Samborski *et al.*, 1958; Bjoerkmann, 1960; Lumbroso *et al.*, 1977) or of other chemicals with similar effects (Wang *et al.*, 1961; Wolfe & Macer, 1964).

The aim of this study was to establish a convenient, reliable but economical method of growing rust fungi for the use in physiological and taxonomical experiments.

Materials and Methods

Seedling leaves of cultivars of barley, oats, rye, triticale and wheat, grown in trays in a growth chamber set at 14°C and 16h light at 23000 lux, were used. The varieties used are shown in Appendix 1. The seedling leaves were harvested when they had fully expanded, and in the middle of the light cycle, as it seemed important that the translocation of carbohydrates was interrupted when a high concentration of assimilates was present in the leaves (Samborski *et al.*, 1958). After detachment they were immediately laid onto the surface of the various treatments in petri dishes. The light and temperature conditions of the seedlings before detachment were the same irrespective of the conditions in the detached culture. All experiments were carried out in sterile 100mm square plastic petri dishes.

Three different parameters were varied to find the most suitable conditions for maintaining cereal leaf rusts in detached leaf culture.

i. The concentration of benzimidazole dissolved in the medium: four different concentrations were tried, 0ppm, 40ppm, 80ppm and 100ppm.
ii. The supporting medium: three concentrations of water agar, 0.4%, 0.7% and 1.0%, were poured in two different ways, horizontally and sloping (Fig. 2). Two other support media were used, cotton wool and filter paper.
iii. Temperature and light regimes: temperatures of 10, 14 and 22°C and light at 23000 lux, applied for 8, 16 and 24h per day.

The seedling leaves were inoculated with the appropriate rust spores immediately after they had been transferred to the various culture media using a spore settling tower (Helfer, 1986). For comparison potted cereal seedlings were inoculated with the same isolates and kept under isolation domes in the glasshouse. A list of rust isolates used is given in Appendix 2. An uninoculated control was included in the experiment. A qualitative assessment of the rust development and leaf preservation was made 21 days after leaf detachment and inoculation. The experiments were replicated five times.

Results

The results for rust development are summarized in Table 1.

Benzimidazole concentration:
The highest concentration (100ppm) preserved the leaves best, but very good preservation was also achieved with 80ppm. The fungal development was also very satisfactory at 80ppm, and wheat yellow rust developed better than at 100ppm. At 40ppm barley leaves very soon became chlorotic (after 14 days) and died shortly afterwards. Consequently rust development on this host could not reach any significant level. A similar lack of preservation was observed with all the cereal seedling leaves when no benzimidazole was used. At 80ppm benzimidazole, detached leaves of all the cultivars used in the present experiments exhibited the same resistance responses to the rust isolates as potted plants of these cultivars in the glasshouse.

Supporting medium:
A medium containing 0.7% water agar proved most suitable and convenient. In 0.4% water agar the leaves were too immersed producing areas of

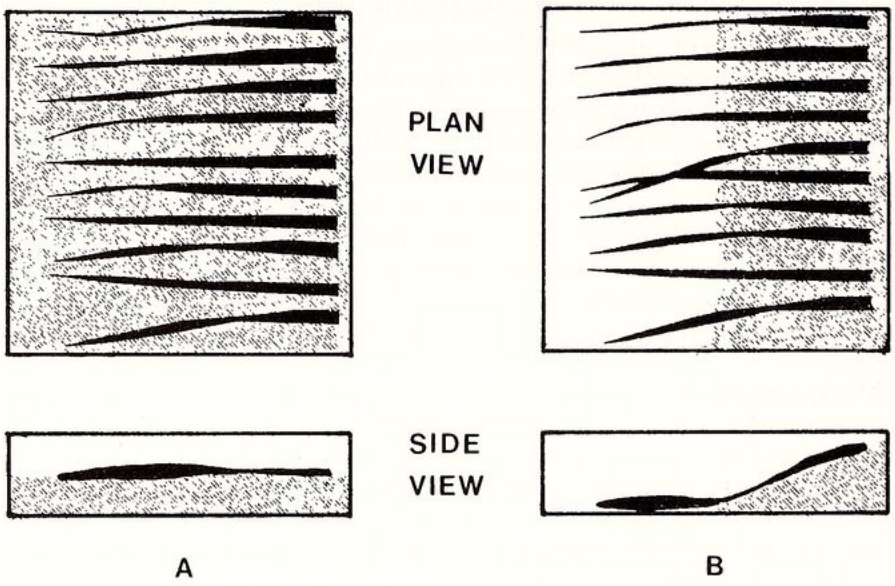

FIG. 2 Two ways in which agar was poured. A, horizontal agar; B, sloping agar.

condensation on their lower surface. This made spore collection difficult. The higher concentration (1%) did not allow sufficient contact between the leaf surface and the medium to enable the preservation by benzimidazole to take place effectively, and the leaves soon wilted (14–21 days). The other media (cotton wool and filter paper) proved far inferior to agar possibly due to an insufficient contact with the leaves. The tilted agar method was very useful for the bulking up of inoculum, as the spores falling from the leaves were not wetted by agar and could be collected easily.

Temperature and light:
The optimal temperature was different for each rust species. Yellow rusts developed best at 14°C, whereas the other leaf rusts had a slight preference for the higher temperature (22°C). Conversely the leaves (both inoculated and uninoculated) were best preserved in the low temperature (10°C). The light regime applied at 16h provided sufficient energy for the photosynthesis of the detached leaves. Both inoculated and uninoculated leaves kept at constant light tended to become chlorotic much more quickly (7–10 days) than those kept in a day/night regime. The rust development was not affected by the different light regimes.

DISCUSSION

Bjoerkmann (1960) found, in his experiments with primary leaves of oats, that the most suitable concentration of benzimidazole for detached leaf culture was 40ppm when the leaves were floated on the solution and 60ppm when the petri dishes were tilted in a similar manner as described here and the leaves, therefore, were only partly in contact with the medium. Wolfe & Macer (1964)

TABLE 1
Rust development on detached host leaves 21 days after inoculation.

EXPERIMENT & TREATMENT	RUST ISOLATES: Oat Crown Rust	Barley Brown Rust	Rye Brown Rust	Wheat Brown Rust	Wheat Yellow Rust	Barley Yellow Rust
Benzimidazole						
0 ppm	+	+ *	+	+	–	– *
40 ppm	+++	+ *	+++	+++	++	+ *
80 ppm	+++	+++	+++	+++	++	+++
100 ppm	+++	+++	+++	+++	+	+++
Support medium						
Agar 0.4%	+++	+++	+++	+++	++	+++
0.7%	+++	+++	+++	+++	++	+++
1.0%	++	+ *	++	++	++	+ *
Cotton Wool	++	+ *	++	++	++	(+) *
Filter paper	++	+ *	++	++	++	(+) *
Temperature						
10°C	++	++	++	++	++	+++
14°C	++	+++	+++	+++	++	+++
22°C	+++	+++	+++	+++	(+)	+
Light						
8h	++	++	++	++	++	++
16h	+++	+++	+++	+++	++	+++
24h	++ *	++ *	++ *	++ *	(+) *	(+) *

– = no growth
\+ = little growth
++ = satisfactory growth
+++ = very good growth
(+) = not consistent
* = host chlorosis

used a solution of 50ppm benzimidazole together with 10ppm kinetin in distilled water to culture wheat and barley yellow rust (*Puccinia striiformis*). A solution of 40ppm benzimidazole in water was used for the completion of life cycles of *P. hordei* and *Uromyces scillarum* on detached leaves of their main and alternative hosts by Lumbroso *et al.* (1977). Browning (1954) and Samborski *et al.* (1958) reported that detached leaves of rust-resistant oats or wheat became susceptible when floated on water, thus rendering the results of resistance studies doubtful. However, wheat leaves regained their resistance if 40ppm benzimidazole was added, and 1% glucose repressed the effect of benzimidazole (Samborski *et al.*, 1958). In work with cereal mildew (*Erysiphe graminis*) concentrations between 10 and 150 ppm benzimidazole have been used (Limpert *et al.*, 1988).

Most researchers have used a liquid medium for detached leaf culture (Clinton & McCormick, 1924; Waters, 1928; Person *et al.*, 1957; Samborski *et al.*, 1958; Bjoerkmann, 1960; Wang *et al.*, 1961; Wolfe & Macer, 1964; Lumbroso *et al.*, 1977) although some used filter paper (Hennessy & Sackstone, 1970. The agar method in the present experiments was chosen mainly for its convenience and reliability.

The most reliable and convenient way of experimenting with cereal leaf rusts in the laboratory arrived at during the present study, was to grow them on detached host leaves maintained on 80ppm benzimidazole in 0.7% water agar. A 16h daily light regime was found to be most suitable, while the temperature of incubation depended on the rust species concerned. The methods described here are now used by the author for propagation and physiological and taxonomical studies in rusts and mildews.

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APPENDIX 1

List of cereal varieties used in the culture experiments:

a) Barley *Hordeum vulgare*:

Astrix	Berac	Bigo	Bolivia
Cebada Capa	CI 1243	Egypt 4	Gold
Keg	Mazurka	Midas	Peruvian
Quinn	Ribari	Simon	Sudan
Varunda			

b) Oats *Avena sativa*:

Anthony	Appler	Bond	Bondvic
Landhafer	Maris Tabard	Saia	Santa Fe
Trispernia	Ukraine	Victoria	

c) Rye *Secale cereale*:

Dominion	Rheidol

d) Triticale:

Bush

e) Wheat *Triticum aestivum*:

Armada	Carstens V	Chinese 166	Clement
Compair	Heines VII	Heines Kolben	Heines Peko
Hustler	Hybrid 46	Lee 1	Longbow
Mardler	Maris Bilbo	Maris Fundin	Maris Huntsman
Maris Ranger	Michigan Amber	Moro	Nord Desprez

Norman	Rapier	Reichsberg 42	Riebesel 47/51
Sappo	Spalding's Prolifique	Sportsman	Strubes Dickkopf
Suwon x Omar	Vilmorin 23		*Triticum spelta*

Appendix 2

List of rust isolates used in the culture experiments:

a) Oat crown rust *Puccinia coronata*:
Field isolate (1)*

b) Barley Brown rust *Puccinia hordei*:

Race A (2)	76–12 (2)	83–1 (1)	83–2 (1)

c) Rye brown rust *Puccinia recondita*: RBR 70–1 (2)

d) Barley yellow rust *Puccinia striiformis*: Race 1 (1)

e) Wheat yellow rust *Puccinia striiformis*:

Race 37E132 (3)	Race 41E136 (3)	Race 104E137 (3)	Race 104E137 W (3)
Race 108E9 (3)	P 631 (4)	P 71–493 (4)	P 72–23 (4)
P 75–27 (4)	P 75–109 (4)	P 76–15 (4)	P 80–21 (4)
P 81–11 (4)			

f) Wheat brown rust *Puccinia triticina* (= *P. recondita* f. sp. *triticina*):

WBRS 74–2 (2)	WBRS 77–22 (2)	WBRS 79–4 (2)	WBRS 79–21 (2)
WBR 83–1 (1)	WBR 83–2 (1)		

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