Effects of Fungi on Barley Seed Germination

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Barley seeds placed in contact with straw buried in soil imbibed water more slowly and had a greater fungal biomass under the husk at germination than did seeds which had been in contact with soil only. The growth of fungi under the husk increased during imbibition and continued when the seed was fully imbibed; such growth resulted in poor seed germination. A common soil saprophyte, Gliocladium roseum, inoculated on to the outer surfaces of the seed husk, suppressed germination by competing with the embryo for available oxygen. The prevention of seed germination by some micro-organisms seems to be related to the microbial affinity for oxygen.

INTRODUCTION

The examination of seeds for colonization by micro-organisms has been limited largely to samples of grain stored before feeding to animals or sowing. The seed microflora are predominantly fungi (Christensen, 1972) and relatively few bacteria are found (Mundt & Hinkle, 1976). The fungi can be identified and their location ascertained by incubating seed or portions of seed on agar (Mulinge & Chesters, 1970; Tempe & Limonard, 1973; Flannigan, 1974) but this does not give a quantitative measure of colonization. Methods of direct observation have been described; the outer layers of seed containing the fungi are removed or seed sections are prepared and the fungi are then stained (Hyde, 1950; Warnock & Preece, 1971; Mulinge & Chesters, 1970). The colonization of barley and oat seed can be studied by removing and then examining the husk. This allows examination of the colonization of the entire husk rather than that of small cross-sections; also a relatively large number of seeds can be examined. The method is based on the assumption that colonization is predominantly on the husk with relatively little on the husk-covered pericarp.

In addition to the colonization of seed on the ear or during storage, micro-organisms can develop during seed germination, and when the supply of oxygen is limited they can compete for oxygen and inhibit germination (Gaber & Roberts, 1969; Harper & Lynch, 1979; Lynch & Pryn, 1977; Matthews & Collins, 1975). The husk may limit the supply of oxygen to barley seed (Roberts, 1969) and fungi beneath the husk are better placed to compete with the embryo for oxygen than are the soil microflora. These fungi also have the first chance to utilize the carbonaceous energy-rich exudates released from the seed during germination (Simon, 1974).

We have now studied the course and effect of colonization beneath the husk by seed-borne fungi and the influence of the husk on oxygen uptake by germinating barley seed. Colonization on the outer surface of the husk by saprophytic fungi, such as Gliocladium roseum, which compete with the seed for oxygen can inhibit germination (Lynch & Pryn, 1977). Since seed is particularly prone to invasion by such primary colonizers when drilled into decomposing mats of crop residues, the effect of seed contact with straw on germination in a simulated drill slit has also been examined.

METHODS

Measurements of colonization. Seeds were dehusked manually, dry seed being first soaked for 1 h in 0-01% aqueous eosin. The husk was stained for 1 min in phenol/acetic acid/aniline blue (Jones et al., 1948), washed for
that the water level was colonization in impairing germination of seed in low oxygen concentrations. Germination after incubation over water for 8 d was end of the seed was removed leaving the rest of the husk intact. When inoculum density was isolated from barley seed were grown in batch culture (1 was 0.8 to drying at (Lynch, 1979). Harpers) and longitudinal sections (2 g 1-') of the was calculated on the basis that the lemma comprised 60% of the surface area of the husk.

Colonization of seed by seed-borne fungi. Barley seed (Hordeum vulgare var. Proctor) was suspended on stainless-steel wire mesh (5 mm) 2 cm above water at 20 °C. Samples of 30 seeds were taken at intervals and germinated on coarse sand (1-0 to 1-5 mm, 1 kg) moistened with water (225 ml) in conical flasks (2 l capacity) at 20 °C. Oxygen concentrations in the flasks were maintained by continuously passing air or 2-5% oxygen in nitrogen at 100 ml min-1. Germination percentage was recorded after 48 h; seed was said to have germinated when the coleorhiza penetrated the husk.

Colonization of seed in soil under laboratory conditions. Plastic columns (40 cm x 10 cm diam.) were sealed at the base where a hole (5 mm) was drilled to allow drainage. The columns were filled within 3 cm of the top with clay loam soil (Denchworth series) sieved to <3-5 mm. The drainage hole was sealed and water was added until the soil was waterlogged; the hole was then opened and the columns were allowed to drain standing in water such that the water level was 30 cm below the soil surface. The columns were incubated for 7 d before planting seed. Ten seeds were placed in each column at a depth of 3 cm. In some columns, straw (10 g) chopped into 5 cm lengths was spread over the surface; a steel plate was then forced into the soil to form a 3 cm deep straw-lined slit into which the seed was planted. The columns were incubated at 20 or 10 °C. The bulk density of soil in columns was 0.8 to 0.95 g cm-3. Seed was sampled at intervals up to germination, and moisture content was estimated by drying at 105 °C for 1 h. All seeds were examined for colonization.

Germination of seed inoculated with a saprophytic fungus. Gliocladium roseum Bain. (IMI 196507) was cultured in malt extract broth at 25 °C and barley seeds were soaked (5 min) in washed suspensions (5 g l-1) of the fungus. Seed was germinated on sand at 20 °C as described above.

Oxygen uptake by saprophytic fungi. Gliocladium roseum, Mucor hiemalis (ATCC 26035) and a Penicillium sp. isolated from barley seed were grown in batch culture (11 culture vessel) on a glucose and mineral salts medium (Lynch & Harper, 1974). The specific rate of oxygen utilization (qO2) was found from culture dry weight and the difference between oxygen in effluent and influent gas streams.

Respirometric studies. Oxygen uptake was measured in a Gilson respirometer as described earlier (Harper & Lynch, 1979). Husks were removed from some seeds manually or a small part of the husk (the plug) at the embryo end of the seed was removed leaving the rest of the husk intact. When Gliocladium roseum was added to seed the inoculum density was 7 g dry wt l-1.

Histology of seeds. Generally, seeds were embedded in wax and longitudinal sections (15 μm thick) were stained with safranin and fast green. Alternatively, the seeds were embedded in plastic resin ('Spurr's'; Taab Laboratories, Reading) and longitudinal sections (2 μm thick) were stained with toluidine blue.

RESULTS

Colonization and germination of seed incubated at high relative humidity. When seed was incubated over water the weight of fungus colonizing each seed increased with seed moisture content. Colonization increased linearly with time after the moisture content had reached a maximum (Fig. 1). The experiment was terminated after 10 d since some seed had germinated. The germination percentage of seed removed at intervals and planted on moistened sand declined progressively as colonization proceeded, particularly if the oxygen supply to seed was limited (Fig. 2). The maximum depression of germination percentage in 2-5% (v/v) oxygen was reached after 4 d incubation; more than 80% of these seeds carried 100 μg fungus under the husk.

This experiment also showed the importance of the husk together with associated fungal colonization in impairing germination of seed in low oxygen concentrations. Germination after incubation over water for 8 d was <30% in 2-5% (v/v) oxygen, compared with 75% in
Table 1. Percentage of seed carrying more than 100 μg fungal biomass at germination in contact with soil or separated from soil by straw

<table>
<thead>
<tr>
<th>Incubation temperature (°C)</th>
<th>Seed planted in contact with soil only</th>
<th>Seed planted in contact with straw</th>
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<tbody>
<tr>
<td>10</td>
<td>25</td>
<td>75</td>
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<td>20</td>
<td>10</td>
<td>70</td>
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Colonization of seed separated from soil by straw. When seed was separated from soil by a layer of straw the rate of imbibition was slowed and germination was delayed at both 10 and 20 °C (Fig. 3). In addition, fungal colonization at germination was greatly promoted by the presence of straw (Table 1).

Germination and respiration of seed inoculated with Gliocladium roseum. The uptake of oxygen by seeds inoculated with the fungus was 8 μl h⁻¹ seed⁻¹ (Fig. 4). Oxygen uptake by control seed increased slowly to reach 3.5 μl h⁻¹ seed⁻¹ after 16 h when seed germinated. Oxygen uptake by inoculated seed did not increase and germination was completely suppressed.

Affinity of fungi for oxygen. The q₀ values for Gliocladium roseum and Mucor hiemalis were 3.0 and 6.6 mmol (g dry wt)⁻¹ h⁻¹, respectively, and that for a Penicillium sp. isolated from seed was 1.1 mmol (g dry wt)⁻¹ h⁻¹.

Histological examination. We found no evidence of fungal penetration through the pericarp-testa (the layer beneath the husk). When seed was inoculated with Gliocladium roseum, the fungus grew predominantly at the tip of the seed containing the embryo. Here, a continuation of the inner layer of the husk forms a 'plug' that seems to be the point of oxygen entry into the seed (Lynch & Pryn, 1977) (Fig. 5). The plug is fragile and we have never been able to prepare a section where the plug remained attached to both sides of the outer layer of the husk. No evidence has been found for fungal mycelium penetrating the plug.

Respiration of seed after removal of the husk or plug. All seed germinated when oxygen uptake had reached 3 to 4 μl h⁻¹ seed⁻¹. Since oxygen uptake by the seed increased after
Fig. 3. Imbibition of barley seed in contact with soil or separated from soil by straw: ○, 10 °C in contact with soil; ●, 20 °C in contact with soil; □, 10 °C in contact with straw; ■, 20 °C in contact with straw. Arrows indicate time of germination.

Fig. 4. Oxygen uptake by barley seed inoculated with Gliocladium roseum: ○, control; ●, inoculated.

Fig. 5. Longitudinal section of barley seed in the region of the embryo. Bar marker represents 0.1 mm.

Fig. 6. Oxygen uptake by barley seed: ○, control (husk present); □, husk removed; △, plug removed. Arrows indicate time of germination.
removal of the husk or of the plug at the embryo end of the seed, these treatments resulted in earlier germination (Fig. 6).

**DISCUSSION**

Our observations show that when fungi colonize barley seed beneath the husk, germination can be impaired. Similar effects observed with wheat were attributed to damage by seed-borne species of *Aspergillus* and *Penicillium* (Griffin, 1966), but long periods of incubation were necessary before germination was affected. Competition for oxygen between the embryo and fungal population seems to provide the most likely explanation for impaired germination during the early stages of colonization by seed-borne fungi examined here. When seeds are separated from soil by straw, fungal colonization is promoted and hence the susceptibility to low concentrations of oxygen is increased. We have found that seeds recovered from the field and which have not germinated are most heavily colonized by fungi (Lynch *et al.*, 1980).

The present respirometric results add further support to the hypothesis that competition between the seed and fungus for oxygen is probably the mechanism by which germination is suppressed. In our experience aspergilli and penicillia, which have small specific rates of oxygen uptake, produce relatively little effect on germination when colonizing the outer husk, but when these fungi colonize the seed beneath the husk they might compete with the seed for the limited supply of oxygen that penetrates the 'plug'. The maximum reduction in germination percentage in low concentrations of oxygen was recorded when more than 80% of the seed carried 100 μg fungus or more beneath the husk. If this fungus was *Penicillium*, it would account for a maximum oxygen uptake of 2.5 μl h⁻¹ seed⁻¹, closely comparable to the 3.5 μl h⁻¹ seed⁻¹ uptake by uncolonized seed at germination. However, if *Mucor* were the colonist, the oxygen uptake by the fungus would be 15 μl h⁻¹ seed⁻¹, greatly in excess of the seed uptake.

These observations are consistent with the effect of the bacterium *Azotobacter chroococcum* on barley seed (Harper & Lynch, 1979). Among the bacteria the *q₀* values of *Azotobacteraceae* are large, up to 223 mmol (g dry wt)⁻¹ h⁻¹ (Williams & Wilson, 1954), more than an order of magnitude greater, for example, than that of *Escherichia coli* and *Klebsiella aerogenes* (Harrison, 1972). Again *A. chroococcum* is notable amongst the bacteria we have tested in preventing seed germination.

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**REFERENCES**


