SYMPTOMS OF SCALD DISEASE INDUCED BY TOXIC METABOLITES OF *RHYNCHOSPORIUM SECALIS**

By E. N. Ayesu-Offei† and B. G. Clare†

Scald is a disease of barley and some grasses caused by the fungus *Rhynchosporium secalis* (Oud.) Davis. Lesions on naturally or artificially infected leaves begin as grey, water-soaked areas 8–12 days after inoculation. Frequently, lesions at this stage become dry but retain a grey-green colour which persists even when the uninfected parts of a leaf have become senescent and straw-coloured. More usually the lesions become oval-shaped with dry, pale-brown or white centres surrounded by dark-brown margins. We have also observed signs of hypersensitive reactions in artificially and naturally infected barley leaves. The hypersensitive reactions result in the formation of small dark-brown patches on the leaves where infection has been unsuccessful.

When infected leaves were examined microscopically it was found that in lesions showing the initial, grey, water-soaked symptoms mesophyll and epidermal cells had collapsed (Ayesu-Offei and Clare 1970). At this stage hyphal growth was scanty and confined between the cuticle and the outer cell walls of the epidermis. It appeared that toxin(s) from the fungal hyphae were responsible for the collapse of the host cells.

In preliminary experiments we have also found that lesion development is associated with increased respiration and decreased water content of leaves.

This paper reports the production of scald disease symptoms on leaves of barley, oats, wheat, and *Dactylis glomerata* L. when they were treated with culture filtrates of *R. secalis*.

Materials and Methods

(i) *Preparation of Culture Filtrates.*—Clipper barley (WI 2200) was inoculated with *R. secalis* (SA-1) (Ayesu-Offei and Clare 1970) and the fungus was re-isolated and grown on PSP agar (22 g Deb dehydrated potato, 10 g sucrose, 10 g peptone, 10 g agar in 1 litre water) at 15°C in the dark. Colonies were macerated and used to inoculate PSP liquid medium or MY liquid medium (10 g malt extract, 10 g yeast extract in 1 litre water) and incubated, without agitation, at 15°C in the dark for 14 days. The cultures were than strained through a double layer of muslin, centrifuged at 110,000 *g* for 1 hr at 15°C, and the supernatant was sterilized by being passed through a Millipore filter of 0·5 μm pore size. Uninoculated PSP and MY liquid media were sterilized in the same way.

(ii) *Treatment of Seedlings with Culture Filtrates.*—Test plants were barley [cultivars WI 182; WI 186 (Atlas 46); WI 492 (Tennesse Winter); WI 567; WI 722 (La Mesita); WI 729; WI 768; WI 800; WI 958; WI 1237 (Prior); WI 2142; WI 2200 (Clipper)], wheat (cv. Heron), oats (cv. Scotch Grey), and *D. glomerata* (cv. Currie). Of these, only barley is susceptible to infection by *R. secalis*.

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† Department of Plant Pathology, Waite Agricultural Research Institute, Glen Osmond, S.A. 5064.

Clipper barley seeds were surface-sterilized with 5·5% sodium hypochlorite solution and placed aseptically on PSP agar in large test tubes (20 by 5 cm) which were then placed in a growth cabinet at 25°C and illuminated for 12 hr daily at 1000 f.c. The other test plants were grown in pots in a glasshouse at 20–25°C. When seedlings had reached the three-leaf stage the stems were cut 1 cm below the bottom leaf and were immersed in 20-ml portions of either sterile culture filtrate or sterile PSP or MY liquid medium. In addition, Clipper barley seedlings were cut and immersed in autoclaved (20 min at 15 lb/in^2) culture filtrate or dialysed (in distilled water for 16 hr at 5°C) culture filtrate.

Clipper barley seedlings were grown in pots in a glasshouse until they reached the three-leaf stage and were then sprayed with culture filtrate or liquid PSP or MY medium. The sprayed plants were placed in a humid chamber for 48 hr at 15°C and illuminated for 12 hr a day at 1000 f.c. They were then placed in a glasshouse at 20–25°C.

(iii) *Sectioning of Leaves.*—Sections were prepared from inoculated Clipper barley leaves which had dark-brown hypersensitive spots or which had lesions with dark-brown margins or uninoculated leaves which had developed dark-brown spots after being sprayed with culture filtrates. Sections were also made of leaves showing scald symptoms from seedlings whose stems had been immersed in culture filtrates.

Pieces 0·5 cm^2 were cut from the leaves, cleared in formalin–acetic acid–alcohol, sectioned with a freezing microtome, and stained with cotton blue lactophenol.

(iv) *Measurement of Respiration.*—Measurement of oxygen uptake was made at 30°C using a Warburg respirometer with 300 mg (fresh weight) of leaf tissue and 3 ml of 0·1M KH_2PO_4 in each flask. The flasks were covered with black cloth to prevent photosynthesis and after equilibration for 1 hr measurements were made at intervals of 5 min for 50 min. Measurements were made on triplicate samples of leaf tissue. The leaf tissue was then removed and dry weights of the samples determined. Respiration rates were calculated on a dry weight basis.

Respiration measurements were made with (1) healthy tissue; (2) leaves inoculated by spraying with spore suspensions (150,000–200,000 spores/ml) or by smearing with sporulating colonies growing on pieces of agar medium; (3) leaves from seedlings whose stems had been immersed in either sterile culture filtrate or sterile PSP or MY liquid medium for periods of up to 26 hr as described in (ii) above.

Clipper barley seedlings, grown in pots in a glasshouse at 20–25°C, were used in these experiments from the three- to six-leaf stages of development. Inoculated and uninoculated plants were placed in a humid chamber for 48 hr and then in a glasshouse, as described in (ii) above. Samples were taken from the distal halves of the upper three or four leaves at periods between 7 and 13 days after inoculation.

Results and Discussion

When cut stems of Clipper barley seedlings were immersed in sterile culture filtrate, leaves of the seedlings developed grey, water-soaked patches similar to those produced on infected leaves. These patches, which often appeared within 1 hr of treatment with culture filtrate, gradually coalesced to form large, dry, grey-green areas, usually towards the leaf tips (Fig. 1*B*), which retained their colour when the rest of the leaf had dried to a straw colour. Similar lesions also develop on infected plants. These effects were not produced in seedlings treated with sterile, uninoculated culture medium and the effectiveness of sterile culture filtrates was not reduced by dialysis or autoclaving. The other test plants reacted in a similar manner to Clipper barley when treated with sterile culture filtrate and sterile culture media (Fig. 1*A*).

When the affected parts of leaves treated with culture filtrate were examined microscopically it was found that the mesophyll and the anticlinal walls of the epidermis had both collapsed so that the outer and inner periclinal walls were in contact (see Fig. 3). This damage is similar to that observed in infected leaves in the initial stages of infection (Ayesu-Offei and Clare 1970).

When Clipper barley was sprayed with sterile culture filtrate, brown spots appeared on the leaves 3 days later (Fig. 2). Plants sprayed with uninoculated culture medium did not react in this way. The intensity of the colour gradually increased and the margins of the spots became necrotic. When these spots were examined microscopically it was found that the dark pigment was confined to epidermal cells,

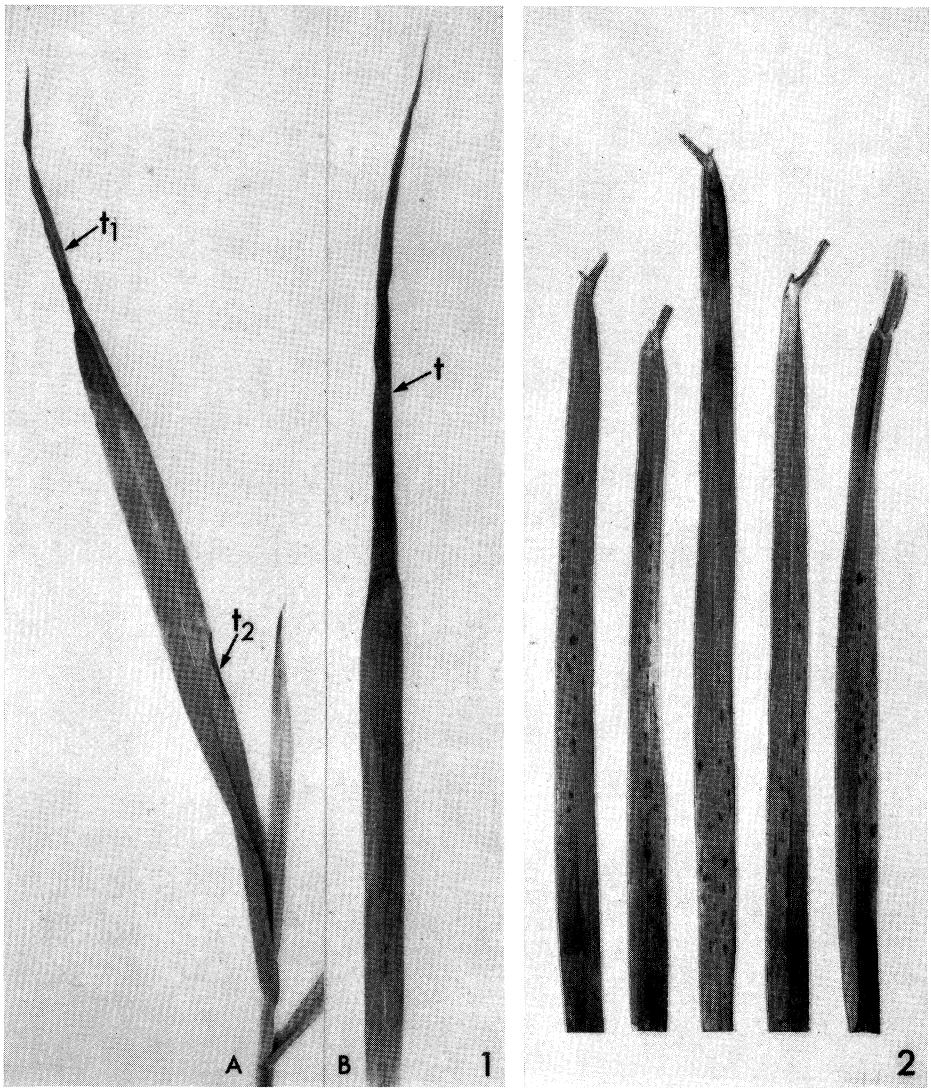
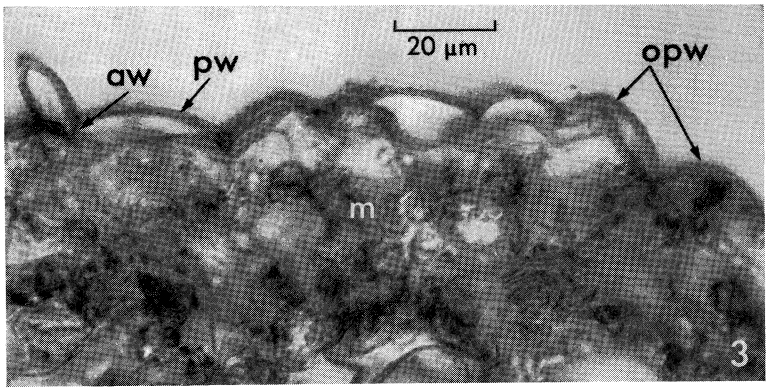
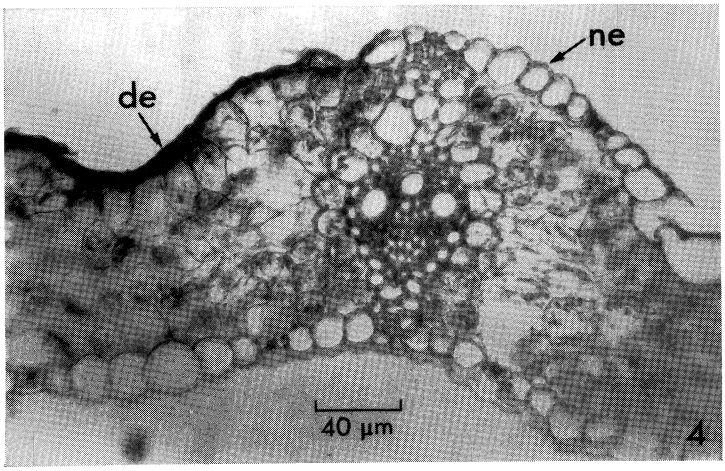


Fig. 1.—Leaves of wheat (*A*) and barley (*B*) 24 hr after cut stems of seedlings had been inserted into tubes containing sterile culture filtrate. t, t_1, and t_2, affected parts of the leaves.

Fig. 2.—Leaves of Clipper barley showing brown spotting 5 days after being sprayed with sterile culture filtrate.

which had collapsed (Fig. 4). This was also true of the darkened margins of lesions on infected plants (Fig. 5) and the dark-brown patches on leaves reacting hypersensitively when inoculated.





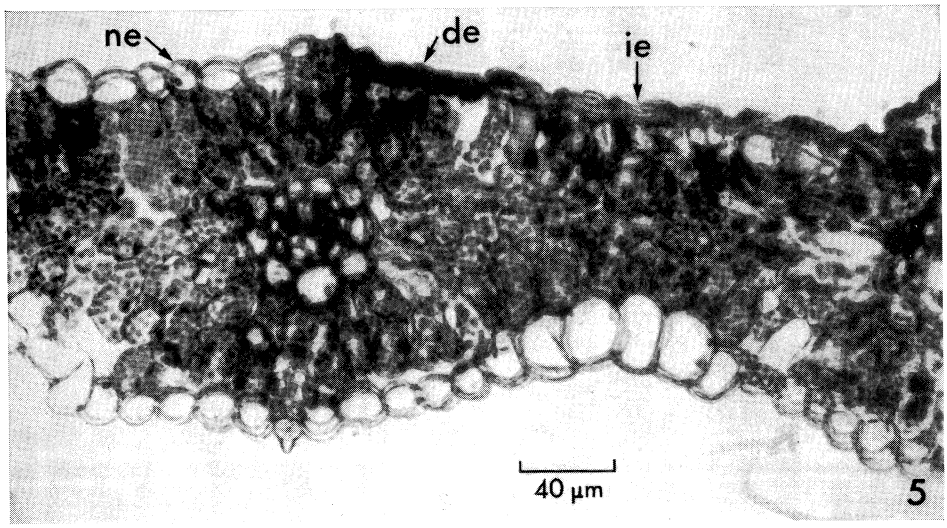


Fig. 3.—Transverse section of barley leaf 24 hr after treatment with toxin showing stages in the collapse of mesophyll cells and the anticlinal cell walls of the epidermis. *aw*, collapsing anticlinal cell wall; *pw*, periclinal cell wall; *opw*, outer periclinal cell walls collapsed against inner walls; *m*, collapsed mesophyll cells.

The respiration rates of Clipper barley leaves varied markedly depending upon the stage of seedling development. However, there was a consistent increase in the respiration rate of leaves showing symptoms of infection, until the lesions began to bleach and lose appreciable amounts of water at which stage respiration rates fell below those of healthy leaves. Typical results are given in Table 1.

TABLE 1

RESPIRATION OF BARLEY (CLIPPER) LEAVES* INFECTED BY *R. SECALIS*

Time after Inoculation (days)	Symptoms	Water Content (%)†	Respiration Rate (%)†
7·0	Slight greying	100	158
7·5	Slight wilting	100	174
8·0	Lesions yellowing	96	168
13·0	Lesions dry and bleached	68	15

* Seedlings at three-leaf stage. † Compared with healthy leaves.

The respiration rates of leaves treated with sterile culture filtrates were also higher than those of untreated leaves or leaves treated with sterile, uninoculated, liquid medium. These increases began between the second and fifth hours of the treatment period and were maintained until wilting became severe. Typical results are given in Table 2. The respiratory increases were not as great as those in infected

TABLE 2

RESPIRATION OF BARLEY (CLIPPER) LEAVES* TREATED WITH *R. SECALIS* CULTURE FILTRATE† OR WITH UNINOCULATED LIQUID MEDIUM†

Treatment	Time of Treatment (hr)	Symptoms	Water Content (%)‡	Respiration Rate (%)‡
Culture filtrate	2	Nil	100	110
Medium	2	Nil	100	111
Culture filtrate	5	Nil	98	132
Medium	5	Nil	100	112
Culture filtrate	7	Slight greying	97	121
Medium	7	Nil	100	109
Culture filtrate	26	Grey and wilting	90	103
Medium	26	Nil	100	92

* Seedlings at four-leaf stage.

† Malt–yeast liquid medium in both cases.

‡ Compared with untreated leaves.

Fig. 4.—Transverse section of Clipper barley leaf, cleared with formalin–acetic acid–alcohol and stained with cotton blue lactophenol, showing dark-brown pigments in the epidermis of a leaf which had been sprayed with sterile culture filtrate 5 days previously. *de*, dark-brown and collapsed epidermis; *ne*, normal epidermis.

Fig 5 —Transverse section of *R. secalis* lesion on Clipper barley leaf, cleared in formalin–acetic acid–alcohol and stained with cotton blue lactophenol. *ne*, normal epidermis; *ie*, infected and collapsed epidermis; *de*, dark-brown and collapsed epidermis.

leaves. This was probably due to the fact that symptoms were not apparent in the early stages of treatment, and the samples used would have contained a mixture of affected and unaffected tissue. In addition, the metabolic effects of culture filtrates on leaf tissue are so rapid that is it probable that respiratory stimulation would soon be lost as cells became metabolically disorganized.

Sterilized culture filtrates of *R. secalis* are thus able to reproduce visible and physiological symptoms of scald disease in barley. It appears, therefore, that toxic metabolites of *R. secalis* are responsible for disease symptoms in infected plants. Toxin production by plant pathogens is involved in a number of plant diseases (Owens 1969). Most of the toxins which have been investigated are polypeptides or glycopeptides and the *R. secalis* toxins may be of this type, since the toxic effect is not removed from culture filtrates by dialysis or autoclaving. Some toxins of plant pathogens, such as victorin and *Helminthosporium carbonum* toxin, are host-specific but this is not true of the *R. secalis* toxin(s) since a range of plants, including ones not susceptible to *R. secalis*, will produce some of the visible symptoms of scald disease when treated with culture filtrates.

Dark-brown pigmentation and collapse of epidermal cells resulted when culture filtrates were applied to barley leaf surfaces but not when cut stems of barley seedlings were placed in culture filtrates. This probably is a result of the rapid collapse and necrosis of epidermal cells when stems are immersed in culture filtrates. Culture filtrate applied to leaf surfaces would move relatively slowly through the cuticles and hence the epidermal cells could synthesize pigments before the cells were inactivated. The production of these pigments appears to be a protective reaction in the host since lateral spread of *R. secalis* hyphae, which takes place between the cuticle and epidermis, does not proceed through the darkened margins of lesions nor through darkened epidermal areas resulting from hypersensitive reactions to attempted infection.

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References

Ayesu-Offei, E. N., and Clare, B. G. (1970).—Processes in the infection of barley leaves by *Rhynchosporium secalis*. *Aust. J. biol. Sci.* **23**, 299.

Owens, L. D. (1969).—Toxins in plant disease: structure and mode of action. *Science, N.Y.* **165**, 18.