



Fungal associations with weather stained barley in Western Australia

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Abstract

Barley grain produced along the south coast of Western Australia is often affected by kernel discolouration (KD) due to weather staining. Conditions that favour weather staining may be expected to enhance fungal activity of grain. This study aimed to determine if fungal infection varied with susceptibility to KD and identify principal species of fungi that colonise grain in this environment.

Replicated grain samples were collected from 5 dates when flowering coincided between Stirling (KD susceptible) and Kino Nijo 7 (KD resistant) in a time of sowing experiment at Esperance in 1999 in which plots were either untreated or irrigated overhead late in grain filling.

Fungal ergosterol levels were consistently lower in Kino Nijo 7 with a mean grain content of 1.7 µg ergosterol/g grain compared to Stirling 2.5 µg/g ($P < 0.05$). A concentration of < 3 µg/g has been proposed as suitable for food quality in cereals. Levels were higher under irrigation (1.2 vs 3.1 µg/g, $P < 0.05$). There was a linear relationship between grain colour and ergosterol level. Grain of Kino Nijo 7 is about two Minolta L units brighter than Stirling at the same level of ergosterol.

Nineteen species of fungi were identified colonising barley grain. *Alternaria alternata* was most frequently isolated (84% of all isolates). *Alternaria infectoria*, *Stemphylium botryosum*, *Ulocladium atrum* and *Ulocladium chartarum* were common. *Fusarium spp.*, *Aspergillus spp.* and *Cladosporium spp.* were rarely detected.

The percentage of grain from which no fungi were isolated was consistently higher in Kino Nijo 7 and lower under irrigation. This is consistent with the levels of ergosterol. The frequency and range of species was not affected by variety, flowering time or irrigation.

We hypothesise that Japanese two row malting barleys like Kino Nijo 7, when used to breed varieties with improved grain colour, may also contribute to reduced fungal activity.

Introduction

Weather staining of barley can take three main forms. One common form is kernel discolouration, the colour change from the light straw colour of bright grain to a deep yellow/tan coloured grain (or caramel colour when extreme). Another common form is a dark brown or black staining of the germ end, commonly referred to as either black point or germ end staining. A third extreme form is where a greyish hue or distinctive spots appear on the grain as visible mould formation.

Barley produced along the south coast of Western Australia is often affected by the kernel discolouration (KD) form of weather staining. This study considers the association between KD and grain microflora as conditions that favour weather staining may be expected to enhance fungal activity of grain. Grain that is visibly affected by weather staining is less preferred in the malthouse and brewery because of possibly larger populations of microorganisms that can cause a range of problems.

An extensive study on the suitability of weathered barley for malting and brewing was conducted by the Malt Research Institute 1955 (reference is listed below). The authors concluded that weathered barley steeped at a faster rate, tended toward prolonged post-harvest dormancy and had poor germination energy and vigour. Malt from weathered barley had abnormally high protein modification, high wort colour, and tended to be lower extract. The beers were abnormally high in nitrogen, dark in colour and less desirable in taste and colour.

Increased water sensitivity may be the result of microorganisms living on the surface of the grain, which compete with the embryo for available oxygen (Major and Roberts 1968, Kelly and Briggs, 1992).

Gushing in beer has long been associated with substances produced by weathered barley (Gjertsen 1963) and a number of microflora associated with grain are now understood to produce this negative effect on beer quality. Enari 1995, reported that a group of Japanese scientists had isolated gushing inducing peptides formed by species *Fusarium*, *Penicillium*, *Nigrospora* and *Stemphylium*. Once formed in the field or the malthouse nothing can be done to destroy the gushing-inducing substances.

Microflora associated with grain can sometimes produce harmful mycotoxins. The toxins, causative fungi and biological actions have been summarised (Burger and LaBerge 1985).

| Mycotoxin | Fungus | Type of action |
|-----------------|------------------------------|------------------------|
| Ergot alkaloids | <i>Claviceps pupurea</i> | Ergotism |
| Ochratoxin | <i>Aspergillus ochraceus</i> | Kidney toxin |
| Sterigmatocysin | <i>Aspergillus flavus</i> | Liver carcinogen |
| Vomitoxin | <i>Fusarium spp.</i> | Alimentary canal toxin |
| Aflatoxin | <i>Aspergillus flavus</i> | Liver carcinogen |

Microorganism-produced enzymes can contribute to an over estimation of the enzyme potential of the malt. Endo-(1→4)-β-glucanase produced by microorganisms can solubilize β-glucan, however it is unlikely that surface growing organisms have a function in cell wall modification during germination (Bamforth and Barclay 1993).

Preliminary studies on the enzymes causing black point in both wheat and barley (Williamson 1997) indicate the involvement of peroxidase enzymes and these same enzymes may be involved in the KD of barley. Peroxidases are thought to have a role in protection against fungal pathogens (Shewry 1993), they may also be responsible for the breakdown of polyphenols. Astringency and perhaps body in beer may be due to polyphenols (tannins) originating in the husk of barley (Eastmond and Gardner, 1974). In the brewing process polyphenols from the addition of hops add the bitterness units required. Non specific peroxidases are probably very important in eliminating peroxide by reacting with it and polyphenols (Bamforth *et al*, 1991). As such they are more important in oxidising polyphenol than polyphenol oxidase which is extensively lost during malting (Clarkson *et al*, 1992). The elimination of peroxidases by plant breeding may have significant benefits to the brewing industry, however it may also reduce the grain's ability to defend itself from invasion by fungal pathogens.

Research conducted at the University of Minnesota (Miles *et al*, 1989) showed that it was possible to breed for resistance to weather staining. Screening of a wide range of barley germplasm in Western Australia (Young 1996) revealed some promising sources of tolerance to kernel discolouration, especially among lines originating from Japan. No information is available on the level of fungal infection of grain that is significantly brighter. Furthermore the principal species of fungi that occur on weather stained barley in Western Australia are poorly understood. The aim of this study was to determine if fungal infection varied with susceptibility to KD and identify principal species of fungi that colonise grain in this environment. By breeding for inherently brighter barleys and selecting bright grain for malting grade do we also reduce the number or amount of microflora in barley grain?

Materials and Methods

Replicated grain samples were collected from 5 dates when flowering coincided between Stirling (KD susceptible) and Kino Nijo 7 (KD resistant) in a time of sowing experiment at Esperance in 1999 in which plots were either untreated or irrigated overhead late in grain filling. Grain brightness was measured as the 'L' value for lightness with a Minolta colorimeter model CR310.

Fungal biomass in grain samples was assessed using ergosterol assay techniques (Seitz *et al*, 1977). Ergosterol is a component of fungal cell walls and is thus correlated with hyphal growth and biomass. Ergosterol is the major sterol produced by fungi but at most is a very minor component of plant sterols (Pitt and Hocking, 1997).

Identification of grain microflora was undertaken by the Queensland Department of Primary Industries. Seed was surface sterilised by immersing for 2 min in 70%

ethanol followed by 2 min in 0.4% sodium hypochlorite. The surface sterilised seed was then plated onto half-strength potato dextrose agar with streptomycin (PDA+S) as well as dichloran rose bengal chloramphenicol agar (DRBC) at the rate of five seeds per plate (Pitt and Hocking, 1997). PDA+S was chosen to select *Fusarium* and DRBC to select *Alternaria* and other dematiaceous hyphomycetes. Twenty seeds of each barley sample were plated onto each medium. The plates were incubated for 5 days at 25°C in darkness.

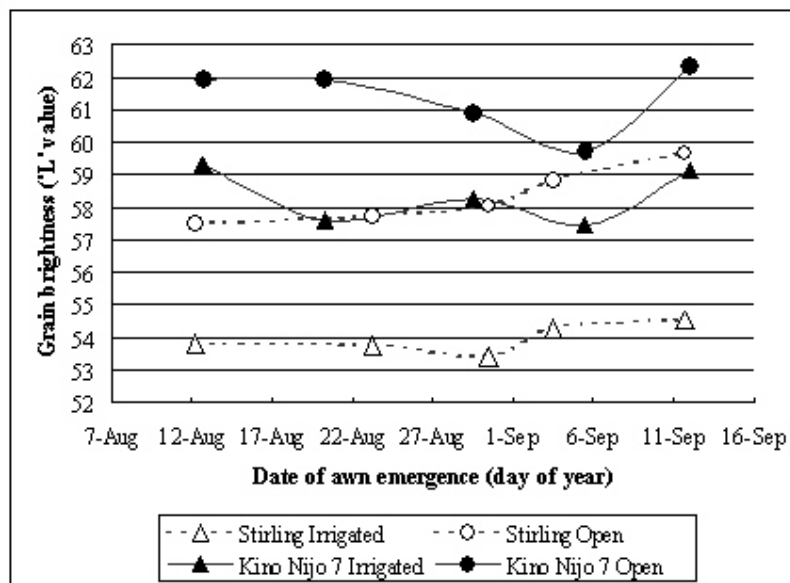
After incubation, the fungi growing from each seed were identified. To aid identification, 197 isolates were brought into pure culture on full-strength PDA.

General statistical analysis was conducted in GENSTAT (Payne *et al.* 1998).

Results and Discussion

Grain brightness for variety Kino Nijo 7 at each heading date was significantly greater than Stirling (on average by three units). The difference was even greater (4.3 units brighter on average) in the irrigation treatment (Figure 1.)

Figure 1. The effect of variety and irrigation on grain brightness at a range of dates of ear emergence.



Fungal ergosterol levels were consistently lower in Kino Nijo 7 with a mean grain content of 1.7µg ergosterol/g grain compared to Stirling 2.5 µg/g ($P<0.05$). Levels were higher under irrigation (1.2 vs 3.1 µg/g, $P<0.05$). There was a weak but statistically significant relationship between grain colour and ergosterol level (Figure

frequently reported from barley seed in overseas studies were not detected. In particular, *Fusarium spp.* and *Bipolaris sorokiniana* were infrequently isolated.

Results are expressed as the percentage of samples colonised by particular fungi on PDA+S as well as the overall percentage of seeds in each sample colonised (Table 1). The number and identity of fungi isolated from barley seed on PDA+S is similar to that on DRBC.

It is promising that the species that are commonly associated with the production of mycotoxins and gushing of beer were either absent or very infrequent in this study, even under irrigation induced weather damage.

Table 1. Frequency of fungal species by 20 seed samples and by individual seeds of Kino Nijo 7 and Stirling at five sowing dates and two irrigation treatments

| Fungus | % Samples detected | % Seeds colonised within sample |
|-------------------------------------|---------------------------|--|
| <i>Alternaria alternata</i> | 100.0 | 82.6 |
| <i>Ulocladium atrum</i> | 47.1 | 4.4 |
| <i>Alternaria infectoria</i> | 34.3 | 2.5 |
| <i>Sterile mycelium</i> | 34.3 | 2.5 |
| <i>Ulocladium chartarum</i> | 18.6 | 1.3 |
| <i>Stemphylium botryosum</i> | 12.9 | 1.2 |
| <i>Fusarium equiseti</i> | 11.4 | 0.7 |
| <i>Rhizoctonia sp.</i> | 10.0 | 0.5 |
| <i>Fusarium graminearum</i> | 4.3 | 0.2 |
| <i>Bipolaris sorokiniana</i> | 2.9 | 0.1 |
| <i>Cladosporium cladosporioides</i> | 2.9 | 0.1 |
| <i>Epicoccum nigrum</i> | 2.9 | 0.1 |
| <i>Aspergillus spp.</i> | 1.4 | 0.1 |
| <i>Curvularia brachyspora</i> | 1.4 | 0.1 |
| <i>Phoma sp.</i> | 1.4 | 0.1 |
| <i>Ulocladium botrytis</i> | 1.4 | 0.1 |

Conclusions

We suggest that Japanese two row malting barleys like Kino Nijo 7 may be used to breed varieties with improved grain colour and may also contribute to reduced fungal activity.

In order to ascertain that grain is received at an ergosterol level below that suggested for human consumption an ergosterol assay could be used to determine the relationship between fungal activity and grain brightness for any new varieties with resistance to kernel discolouration.

Fungi commonly associated with the production of mycotoxins and gushing were not present at significant levels in grain analysed in this work and are likely to be absent in Western Australian grain that meets the brightness standard of exceeding an 'L' value of 55 units.

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