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RESPIRATION OF WHEAT GRAIN STORED IN DIFFERENT ENVIRONMENTS

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ABSTRACT

Respiratory activity in grain is usually measured by the the release of carbon dioxide (CO_2) or the uptake of oxygen (O_2) in a closed system. An automatic electrolytic respirometer, which constantly monitors O_2 uptake and allows overall measurement of CO_2 production, has allowed replicate determinations of respiration rates in 25-g samples of grain at different constant temperatures (15–35°C) and water activities (0.65–0.95 a_w). O_2 uptake increased linearly with temperature up to 35°C and with time at water activities above 0.90 a_w, but not at lower water activities. With a_w and high germinability, most respiration could be attributed to respiration by the grains themselves. However, with 0.90 a_w, germinability decreased and microbial respiration predominated. Autoclaved grain inoculated with either *Eurotium amstelodami* or *Penicillium aurantiogriseum* respired at similar rates at 0.85 a_w and 20°C, but O_2 uptake by *P. aurantiogriseum*-inoculated grain was more than ten times that of *E. amstelodami*-inoculated grain at 0.90 a_w. Comparisons of O_2 consumption and CO_2 production generally yielded respiratory quotients less than 1.0 except at 15°C. No visible mould developed after 7 d at 15°C but the amount of visible mould increased from 20 to 35°C. Up to 0.13% of the dry matter was lost before the grain was visibly mouldy whereas 0.13–1.24% of the dry matter was lost from visibly mouldy grain, the exact percentage depending on the temperature and water content.

INTRODUCTION

Respiration is a fundamental process, common to all living organisms, which provides energy for metabolism and growth. By aerobic respiration, carbohydrates are oxidised into carbon dioxide (CO_2) and water with the release of energy as per the equation: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 2835$ kJ. A 1% loss of carbohydrate is thus accompanied by the production of 14.7 g CO_2 kg^{-1} dry matter. Respiration over time can be measured by the uptake of oxygen (O_2), or the production of CO_2, or even as the loss of dry matter or

temperature change. The grain itself, its microflora and its insect infestations all contribute to the total respiration of stored grain, and the extent of respiration is a measure of the total metabolic activity of the system. The intensity of the process is governed by the following physical parameters: water availability, water temperature, and, to a lesser extent, O_2 concentration, the degree of microbial contamination, mechanical damage and the conditions and length of previous storage.

Microorganisms, especially fungi, are important factors in the deterioration of stored grain. They contribute greatly to total respiration at water activities (a_w) which are insufficient to support germination. However, the relative contribution of grain and microorganisms to total respiration remains controversial. Respiration of maize with 22–27% water content was reported to be considerably greater than that of its microflora (Seitz *et al.*, 1982). By contrast, relatively low and constant respiration rates were reported from mould-free wheat with 12–35% water content (Larmour *et al.*, 1935; Hummel *et al.*, 1954). However, the sterilants used might have affected physiological processes in the seed. Woodstock and Coombs (1965) found that respiration decreased by 10% when fungi were eliminated from up to 80% of the seeds by using combinations of sodium hypochlorite, phenacridine chloride and gamma irradiation.

Respiration of naturally colonised barley and maize grains with high water content has been utilised to preserve the grain for animal feed during its storage in sealed steel bins and in unsealed concrete staved silos and also during storage in underground pits (Lacey, 1971, 1972, 1988; Clarke and Hill, 1981; Hill *et al.*, 1983).

METHODS USED TO MEASURE RESPIRATION

Respiration has usually been measured by titration after absorbing CO_2 in ascarite or alkali, by using infra-red gas analysers or by monitoring O_2 production. Milner and Geddes (1945) drew 150–2000 ml of air at a constant rate over 23.5 h into a respirometer. The air was freed of CO_2 and humidified with soda lime in sulphuric acid and saturated salt solutions before being passed through grain with an appropriate water content which had been placed in a jar standing in a constant-temperature water bath. Afterwards, a gas sample was withdrawn for analysis using Haldane-Henderson gas analysis. Essentially similar methods were used by Al-Yahya *et al.* (1993) and Aljinovic *et al.* (1995), except that the CO_2 was first absorbed with KOH, the grain was stored in plexiglass tubes, the air was subsequently dried with anhydrous $CuSO_4$ and $Mg(ClO_4)_2$ (Mg perchlorate) and CO_2 was absorbed onto Sulaimanite, a mixture of KOH solution and vermiculite.

Wilcke *et al.* (1993) used a more complex apparatus to continuously monitor CO_2 evolution from fungicide-treated maize. Sample bottles, containing maize grain conditioned to the required water content and standing in temperature-controlled water baths, were connected to a compressed air supply and an infra-red spectrometer by airlines with computer-controlled valves. Airlines and bottles were individually purged with compressed air conditioned to the appropriate relative humidity by being passed through glycerol solutions for 4 min every 20 min, to prevent CO_2 from accumulating and inhibit-

ing fungal growth. The current rate of CO_2 production in each bottle was calculated from three measurements of CO_2 concentration taken for 3 min every 6 h. The resulting rates of CO_2 production were then integrated to give a rate for cumulative CO_2 production and to allow the calculation of dry-matter loss.

We used an electrolytic respirometer (Tribe and Maynard, 1989) to continuously monitor O_2 uptake by the respiring grain. When CO_2 from grain respiration was absorbed into the alkali, there was a decrease in the air pressure within the glass leaching tube mounted in a temperature-controlled water bath. This caused a saturated $CuSO_4$ solution to rise in a U-tube until the solution came into contact with a platinum anode, thus closing a circuit with a copper cathode immersed in the solution. O_2 was produced at the anode until pressure was equalised, breaking the contact with the cathode. Periods of operation were recorded electronically and converted into volumes of O_2 produced. At the end of the experiment, the amounts of CO_2 absorbed into the alkali were determined by titration, and the respiratory quotient (CO_2 evolved/O_2 uptake) and dry matter loss were calculated. In theory, the apparatus and software allowed up to 128 individual treatments to be monitored simultaneously, although only a maximum of 32 tubes was found to be manageable in practice (one or two racks of 16 tubes each per water bath). The data obtained by this method, using winter wheat grain, is reported in this paper.

RESPIRATION OF GRAIN AT DIFFERENT TEMPERATURES AND WATER ACTIVITIES

Respiration was measured during the incubation of naturally contaminated winter wheat grain, cv Avalon, at water activities ranging from 0.70 to 0.95 a_w, in 0.05 a_w steps, and at temperatures ranging from 15 to 35°C, in 5°C steps, over 160–165 h. All experiments, except those at 35°C, were repeated at least twice. The results are shown in Figs. 1 and 2.

Cumulative O_2 consumption generally increased with both a_w and temperature. However, there was an initial lag in O_2 consumption at 15–25°C and a_w followed by a period of increased activity until the end of the experiment. Otherwise, O_2 consumption increased linearly with time. Respiration was most rapid at 0.95 a_w and 25–35°C and least rapid at 0.80 a_w and 15°C. However, there was little difference in O_2 consumption between 20 and 25°C. About 100 ml O_2 were utilised by 25 g wheat grain over 160 h at 0.95 a_w/25°C and at 0.90 a_w/35°C. Measurement of CO_2 production enabled respiratory quotients to be calculated (Table 1). Except at 15°C, respiratory quotients (*RQ*) were generally in the range 0.5–1.5, especially at low a_w where *RQ* up to 5.13 were found. The mean *RQ* from all treatments was 1.11 ± 0.228, agreeing closely with other published data.

Assuming an *RQ* of 1.0, dry matter losses, calculated using O_2 uptake data, generally increased with increasing a_w and temperature (Table 2). They were greatest at 0.95 a_w/25–35°C and least at 0.80 a_w/15°C. In many of the treatments (marked in bold in Table 2), more than 1% of the grains were visibly mouldy, but this did not necessarily indicate large dry matter losses. For instance, only 0.13% of the dry matter was lost from visibly

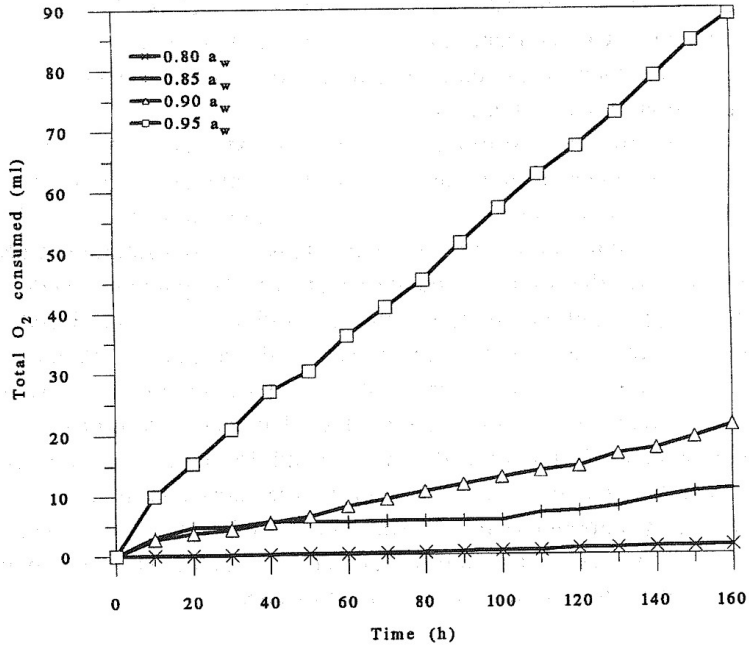

Fig. 1. Respiration of wheat grain cv Avalon at different water activities (a_w) at 20°C.

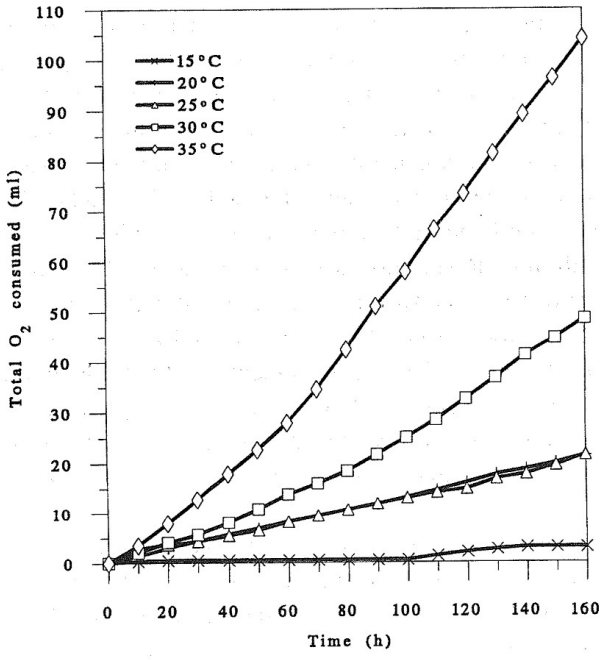

Fig. 2. Respiration of wheat grain cv Avalon at different temperatures at 0.9 a_w.

TABLE 1
Respiratory quotients calculated from the respiration of wheat cv Avalon over 160 h incubation at different a_w and temperatures

	Mean respiratory quotient ± SEM at stated temperature (°C)				
a_w	15	20	25	30	35
0.80	5.13 ± 0.91	0.68 ± 0.13	0.45 ± 0.04	0.61 ± 0.04	0.84 ± 0.04
0.85	2.50 ± 0.70	2.50 ± 0.70	0.54 ± 0.21	0.87 ± 0.07	0.67 ± 0.11
0.90	1.81 ± 0.39	0.75 ± 0.11	0.54 ± 0.17	0.90 ± 0.01	0.82 ± 0.03
0.95	1.18 ± 0.24	0.73 ± 0.02	0.59 ± 0.09	0.90 ± 0.07	1.02 ± 0.09

TABLE 2
Calculated dry matter losses in wheat cv Avalon over 160 h incubation at different a_w and temperatures

	Calculated dry matter loss (%) at stated temperature (°C)				
a_w	15	20	25	30	35
0.80	0.007	0.020	0.039	0.061	**0.133**
0.85	0.018	0.027	**0.130**	0.161	**0.372**
0.90	0.085	**0.226**	0.436	**0.347**	**0.774**
0.95	**0.517**	0.762	**1.210**	**1.187**	**1.239**

Bold text indicates visible moulding after 160 h incubation.

moulded grain at 0.85 a_w/25°C. The range of a_w supporting visible moulding increased with increasing temperature and, conversely, the range of temperatures supporting moulding increased with increasing a_w.

FUNGAL RESPIRATION

Respiration of autoclaved grain inoculated with *E. amstelodami* and *P. aurantiogriseum*, either separately or in combination, was compared at 0.85 and 0.90 a_w and at 20°C. Respiration over 350 h followed a sigmoid pattern for the fastest respiring treatments (*P. aurantiogriseum*/0.90 a_w and mixed inoculum/0.90 a_w) but failed to reach the plateau phase for others. The least O_2 was consumed by *E. amstelodami* alone at 0.90 a_w and the most by the mixed culture at 0.90 a_w, although there was no significant difference from *P. aurantiogriseum* alone. Respiration of *E. amstelodami* over 350 h incubation at 0.85 a_w was more than twice that at 0.90 a_w, although there was little difference up to 250 h. Respiration of *P. aurantiogriseum*-inoculated grain differed little from that with *E. amstelodami* at 0.85 a_w but increased more than tenfold at 0.90 a_w. Respiratory quotients

ranged from 0.75 to 0.89 and calculated dry matter losses after 14 d ranged from 0.06% (*E. amstelodami*/0.90 a_w) to 0.80% (mixture/0.90 a_w).

DISCUSSION AND CONCLUSIONS

Respiration has frequently been used to measure grain deterioration (Paster *et al.*, 1992) and the effectiveness of physical and chemical methods of mould prevention (Al-Yahya *et al.*, 1993; Wilcke *et al.*, 1993; Aljinovic *et al.* (1995). It has also been used to assess dry matter losses during storage. Only 0.5% dry matter loss in maize is sufficient to indicate that the grain is unfit for use (Saul and Lind, 1958; Saul and Steele, 1969), and such losses may occur before moulding is visible (Seitz *et al.*, 1982). Storage life before quality loss becomes unacceptable is a function of kernel damage, water content and temperature, expressed by the following formula: $\theta = \theta_R \times M_T \times M_W \times M_D$, where θ is the allowable storage time before 0.5% dry matter loss, θ_R is the elapsed time for maize grain with 25% water content and 30% of the kernels mechanically damaged to lose 0.5% dry matter at 15.5°C. M_T, M_W and M_D are multipliers used to correct for actual temperature, water content and mechanical damage determined experimentally. Storage life decreases as temperature, water content and mechanical damage increase. Such multipliers and the respiration data on which they are based have been incorporated into models of ambient air drying of cereal grains (Thompson, 1972; Stroshine and Yang, 1990). Allowable dry matter losses for wheat have ranged from 0.1 to 2% (Kreyger, 1972; Hall and Dean, 1978; White *et al.*, 1982). However, although White *et al.* (1982) predicted that wheat with 18.4% water content could be stored safely for 55 d, visible moulding appeared after only 23 d, suggesting that 0.04% dry matter loss was the limit for acceptability. Bailey (1940) suggested that respiration rate might be proportional to kernel size. Brook (1987) calculated that 0.085% dry matter loss in wheat was equivalent to 0.5% loss in maize. Our results tend to support the conclusions of Brook (1987).

The results obtained with the automatic electrolytic respirometer agree well with those reported previously by different authors (Fig. 3). Although respiration rates described by Milner *et al.* (1947), Scholz (1962) and Kittock and Law (1967) were much faster than those found in our experiments, they were also outside the range of other data. Kittock and Law (1967) were studying seed germination, which may account for the faster respiration in their experiments, but the reasons for other deviations are not known. Respiration rates determined by White *et al.* (1982) were slower than those in our experiments, but they sampled only three to five times per week, depending on temperature, and it is possible that CO_2 concentrations became inhibitory to respiration. The mean repiratory quotient agrees well with previous reports, but the high value at low temperatures needs to be explained. Allowance may need to be made for the absorption of CO_2 by the grain (Cofie-Agblor *et al.*, 1995).

In a personal communication, Nellist and White have taken our respiration data and calculated the results as mg O_2 d^{-1} kg^{-1} dry grain (R) divided by incubation temperature (θ) to give R/θ with units of mg O_2 d^{-1} kg^{-1} dry matter $°C^{-1}$. There was a linear relationship

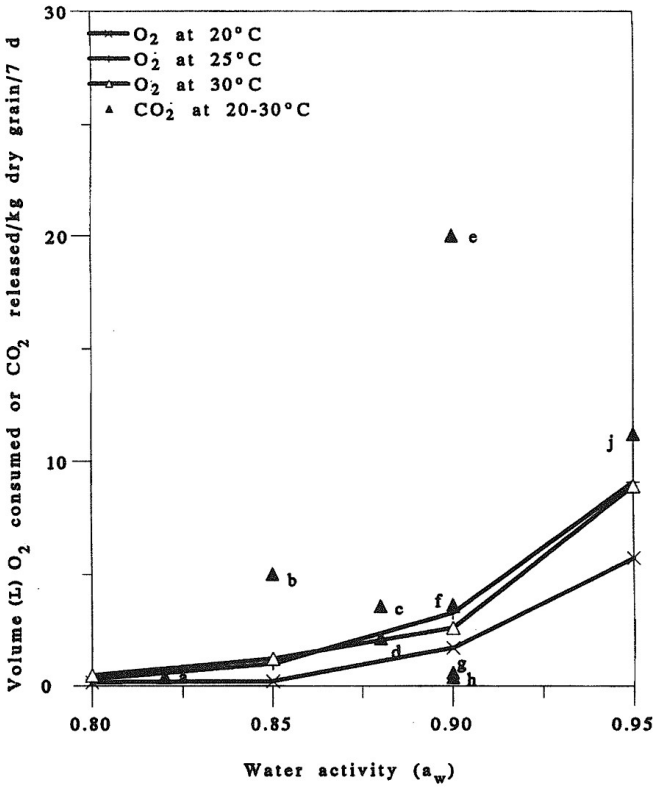

Fig. 3. Respiration of wheat grain cv Avalon measured in electrolytic respirometer over 7 d compared to published data on wheat grain respiration: a, Bailey (1940); b and e, Scholz (1962); c and f, Milner *et al.* (1947); d, Larmour *et al.* (1935); g and h, White *et al.* (1982); j, Woodstock and Justice (1967).

(r^2 = 0.9594) between R/θ and a_w (Fig. 4), corrected for the effects of temperature following Chen and Morey (1989), even though the calculated a_w at 0.90 and 0.95 a_w and 25°C was greater than that indicated by the water content/water activity isotherms determined experimentally for the grain used. This relationship was then expressed in the following equation, which is to be inserted into models of ambient air drying of wheat grain in predicting storage life and dry matter losses:

$$C = \frac{a_1 + a_2}{Y\,(1 + \exp(-(a_5 + a_6 t + a_7\theta)\,(w - a_8)))}$$

where C = cumulative O_2 consumption (mg O_2 kg^{-1} dry matter), t = time (h), w = water content (% wet basis), θ = temperature (°C), $Y = 1 + \exp(a_3(a_4 - \theta))$, $a_1 = 345.83$, $a_2 = 125.2$, $a_3 = 0.1737$, $a_4 = 20.33$, $a_5 = 0.9143$, $a_6 = -0.001036$, $a_7 = -0.013634$, $a_8 = 24.38$.

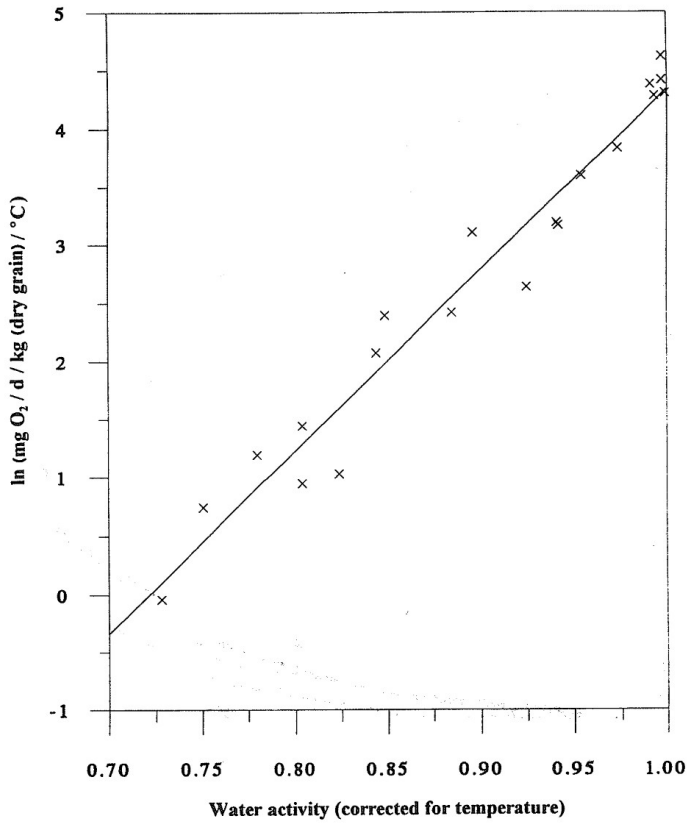

Fig. 4. The ratio of O_2 consumption rate to temperature at different a_w, corrected for temperature, in wheat grain cv Avalon after 7 d incubation.

This data could also be used in controlled atmosphere storage to predict how much the grain contributes to overall CO_2 concentrations and the time needed to produce inhibitory atmospheres in high moisture grains stored for animal feeds. However, further work is necessary to determine the concentrations of CO_2 at which respiration is inhibited and the effects of diurnal temperature fluctuations.

REFERENCES

Aljinovic, S., Bern, C.J., Dugba, P.N. and Misra, M.K. (1995) Carbon dioxide evolution from high-moisture shelled corn treated with iprodione. *J. Food Prot.* **58,** 673–677.

Al-Yahya, S.A., Bern, C.J., Misra, M.K. and Bailey, T.B. (1993) Carbon dioxide evolution of fungicide-treated high-moisture corn. *Trans. Am. Soc. Agric. Eng.* **36,** 1417–1422.

Bailey, C.H. (1940) Respiration of cereal grains and flaxseed. *Plant Physiol.* **15,** 257–274.

Brook, C. (1987) *Modelling Grain Spoilage during Near-Ambient Grain Drying*. Divisional Note 1388. AFRC Institute of Agricultural Engineering Research, Silsoe, UK.

Chen, C.-C. and Morey, R.V. (1989) Comparison of four EMC/ERH equations. *Trans. Am. Soc. Agric. Eng.* **32,** 883–890.

Clarke, J.H. and Hill, S.T. (1981) Mycofloras of moist barley during sealed storage in farm and laboratory silos. *Trans. Br. Mycol. Soc.* **77,** 557–565.

Cofie-Agblor, R., Muir, W.E., Sinicio, R., Cenkowski, S. and Jayas, D.S. (1995) Characteristics of carbon dioxide sorption by stored wheat. *J. Stored Prod. Res.* **31,** 317–324.

Hall, C.W. and Dean, P.E. (1978) Storage and preservation of cereal grains. In: *Cereals '78: Better Nutrition for the World's Millions, Sixth Int. Cereal and Bread Congress* (Edited by Pomeranz, Y.), American Association of Cereal Chemists, St Paul, MN, 223–243.

Hill, R.A., Lacey, J. and Reynolds, P.J. (1983) Storage of barley grain in Iron Age type underground pits. *J. Stored Prod. Res.* **19,** 163–171.

Hummel, B.C.W., Cuendet, L.S., Christensen, C.M. and Geddes, W.F. (1954) Grain storage studies XIII, Comparative changes in respiration, viability, and chemical composition of mold-free and mold-contaminated wheat upon storage. *Cereal Chem.* **31,** 143–150.

Kittock, D.L. and Law, A.G. (1967) Relationship of seedling vigour to respiration and tetrazolium reduction in germinating wheat seeds. *Agron. J.* **60,** 268–288.

Kreyger, J. (1972) *Drying and storing grains, seeds and pulses in temperate climates*, Publication 205. IBVL, Wageningen, Holland.

Lacey, J. (1971) The microbiology of moist barley storage in unsealed silos. *Ann. Appl. Biol.* **69,** 187–212.

Lacey, J. (1972) The microbiology of grain stored underground in Iron Age type pits. *J. Stored Prod. Res.* **8,** 151–154.

Lacey, J. (1988) The microbiology of cereal grains from areas of Iran with a high incidence of oesophageal cancer. *J. Stored Prod. Res.* **24,** 39–50.

Larmour, R.K., Clayton, J.S. and Wrenshall, C.L. (1935) A study of the respiration and heating of damp wheat. *Can. J. Res.* **12,** 627–645.

Milner, M. and Geddes, W.F. (1945) Grain storage studies II, The effect of aeration, temperature, and time on the respiration of soybeans containing excessive moisture. *Cereal Chem.* **22,** 484–501.

Milner, M., Christensen, C.M. and Geddes, W.F. (1947) Grain storage studies VII, Influence of certain mold inhibitors on respiration of moist wheat. *Cereal Chem.* **24,** 504–517.

Paster, N., Menasherov, M., Lacey, J. and Fanelli, C. (1992) Synergism between methods for inhibiting the spoilage of damp maize during storage. *Postharvest Biol. Technol.* **2,** 166–170.

Saul, R.A. and Lind, E.F. (1958) Maximum time for safe drying of grain with untreated air. *Trans. Am. Soc. Agric. Eng.* **1,** 29–33.

Saul, R.A. and Steele, J.L. (1969) Why damaged shelled corn costs more to dry. *Agric. Eng.* **47,** 326–329.

Scholz, B. (1962) Atmungsverluste bei Weizen in Abhängigkeit von Temperatur, Lagerzeit und Wassergehalt. *Landtech. Forsch.* **12,** 48–52.

Seitz, L.M., Sauer, D.B. and Mohr, H.E. (1982) Fungal growth and dry matter loss during bin storage of high moisture corn. *Cereal Chem.* **59,** 9–14.

Stroshine, R.L. and Yang, X. (1990) Effects of hybrid and grain damage on estimated dry matter loss for high-moisture shelled corn. *Trans. Am. Soc. Agric. Eng.* **33,** 1291–1298.

Thompson, T.L. (1972) Temporary storage of high-moisture shelled corn using continuous aeration. *Trans. Am. Soc. Agric. Eng.* **15,** 333–337.

Tribe, H.T. and Maynard, P. (1989) A new automatic electrolytic respirometer. *Mycologist* **3,** 24–27.
White, N.D.G., Sinha, R.N. and Muir, W.E. (1982) Intergranular carbon dioxide as an indicator of biological activity associated with the spoilage of stored wheat. *Can. J. Agric. Eng.* **24,** 35–42.
Wilcke, W.F., Meronuck, R.A., Morey, R.V., Ng, H.F., Lang, J.P. and Jiang, D. (1993) Storage life of shelled corn treated with a fungicide. *Trans. Am. Soc. Agric. Eng.* **36,** 1847–1856.
Woodstock, L.W. and Coombs, M.F. (1965) Effects of gamma-irradiation of corn seed on the respiration and growth of the seedling. *Am. J. Bot.* **52,** 563–569.