Effect of modified atmosphere storage on wheat seed germination vigour and on physiological criteria of the ageing process

F. Fleurat-Lessard,* D. Just,† P. Barrieu,† J.-M. Le Torc'h,* P. Raymond† and P. Saglio†

Abstract

It has not been clearly proved whether modified atmospheres (MA) adversely affect seed germination and seedling vigour after a prolonged storage period. An experiment was therefore conducted to study the comparative biochemical changes that take place with respect to germinative power when wheat is stored at each of two temperatures (30° and 15°C) under various modified atmospheres: air (control) ; N_2 (100%) ; N_2/ O_2(50/50) ; CO_2/air (60/40) and CO_2 (100%).

Samples were taken at various times over a 20-month storage period. Tests performed during germination included energy charge kinetics (EC), adenylic nucleotide content, malondialdehyde content and dehydrogenase activity. At the higher temperature (30°C) there was significant and rapid ageing of seed with respect to physiological criteria and germinative power. At 15°C, only EC and nucleotide content changed during several months in MA. A hyperoxygenated atmosphere seemed to have the most injurious effect. Nevertheless, neither high levels of CO_2 nor nitrogen could prevent biochemical deterioration.

Introduction

The goal of modified atmospheres (MAs) as a means of grain preservation is often described in terms of achieving complete reduction of insect damage or of losses induced mainly by storage fungi. In the same way the inhibition of insect reproduction and microorganisms multiplication by MAs have been extensively studied. A lot of papers deal with the subject which allows easy forecasting of the biological efficiency of MA within the possible range of gas mixture compositions against the main insect pest species or storage mycoflora species and strains (Jay 1980; Annis 1987; Banks and Annis 1990; Richard-Molard 1990). Nevertheless, inhibition of grain pest infestation or contamination by fungi should not be sensible criterion if this inhibition is achieved only after a great deal of damage has occurred to the germination power or if practical difficulties prevent a current use of the appropriate MA storage conditions already perfected (e.g. adsorption of CO_2 or leakage rate in storage structures or high temperature).

The most studied quality parameter in grain storage is the viability of the seed, not only because of direct importance, for instance with malting barley, but because it is generally considered the best direct criterion of grain soundness (Aspinall and Paleg 1971; Pomeranz 1982).

* Institut National de la Recherche Agronomique, Laboratoire des Insectes des Denrées Stockées, –BP 81–F 33883 Villenave d'Ornon cédex, France.

† INRA, Station de Physiologie Végétale,–BP 81–F 33883 Villenave d'Ornon cédex, France.

Very few experiments have been conducted to study comparative biochemical or metabolic changes that take place in cereal seeds during long-term storage under MA (Blowers et al. 1980; Standard et al. 1983; Petruzzelli and Taranto 1989). Hence, a greater understanding of the metabolic changes associated with aging of seeds is essential for the choice of MA as a safe technique for seed quality preservation on long-term storage periods of more than one year.

Recently, the loss in cereal seed vigour during the germination process has been correlated with the rate of ethylene production (Khan and Seshu 1986; Jilani et al. 1989). Many small biochemical changes have been described after prolonged storage of cereals without any loss of germinative power. On the other hand, the biochemical criteria associated with aging of seeds during storage under MA could be eventually used in different ways:

- To predict the loss of germination rate and germinative vigour (= capacity) observed later on grain seedlings.
- To assess the susceptibility of cereal seeds to be injured by atmospheres modified by a high concentration of oxygen (O_2) or carbon dioxide (CO_2) balanced with nitrogen (N_2).
- To determine quickly the suitability of a seed lot of cereals for long-term MA storage.

The goal of this comparative study was to discover which physiological indices change significantly under different MA compositions and at different temperature levels and to link these changes with a final decrease in germination vigour.

Materials and Methods

Plant materials

Wheat seeds (*Triticum aestivum*, var. 'top' 1991 harvest) were obtained directly from the crop at harvest in the south-west of France and stored temporarily at a low temperature level (7°C) before the experiments.

Experimental storage conditions

About 1.2 kg samples of wheat seeds were stored inside 2 L airtight vessels. Each of them was equipped with inlet and outlet gas port closed with an automatic valve. A circular hole in the lid of the container was tightly plugged with a rubber septum, allowing gas sampling with a gas syringe for injection into a gas chromatograph. Five different gas compositions were prepared by mixing pure nitrogen (N_2), oxygen (O_2), and carbon dioxide (CO_2) from cylinders. The expected composition was obtained using 3 mass-flow meters (Air Liquide Alphagaz RDM) monitored by a electronic regulator (mass-flow regulator 'Alphagaz') (Fleurat-Lessard and Le Torc'h 1991). The five different gas compositions used in the experiment are listed in Table 1.

Storage of seeds in airtight containers under MA was done in two climatic rooms maintained 15 and 30°C. Each gas composition was replicated 3 times in each room. During the initial purge of storage containers by MA the inlet valve was connected to the exit tube of the gas mixer (flow-rate 1

Table 1. Different composition of modified atmosphere used and conditions of storage of wheat seeds lots

	Control air	Nitrogen (N_2)	N_2 / O_2	CO_2 / air	CO_2
N_2	80	100	50	32	0
O_2	20	0	50	8	0
CO_2	0	0	0	60	100
30°C	+	+	+	+	+
15°C	+	+	+	+	+

3 replicates for each condition

L/minute) and the outlet valve was connected either to a paramagnetic O_2 analyser (HM16G oxymeter) or to CO_2 analyser (HM Box 40) or to the two, connected together on the same derivative gas-flow line. The purge ended when the gas composition measured at the outlet port of the container was the same as the injected gas mixture.

At the beginning of the incubation period the same operation of gas injection was done each week to adjust the different gas compositions to the right levels. After the first month of incubation, the periodicity of gas composition adjustments was reduced to once per month.

Periodically sampling and incubation conditions for germination tests

Every two months approximately, a sample of 50–60 g of grain was withdrawn from the different containers for different tests and analytical measures. For the determination of average time for germination (ATG), two replicates of 50 seeds were soaked in 15 mL disinfection solution of calcium hypochlorite 10% (7% active Cl^-) for 5 minutes followed by rinsing twice with distilled water before plating in a Petri dish on a germination paper already humidified with 10 mL of distilled water. Incubation was conducted in an air-conditioned oven at 25°C in the darkness. Daily counts of germinated seeds were made for one week. The time at which 50% of the seeds germinated (t_{50}) was determined following the ISTA method (1976).

The other part of the sample was used to assess:

i) Biochemical indices of metabolic changes during germination process :

- Amount of ethylene produced during the first 3 days of germination (gas chromatographic measurement of the headspace sample, FID, alumina column, temperature program, N_2 carrier gas);

ii) Assessment of physiological criteria related to early aging of seeds:

- Adenylic nucleotides content after hydration of seeds (measured at the same time after germination started), in other respects used for energy charge (EC) ratio calculation (Raymond and Pradet 1980):
  EC = [ATP] + 1/2 [ADP] / [ATP] + [ADP] + [AMP];
- Dehydrogenase activity measured through tetrazolium test (Moore 1962);
- Malondialdehyde content (Peever and Higgins 1989).

In addition, a qualitative determination of mould microflora was performed on seeds after one year of storage. The International standard method (ISO 7954: 1987 (F)), Ulster technique was used by the specialised laboratory of INRA (Richard-Molard and Cahagnier, LMTC Nantes France) to determine the rate of each fungus species contaminating the seeds.

Results and Discussion

Evolution of gas composition inside the containers during storage

In spite of accurate control of airtightness before the experiment started, the containers did leak slightly and the gas composition inside the containers changed regularly during the time interval between two sampling operations. The average gas composition deviated from the initial one at a rate of about 0.2% per day in some containers as observed on the kinetics recorded during an 18-day cycle of incubation (Fig. 1).

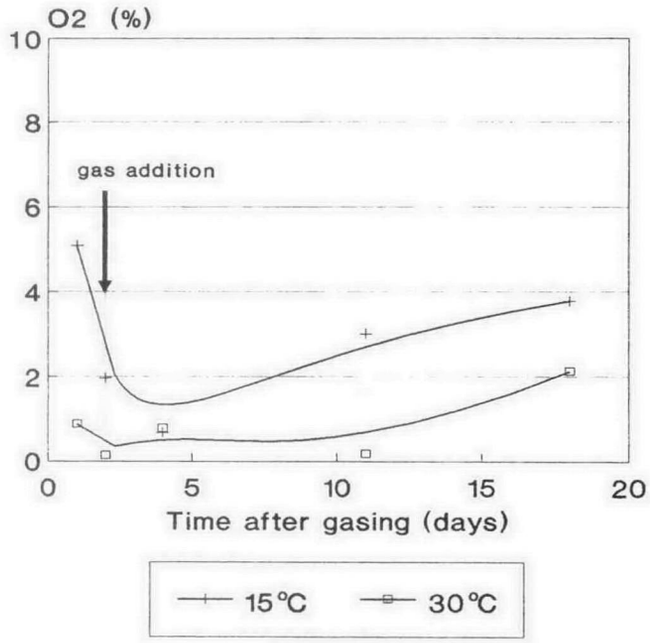

Fig. 1. Changes in O_2 content with time in containers of wheat seeds stored under CO_2 atmosphere (i.e. leakage rate).

Germination performances after storage under MA

We could not compare MA effects observed on lots stored in these conditions with the control batch of seeds stored in air because of a significant difference in the water content between the latter stored under slight air flow (5 mL/minute) and the other lots stored in airtight vessels convenient for MA. Even with a strict control of relative humidity of air injected through the control batch of seeds this technique induced a decrease in water content of the seeds.

Nevertheless, at 30°C a reduction of germination capacity was observed following an increase of the average time for germination during the first 4 months of the storage period (Fig. 2). The germination capacity fell to zero after 6 months storage in all the MA conditions. None of the modified atmos-

pheric compositions could limit either the decrease of the germination capacity or the increase in the average time for germination. However, the most rapid effect was obtained with hyperoxygenated MA (N_2 / O_2 in equal parts).

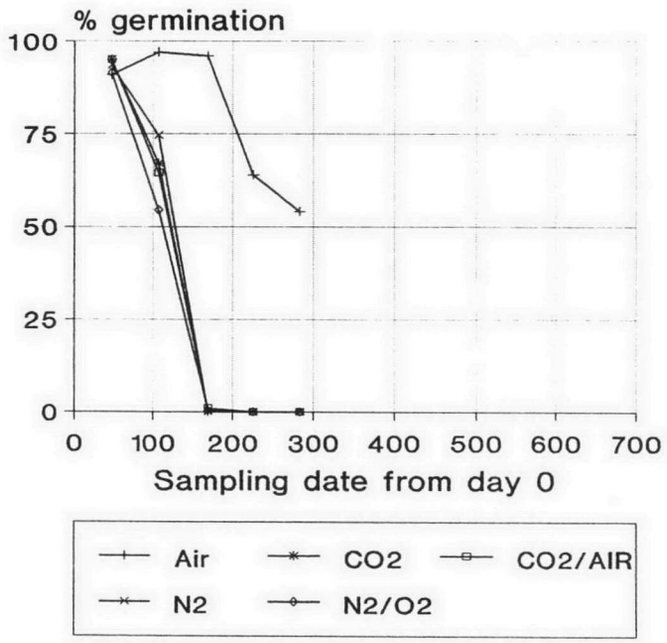

Fig. 2. Seed germination (%) of wheat seeds during 10 months storage at 30°C under various modified atmospheres.

At 15°C no significative reduction of germination capacity was observed during the first part of the storage period (Fig 3). After one year of storage, air (control) and the N_2/O_2 mixture gave an insignificant reduction of germinative capacity. At the end of 20 months storage, two compositions (CO_2/air mixture and N_2) can be considered as 'preservative' for germination. In contrast, the three other MA compositions significantly reduced germination capacity. In order of decreasing germination capacity, they were: CO_2, N_2/O_2, and air (control), the last coming close to 77% germination capacity at the final check (but at H_2O content slightly lower in air than in MA).

The average time for germination was determined in the same control operation. Unfortunately, some improvements in the incubation protocol occurred soon after starting the experiment and unexpected reduction of average time for germination was observed first after the second check (checks 2–6 = constant ATG assessed at 3.3 days < check no. 1 = 4 days) and again after check 7 (checks 7–9 = constant AGT at 2.8 days). In the meantime, using the standard ISTA method to determine t_{50} in constant conditions we could obtain a comparison between seeds stored under MA conditions over a 4-months storage period. At the beginning of the experiment the time at which 50% of seeds germinated (t_{50}) was about 26 hours. After 4 months at 30°C (Fig. 4a) (except for the control in air which is not comparable with the other conditions because of lower H_2O content) all MAs destroyed the germinative capacity of the seeds. For MA compositions other than N_2/O_2 which killed the seeds, the average time for germination was in the range 40–50 hours and germinative capacity fell to below 50%. At 15°C, average time for germination (measured in the same conditions) remained below 30 hours and germinative capacity above 90% (Fig. 4b).

Consequently, we can state that the most drastic reduction in germination capacity and vigour occurs in seeds stored in hyperoxygenated atmosphere (N_2/O_2), and additional physiological injury occurs particularly early at 30°C. However, if high storage temperature is the main active factor inducing a rapid decrease in germination capacity of wheat seeds, dry seeds (as in the control) can survive for a longer period and retain a higher germination power than under presumably protective MA (e.g. N_2) and higher moisture content.

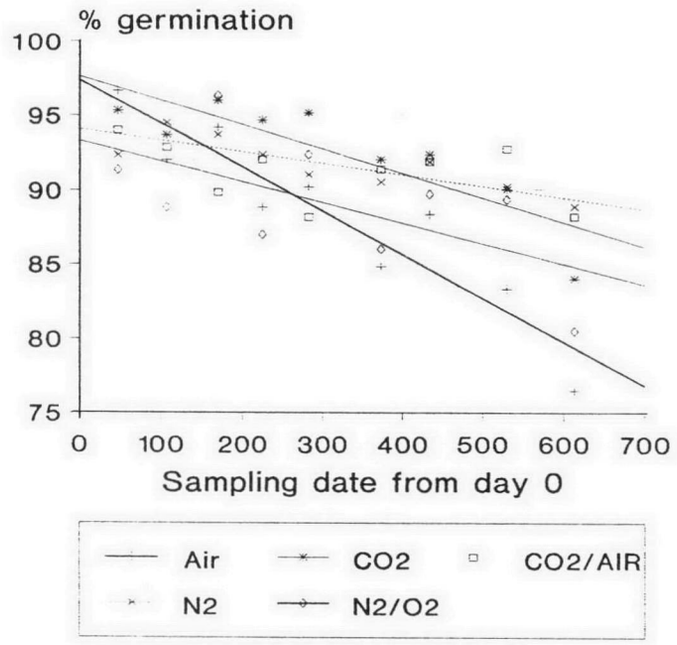

Fig. 3a. Seed germination (%) of wheat seeds during 20 months storage at 15°C under various modified atmospheres.

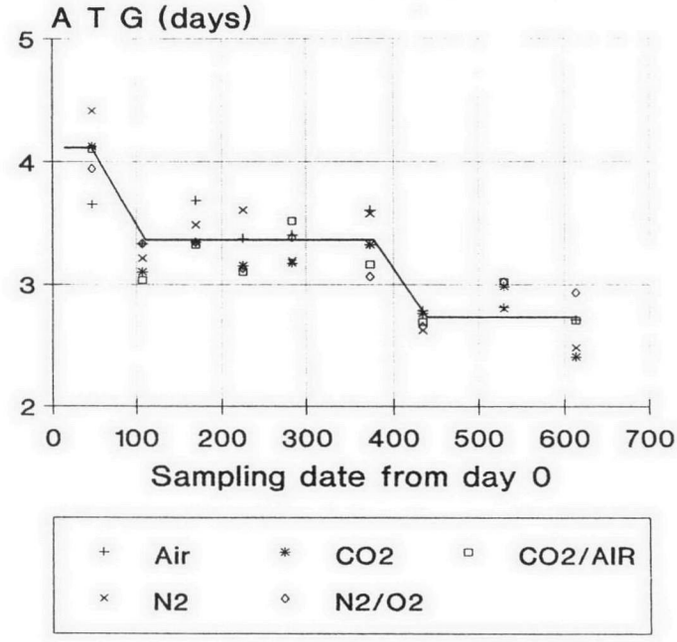

Fig. 3b. Average time for germination of wheat seeds during 20 months storage at 15°C under various modified atmospheres.

Physiological indices of seed ageing

Extraction and determination of adenylic nucleotides from wheat seed embryos after 4 months storage highlighted a significant difference between MA compositions (Table 2) especially at 15°C storage temperature. The starting batch of seeds has the highest content of nucleotides (1420 pmol/seed). At 15°C, the nucleotide content was lower than in the control

but remained at a high level, except with storage under N_2 (airtightness was not perfect and oxygen concentration was not zero).

In all seeds stored at 30°C a drastic decrease in the sum of adenylic nucleotides was observed, revealing a general effect of temperature much more important than the MA composition effect, if any.

The same result was obtained for EC ratio with a significant decrease at 30°C and without modification at 15°C (data not shown).

Key metabolic changes

The amount of ethylene produced during the first 3 days of germination is close to the limit for detection by gas chromatography (below 0.2 ppm). Dehydrogenase activity linked with tetrazolium test was not affected by any of the storage conditions. Malondialdehyde content, a criterion associated with lipid peroxidation, is not modified under any condition after 4 months of storage (Table 3).

Microbiological analysis after 7 months of storage

Microbiological qualitative and quantitative analysis carried out on seeds after 7 months storage (218 days, 4th check date) revealed a high level of external grain contamination either by field fungi species (*Fusarium*, *Alternaria*) or storage fungi (*Aspergillus*, *Penicillium*) (Table 4).

At a storage temperature of 30°C, the first obvious change is the reduction of *A. glaucus* contamination rate in seeds stored under inert MA compositions (N_2 and CO_2) or in a hypercarbic atmosphere (CO_2/air). If airtightness had been perfect, a complete decrease in fungi contamination should be expected at least in the inert atmosphere. We can associate incomplete reduction of mould flora in these conditions with residual oxygen inside the storage vessels. Of course, this is more obvious at 30°C than at 15°C. In the latter situation, the lower moisture content in grain stored with aeration is associated with a different range of mould species. Contamination by *A. glaucus* is high in the aerated condition only. *Alternaria* is the main genus observed in grain stored under MA, without differences between the different gas compositions. Similarly with the series at 30°C, an incomplete airtightness of containers during storage should explain the lack of difference in type of mould contamination between the hyperoxygenated atmosphere and the inert other ones.

Table 2. Sum of adenylic nucleotides concentrations in one seed (pmol) after 1 hour hydration observed on wheat seed embryos following 4 month storage time under various modified atmospheres.

Atmospheric composition	Storage temperature	
	30°C	15°C
Air	338	1108
N_2	237	725
N_2 / O_2	137	946
CO_2 / air	157	1110
CO_2	236	1319
Day 0 Control	1420	

D 0 = Starting of experiment (day 0)

Table 3. Malondialdehyde content in germinating wheat seeds measured on embryonic axes from seeds stored under various MA conditions during 4 months.

Atmospheric composition	Storage temperature	
	30°C	15°C
Air	0.95 ± 0.08	0.87 ± 0.15
N_2	0.91 ± 0.08	0.98 ± 0.03
N_2 / O_2	0.94 ± 0.05	0.94 ± 0.04
CO_2 / air	0.79 ± 0.11	1.02 ± 0.00
CO_2	0.66 ± 0.06	1.12 ± 0.16
Day 0 (Control)	0.85 ± 0.09	

± Standard error

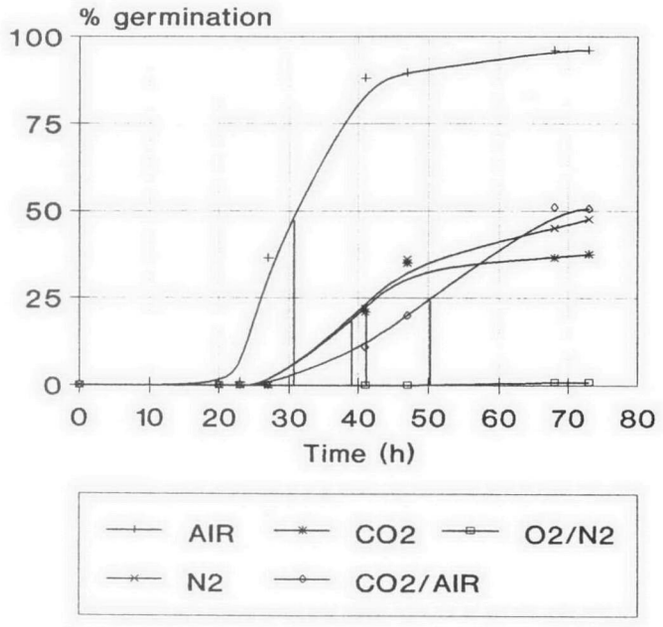

Fig. 4a. Time for 50% germination (t_{50}) of wheat seeds after 4 months storage at 30°C under various modified atmospheres.

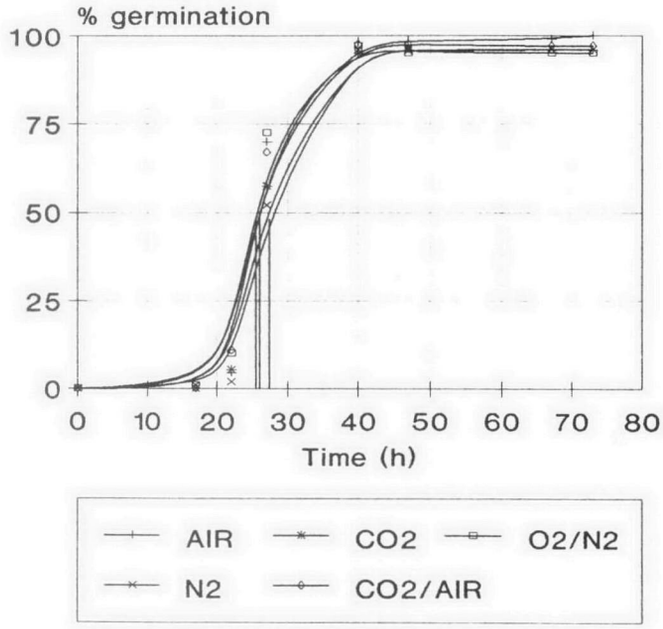

Fig. 4b. Time for 50% germination (t_{50}) of wheat seeds after 4 months storage at 15°C under various modified atmospheres.

Table 4. Percentage of contaminated kernels by fungi species in wheat seeds after a 7 months storage period of grain under various modified atmospheres at 30 or 15°C (rate/100 seeds)

Fungus species	Atmospheric conditions				
	Air[a]	N_2	N_2/O_2	CO_2/air	CO_2
Temperature 30°C					
Alternaria					
Fusarium	2	2			
Epicoccum					
Penicillium	20	30	48	2	8
Cladosporium					
Aspergillus	92[b]	26[b]	100[b]	8[b]	8[b]
Mucorales	2				
Germination %	92	0	0	0	
Temperature 15°C					
Alternaria	40	84	96	76	85
Fusarium	4	2	6	10	
Epicoccum	8	2	10		
Penicillium	96	80	68	12	10
Cladosporium	14	10	14		
Aspergillus	100[b]	14[b]	240[b]	46[b]	
Mucorales	2	2	2		
Germination %	100	96	100	96	96

[a]Grain stored in air has a lower moisture content than grain under MA
[b]*Aspergillus glaucus*

Conclusion

It has been confirmed that climatic conditions of storage are much more injurious for wheat seed senescence than MA storage. The most influent parameter is high temperature followed by high moisture content (even inside legal tolerances). It is observed for the first time that biochemical indices that can be measured on grain during the germination process change a long time before germination capacity is significantly affected. Even if we were not able to correlate these early changes with the delay of time elapsing before germination capacity decreases, it seems that these criteria are more sensitive than methods in current use (e.g. tetrazolium test).

A low level of energy charge is associated with further decrease in the rate of seed germination. It is only with these very precursors of seed senescence that it should be possible to see an effect of MA on seed germination, if any.

Finally, sensitive biochemical criteria change well before a reduction in germination capacity occurs. In the case of appropriate storage conditions, i.e. low temperature and moderate water content of the seeds, significant changes of total adenylic nucleotides content or energy charge are easily observed, although little reduction in germination will occur during the long storage period which follows (several months).

However, in our experiment there was a great influence of the moisture content of the seeds because it is not easy to maintain the same activity of water in seeds stored under different conditions and in applying different means of control of physico-chemical conditions.

Another experiment has already started for a close and accurate control of this parameter. Appropriate indices are now known which can be used to relate seed aging to metabolic perturbations induced by storage under MA. The delay separating the changes in physiological indices from the reduction in germination vigour of the seeds must now be investigated.

Acknowledgments

We thank Dr B. Cahagnier, microbiologist, INRA LMTC, Nantes, for microbial analysis and interpretation advice and Mr B. Fuzeau, INRA LIDS, Bordeaux, for the illustrations.

References

Annis, P.C. 1987. Towards rational controlled atmosphere dosage schedules: a review of current knowledge. In: Donahaye, E. and Navarro, S., ed., Proceedings of 4th International Working Conference on Stored-product Protection, Tel-Aviv, Israel, 21–26 September 1986, 128–148.

Aspinall, D. and Paleg, L.G. 1971. The deterioration of the wheat embryo and endosperm function with age. Journal of Experimental Botany, 22, 925–935.

Banks, H.J. and Annis, P.C. 1990. Comparative advantages of high CO_2 and low O_2 types of controlled atmospheres for grain storage. In: Calderon, M. and Barkai-Golan, R., ed., Food Preservation by modified atmospheres. Boca Raton, Florida, CRC Press, 7, 93–122.

Blowers, L.E., Stormonth, D.A. and Bray, C.M. 1980. Nucleic acid and protein synthesis and loss of vigour in germinating wheat embryos. Planta, 150 19–25.

Fleurat-Lessard, F. and Le Torc'h, J.M. 1991. Influence de la teneur en oxyghne sur la sensibiliti de certains stades juviniles de *Sitophilus oryzae* et *S. granarius* au dioxyde de carbone. Entomologie Experimentalis et Applicata, 58, 37–41.

I.S.T.A. 1976. International rules for seed testing. Seed Science and Technology, 4, 609–743.

Jay, E. 1980. Methods of applying carbon dioxide for insect control in stored grain. In: Shejbal, J., ed., Controlled atmosphere storage of grain. International Symposium Castelgandolfo, Italy, 12–15 May 1980, 225–234.

Jilani, G., Saxena, R.C. and Khan, A.A. 1989. Ethylene production as an indicator of germination and vigour loss in stored rice seed infested by *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Journal of Stored Products Research, 25, 175–178.

Khan, A.A. and Seshu, D.V. 1986. Using ethylene to monitor the influence of adverse climatic factors and to predict plant performance. In: IRRI, Weather and rice, 103–122.

Moore, R.P. 1962. Tetrazolium as a universally acceptable quality test of viable seed. Proceedings of International Seed Testing Association, 27, 795–805.

Peever, T.L. and Higgins, V.J. 1989. Electrolyte leakage, lipoxygenase, and lipid peroxydation induced in tomato leaf tissue by specific and nonspecific elicitors from *Cladosporium fulvum*. Plant Physiology, 90, 867–875.

Petruzzelli, L. and Taranto, G. 1989. Wheat ageing: the contribution of embryonic and non-embryonic lesions to loss of seed viability. Physiologia Plantarum, 76, 289–294.

Pomeranz, Y. 1982. Biochemical, functional, and nutritive changes during storage. In: Christensen, C.M., ed., Storage of cereal grain and their products, St. Paul, Minnesota, American Association of Cereal Chemists, 2, 56–114.

Raymond, P. and Pradet, A. 1980. Stabilisation of adenine nucleotide ratios at various values by an oxygen limitation of respiration in germinating lettuce (*Lactuca sativa*) seeds. Biochemical Journal 190, 39–44.

Richard-Molard, D. 1990. Conservation of humid grains in controlled atmosphere storage. In: Calderon M. and Barkai–Golan, R., ed., Food preservation by modified atmospheres. Boca Raton, Florida, CRC Press, 5, 57–82.

Standard, S.A., Perret, D. and Bray, C.M. 1983. Nucleotide levels and loss of vigour and viability in germinating wheat embryos. Journal of Experimental Botany, 34, 1047–1054.