Testing for Sprout Damage in Malting Barley Using the Rapid Visco-Analyser

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ABSTRACT

The ability of the Rapid Visco-Analyser (RVA) to detect sprout damage has been assessed using seven cultivars of Australian malting barley. The RVA stirring number (SN, viscosity at 3 min) correlated well with falling number, α-amylase levels, and the subsequent life span of the grain. Sprout damage in malting barley can be reliably detected using the RVA SN method. The relationship between SN and retention of acceptable viability in storage for malting was used to devise a method for determining threshold SN values to indicate whether a sample is sound or damaged. Suggested cutoff values, below which a sample could be classified as damaged, ranged between 120 and 150 Rapid Visco units, depending on cultivar.

Preharvest sprouting in barley predisposes it to a relatively rapid loss of viability in storage (Carn 1982, Moor 1987, Munck 1987, Bason et al 1991a). When viability falls below 95%, malting barley is downgraded to feed grade with consequent financial loss.

In Australia, detection of sprouted barley is largely performed by visual inspection. This method is subjective, poorly correlated with subsequent life span (Moor 1987), and lacks the necessary sensitivity to detect minor levels of damage that can significantly reduce storage life (Bason et al 1991a).

A viscometric method for detecting sprout damage in wheat was reported that employed the Rapid Visco-Analyser (RVA, Newport Scientific Pty Ltd., Narrabeen, NSW, Australia) (Ross et al 1987). This instrument was designed for use under receival conditions and offered several advantages over the Hagberg falling number (FN) method (AACC 1983).

The goal of the work reported here was to assess whether and how the RVA could be used to reliably screen for weather damage in malting barley. In particular, sensitivity to sprout damage, effect of cultivar, and repeatability of measurement have been investigated.

MATERIALS AND METHODS

Samples

Fifty-five samples of 1989-90 and 1990-91 harvest malting barley (cvs. Grimmett, Schooner, Clipper, Lara, Skiff, Stirling, and Parwan) were obtained from 31 sites in New South Wales, Victoria, Queensland, South Australia, and Western Australia. Sprout damage is sporadic and cultivars vary by region, so it was not possible to obtain samples of all cultivars at all sites with a full range of damage. Therefore, both sound and weather-damaged samples, obtained from commercial sources and breeders throughout Australia, were selected to represent the widest available range of sites and damage in the major malting cultivars.

To increase the number of damaged samples, grain from all cultivars (except Lara and Parwan) was artificially sprouted to produce 153 samples with FN values ranging from 62 to 643 sec. Grain was artificially sprouted by moistening 1,000-g samples of grain to 40% moisture content (wet basis) and standing grain in beds 1 cm deep under humidified air for one, two, three, or four days at 23°C. In these samples, sprouting was evident in radicle emergence after one or two days. By the fourth day, seminal roots were ~10 mm long and the coleoptile had emerged, which is similar to heavy sprouting sometimes observed in the field. Grain was then dried overnight at 30°C in a static bed dryer (face velocity = 150 mm/sec) to ~11% moisture content.

Testing for Sprout Damage

Duplicate subsamples from each sample were ground in a hammer mill (model 3100, FN, Stockholm, Sweden; sieve size: 0.8 mm). FN was determined using method 56-81B (AACC 1983). The Ceralpha method (McCleary and Sheehan 1987) was used to determine α-amylase values.

The RVA test used was the same as reported in Ross et al (1987), except whole barley meal was used, the test was performed at 95°C, and the initial high-speed stir lasted for 10 sec. The RVA parameters measured were: height of the peak (Rapid Visco units [RVU], where one RVU = ~10 cP), time to peak (min), area under the curve 0-3 min (RVU × min), and the stirring number (SN, viscosity at 3 min) of the pasting curve (Ross et al 1987 and Fig. 1). A subset of 149 subsamples representing all cultivars was also tested in the RVA. $AgNO_3$ (75 μmoles per gram of barley meal) was added to these subsamples to nullify amylase effects (Meredith 1970) and facilitate comparisons between cultivars.

Two other subsets of samples were used to assess the longevity of the grain. The first subset consisted of a single sample of Grimmett subdivided into 10 subsamples and artificially sprouted, in duplicate, for zero, one, two, three, and four days, as described above. The second subset consisted of 48 subsamples, excluding all artificially damaged grain. This subset contained both sound and field-damaged samples from all cultivars (except for Stirling, where no field-damaged grain was available). For both subsets, the samples were conditioned to 10.1 ± 3% moisture content and stored under accelerated aging conditions of 60°C and 50% rh. The samples were continuously flushed with air heated and humidified using techniques previously described (Gras et al 1989). The moisture level selected represented the equilibrium for barley stored under these conditions (Bason and Gras 1988). Subsamples of stored grain were periodically removed, and germination levels were assessed using the germinative energy three-day test (EBC 1963). The life span of each stored sample was calculated as the time for germination to fall to 95% (t_{95}) and to 50% (t_{50}) by interpolation. These two values were selected because barley viability must retain at least 95% to be accepted for malting, and the half-life of populations is commonly used to index susceptibility to harmful substances or treatments. In four of the samples from the second subset of stored grain, t_{95} data were not available because the initial germination was below 95%.

Statistical Analyses

RVA parameters and FN, α-amylase, and life span data were compared by correlation analysis. Life span and α-amylase data were transformed to their natural logarithms for the correlation analyses. Repeatability of measurement using the RVA was determined by one-way analysis of variance (ANOVA) with sample as the factor. The resulting root mean square (RMS) of residuals due to replication was used to estimate repeatability of measurement. Cultivar effects on RVA were first assessed by analysis of covariance (ANCOVA), using FN as the covariate. These effects were further analyzed by one-way ANOVA on the RVA results from $AgNO_3$-treated samples, with cultivar as the factor. RVA SN data were compared to life span data by regression analysis. These procedures were carried out using the MSUSTAT statistical analysis package (Lund 1988), except for the ANOVA to test repeatability, which was performed using MINITAB (Ryan et al 1985).

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RESULTS AND DISCUSSION

Effect of Sprouting on Grain Longevity

The preliminary study using artificially damaged Grimmett was designed to exclude genetic and environmental variations, other than the duration of sprouting. The results indicated a strong relationship between increased sprout damage (reflected in a declining SN) and decreased longevity (Table I). This effect was marked even after one day, when sprouting was still difficult to detect visually. Although there may be other environmental effects influencing this relationship (e.g., late-maturity α-amylase), this result indicates that sprout damage has a major detrimental influence on grain longevity, which is consistent with previous reports (Carn 1982, Moor 1987, Munck 1987, Bason et al 1991a).

Sensitivity to Sprout Damage

The sensitivity of the RVA methods to sprout damage was assessed by correlation with FN, α-amylase level, and life span of corresponding samples (Table II). The correlation data was pooled for the seven cultivars tested.

Of the various RVA parameters tested, SN was best correlated, overall, with the other quality tests (Table II). The SN is therefore considered the most sensitive of the RVA methods tested in detecting sprout damage. SN was also correlated at least as well with storage life (t_{95} and t_{50}) as FN and log α-amylase were (Table II), further indicating its suitability for detecting sprout damage. The good correlation with the other more established methods, and particularly with subsequent storage life, shows that the RVA can be used effectively to screen for weather damage in malting barley. The SN method employed here is essentially the same technique as that developed for use in wheat (Ross et al 1987).

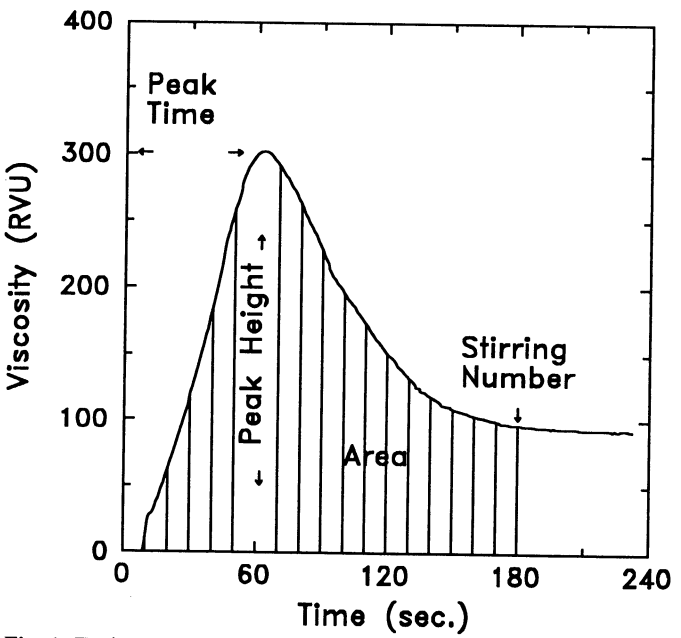

Fig. 1. Typical Rapid Visco-Analyser pasting curve for malting barley, showing the parameters assessed. RVU = Rapid Visco units.

TABLE I
Effect of Artificial Sprouting on Stirring Number and Storage Half-Life (t_{50})[a] for Grimmett Barley

Quality Test	Replicate	Days Sprouted 0	1	2	3	4
Stirring number	1	157	130	23	17	17
	2	167	137	17	19	13
t_{50} (days)	1	9.8	1.7	0.8	0.8	0.8
	2	6.9	1.6	0.6	0.6	0.5

[a] t_{50} = Time to a decline to 50% germination under accelerated aging conditions (60°C and 50° rh).

Methods for measuring the average damage level across an homogenized (ground) sample have been criticized for not reflecting the "actual percentage of seeds affected" (Moor 1987). This argument assumes that only a certain percentage of seeds in a given sample are damaged ("affected") and that they exhibit a reduced storage life more rapidly than the undamaged component. If this were the case, a few very badly damaged seeds could markedly skew the estimate of damage for a ground sample; however, the overall germination percentage would scarcely be affected and the grain would remain suitable for malting. The inevitable result would be a poor correlation between grain life span and estimate of damage using ground sample methods such as the SN.

However, the good correlation of SN with subsequent storage life observed here (using only field samples) indicates its suitability as a screening method to avoid viability loss in storage. Good correlations have also been observed in other cereals between FN, which uses ground sample, and the fluorescein dibutyrate method, which assesses sprouting in individual grains (Munck 1987). It would appear that, although damage may vary from seed to seed within a sample, the underlying level of damage is centered around a mean rather than being bimodal. When visually assessed, individual kernels within a sample are normally scored as sound or shot, giving the impression of bimodality. Visual assessment of the onset of embryo growth also lacks sensitivity and objectivity. The poor correlations observed between visual scores of sprouting and subsequent storage life (Moor 1987) underline the problems with this approach.

Effect of Cultivar

The effect of cultivar on SN was analyzed initially by ANCOVA, using FN as the covariable. The effect of cultivar was significant ($F_{6,292} = 14.3$, $P < 0.001$). A further study of the effect of cultivar was conducted using samples treated with $AgNO_3$ to remove the amylase effect. ANOVA of these results indicated a significant difference between the cultivars tested ($F_{6,142} = 36.2$, $P < 0.001$), presumably reflecting small differences between the starch pasting

TABLE II
Correlation Coefficients (*r*-values)[a]
Rapid Visco-Analyser (RVA) Parameters and Other Measures of Sprouting Damage in Malting Barley

Parameter[b]	PH	PT	AREA	FN	LAA	LT50	LT95
SN	0.87	0.86	0.92	0.97	−0.91	0.90	0.85
PH		0.67	0.97	0.86	−0.89	0.85	0.76
PT			0.71	0.88	−0.83	0.86	0.72
Area				0.89	−0.90	0.85	0.78
FN					−0.91	0.89	0.82
LAA						−0.87	−0.80
LT50							0.92

[a] All correlations are significant ($P < 0.001$).
[b] SN = RVA stirring number, PH = RVA peak height, PT = RVA peak time, Area = RVA area, FN = falling number, LAA = natural logarithm (LN) of α-amylase, LT50 and LT95 = LN days for viability to decline to 50 and 95% (respectively).

TABLE III
Comparison of Mean Stirring Numbers for Different Cultivars of Barley (Samples Treated with $AgNO_3$)

Cultivar	Mean[a]	*n*	Cutoff Value[b]
Skiff	148 a	16	120
Clipper	154 a	17	120
Grimmett	160 b	35	130
Schooner	161 b	29	130
Lara	165 bc	14	130
Stirling	169 c	10	140
Parwan	183 d	28	150

[a] Means are significantly different ($P < 0.05$) where they do not share the same letter.
[b] Suggested cutoff values, below which a sample could be classified as sprout damaged.

properties of different cultivars. Cultivar means are given in Table III and are indicative of expected values in sound grain for each cultivar.

Cultivar effects have been reported for FN results from wheat (Ringlund 1983, Moss 1987). In barley, there are considerable varietal differences in starch and cell-wall polymers (Henry 1987, 1989). Viscometric methods, such as the FN and RVA, detect activity of the enzymes associated with germination on the endogenous substrate; therefore, the observed varietal effects are not surprising.

Adding a pregelatinized substrate and reducing the temperature of the instrument to prevent gelatinization of the endogenous starch has been tested (Bason et al 1991b). This method did reduce the effect of cultivar; however, sensitivity to low levels of enzyme activity was also reduced. Because even low levels of damage are associated with loss in storage life (Table I, Fig. 2), the use of an added substrate to test for weather damage in malting barley was not pursued.

The difference in SN between cultivars, although minor compared to the effect of amylase, should be taken into account when assessing the level of damage in field samples.

Repeatability of Measurement

The repeatability of the measurement of SN was assessed by one-way ANOVA, using the replicate measurements of the 153 samples not treated with $AgNO_3$ (9–198 RVU SN) to assess residual variance. The ANOVA yielded a RMS of residuals of 9.2 RVU, representing about 5% of full-scale variation.

Determination of Screening Cutoff Values

Even minor levels of damage are associated with a loss of subsequent storage life (Fig. 2). It is, therefore, desirable to determine an SN cutoff point, below which samples are deemed to be sprout damaged, that is as stringent as practicable.

Cutoff points for each cultivar tested were determined by establishing sound SN values for each cultivar, and then subtracting a quantity likely to reflect a loss in storage life. First, the mean sound value for each cultivar, derived using the $AgNO_3$-treated samples (Table III), was taken as representative of sound samples. Next, the effect of cultivar on the regression of t_{95} against SN (from samples not artificially damaged and not treated with $AgNO_3$, see Fig. 2) was tested. No significant differences in this regression were observed between the slopes ($F_{5,30} = 0.74$, $P > 0.05$) or intercepts ($F_{5,35} = 1.76$, $P > 0.05$) of the various cultivars, even allowing for Parwan with two low SN values of about 100 (Fig. 2). These two samples had relatively low initial germination levels (just over 95%), which explains their relatively rapid decline to 95% germination.

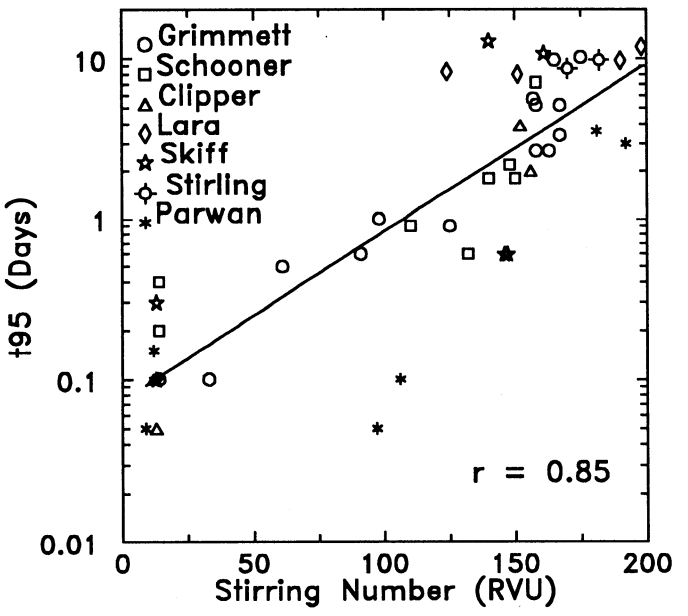

Fig. 2. Relationship between Rapid Visco-Analyser stirring number and grain life span for seven cultivars of Australian malting barley. Life span is days of storage under the accelerated aging conditions of 60°C and 50% rh until a decline to 95% germination.

There was no significant varietal effect, so data were pooled for all cultivars and a common regression of SN was fitted against t_{95}. The RMS of residuals of this regression was 33 RVU. A decline in SN from sound levels by this value would reflect a likely reduction in storage life. This value is also well in excess of the estimate of repeatability of measurement given above. Thus, a decline of this magnitude can be reliably detected. Therefore, the RMS value of the common fit was subtracted from the mean sound value for each cultivar.

With some rounding, this approach yielded the cutoff values given in Table III. Samples with SN values below the appropriate cutoff value could be classified as damaged. These suggested cutoff values could be used as a basis for segregating barley on receival at the silo.

The cutoff values in Table III could be safely lowered where storage managers could guarantee short storage periods (< 3 months) prior to malting, or where grain is to be stored cool (<20°C). Such grain should still have a SN no lower than 100 (~2 × RMS below the sound value).

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