



The effect of modified atmospheres on the survival of the eggs of four storage mite species^{*}

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Abstract. The results of a laboratory investigation into the effects of modified atmospheres (MA) on the eggs of mite pests of grain and cheese are presented. Four species of astigmatid mite were tested; *Acarus farris* (Oudemans), *A. siro* L., *Lepidoglyphus destructor* (Schränk) and *Tyrophagus longior* (Gervais). All are found in many habitats including grain and cheese stores. Three low oxygen (O₂) MA mixtures were used, based on carbon dioxide (CO₂), nitrogen (N₂) or simulated burner gas (0.5 or 2% O₂, 10% CO₂, balance N₂) plus 60% CO₂ in air (8% O₂). The mites were exposed at 15 °C and 80% r.h., a combination of conditions that occurs at the surface of stored grain during the autumn which promotes mite population growth. The exposure periods required to prevent egg hatch for each species in every mixture are given. *Tyrophagus longior* was the most tolerant species, followed by *A. siro* and *A. farris*, with *L. destructor* the most susceptible. Burner gas was the most effective mixture overall with 0.5% O₂ but with an increase in the O₂ level to 2% for all the mixtures, CO₂ became the more effective control agent. With 60% CO₂ in air some loss of efficacy was observed against the three most tolerant species and even more so for *L. destructor*. Sublethal exposures to MAs for at least 4 days in *L. destructor*, 6 days in *A. farris* and *A. siro* and 8 days for *T. longior* caused a delay in egg hatch.

Introduction

Food storage provides the ideal environment for the development of invertebrate pests in an almost limitless food supply. Certain species of mites are able to take advantage of constant cool, moist conditions prevailing in many stores and are becoming important economic pests in temperate regions (White 1995). They are able to complete their development more rapidly than insects, produce more offspring, and tolerate lower temperatures. Stored grain provides an ideal environment and damage is caused by consumption of the grain germ and, to some extent, the endosperm (Parkinson 1990). The grain becomes useless for seed (Zdarkova 1996) or brewing purposes and unacceptable to the miller (Solomon 1946). Mites also contaminate the grain with fungal spores carried in or on their bodies (Lacey 1988). They secrete lipids which taint the grain with an odorous and distinctive smell

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(White 1995). Their allergens have been detected within the food chain and have been shown as a causative agent for asthma, rhinitis and eczema particularly in the occupational environment (Chambers et al. 1999). In cool, damp feed grain populations of up to 1000 mites/kg are often considered as unavoidable (Bell and Armitage 1992). Another food storage area where mites are a problem is the traditional cheese store (Wilkin 1979; Luck and Botha 1987; Lozzia et al. 1989). In this case the cool conditions required for long term cheese maturation are easily exploited by the mites and there is little scope for humidity and temperature manipulation.

The successful storage of grain requires environmental conditions to be manipulated to maintain the quality of the grain and avoid conditions which would allow rapid development of pests. This can be achieved by chilling, drying or altering the gaseous composition of the intra-granular atmosphere (Lacey 1988). Cooling by aeration is unlikely to prevent the build-up of moderate mite infestations and so, for full prevention, the grain has to be dried to 12–13% moisture content (m.c.) (Burrell and Havers 1976). Even under these conditions the grain at the surface will absorb moisture to a point where it becomes favourable for mite population growth (Armitage and Cook 1999). For economic reasons grain is stored at up to 15% m.c. and under such conditions, 'surface populations' of mites such as *Acarus siro* L. and *Lepidoglyphus destructor* (Schrank) persist even with grain temperatures as low as 4–9 °C (Armitage et al. 1994).

Insecticides have been used in the past with success for mite control on grain, both as an admixture (Wilkin and Stables 1985; Tigar and Pinniger 1989; White and Sinha 1990), and as a surface treatment (Armitage et al. 1994), and also for cheese stores (Wilkin 1979). However they are not effective against all species of mites and low temperatures retard the action of most chemicals (Wilkin and Stables 1985). Resistance to many compounds is becoming a world-wide problem (White 1995) and in the UK in particular, pirimiphos-methyl, the main insecticide used for grain (Starzewski 1991) and cheese stores (Wilkin and Stables 1985), now has limited efficacy against mites (Prickett 1997).

With the idea of prevention of damage being superseded by the more stringent requirements of no live pests, there is more pressure on control procedures. All grain for export requires a phytosanitary inspection to check for the presence of pests (Wilkin and Hurlock 1986) and methods of detection are becoming much more sophisticated (Ridgway et al. 1999). Even raw materials intended for incorporation into animal feeds have to be free of mites (Prickett 1992) as they significantly reduce the value of feed (Zdarkova et al. 1993). These requirements preclude or at least hamper the use of several biological control methods that are available (Zdarkova and Feit 1999; Zdarkova and Horak 1999). Some alternatives to insecticides such as diatomaceous earths are available and of acceptable efficacy (Cook and Armitage 2000) but they are an additive to the grain that may require removal.

Modified atmospheres (MA) offer an alternative strategy for mite control especially with the mounting public concern about environmental contamination of any kind and a desire for pesticide-free food (Gibson 1988). This technology involves the alteration of the natural ratio of the atmospheric gases, oxygen (O₂), nitrogen

(N₂) and carbon dioxide (CO₂), to render the atmosphere in stores unfavourable to pests (Banks and Fields 1995). Previous laboratory tests with MAs against mites have shown promising results (Hughes 1943; Mitsura et al. 1973; Lider et al. 1983; Navarro et al. 1985) though only Stepien (1974) included eggs. This stage was certainly the most resistant to fumigants, including methyl bromide and phosphine (Bowley and Bell 1981). White and Jayas (1991, 1993) have carried out successful field trials with CO₂ in small grain bins against mixed populations of several mite species in cool temperatures.

Of the four astigmatid mite species chosen for the tests, *Acarus siro* is the commonest species in UK grain stores throughout the year (Lynch et al. 1991) and is also found in cheese stores (Wilkin 1979). *Lepidoglyphus destructor* appears in farm and commercial grain stores after harvest in the UK, being brought in from the field (Lynch et al. 1991) where it is found in many diverse habitats (Hughes 1976). *Tyrophagus longior* (Gervais) and *Acarus farris* (Oudemans) are other common inhabitants of grain stores (Lynch et al. 1991) which are also found in many other locations including cheese stores (Hughes 1976). These four species are among the most common stored product mites in various countries in Europe and Asia (Sinha 1979). Fifteen °C and 80% r.h. were used for the treatments as these conditions may occur at the surface of grain in storage during the autumn and under these conditions mite population growth will occur. Mobile stages are known to be susceptible to MAs (Hughes 1943; Stepien 1974; Navarro et al. 1985) but eggs are much more tolerant (Stepien 1974; LungShu et al. 1998). Therefore if the use of MAs for mite control is to realise its potential in the field, laboratory tests on eggs are needed to provide the exposures required for their control and to show the differences in susceptibility of the various species.

Materials and methods

Each of the species, *A. farris*, *A. siro*, *L. destructor* and *T. longior*, was reared in 50 mL conical flasks on a heat-sterilised medium consisting of debittered yeast and wheat germ flakes in a ratio of 3 to 1. A bung of non-absorbent cotton wool was used to seal each flask, all cultures being kept in desiccators, one species in each. A relative humidity (r.h.) of 80% in the desiccator was achieved by placing a solution of potassium hydroxide in the bottom (Solomon 1951) with the cultures supported above. These desiccators were placed in a room with controlled temperature at 15°C. The mites were cultured every two weeks with half the culture (1.5 g) being discarded or added to a new flask and then being topped up with an equal quantity of food. All species have been in culture at the laboratory for many years.

Prior to testing with MAs, mean egg hatch was determined for each species at 15 °C and 80% r.h. Three units made of black perspex were set up for each species. Each unit (140 × 20 mm) contained four sub-cells (10 mm diameter) with a black filter paper (Schleicher & Schuell GmbH, Dassel, Germany) stuck to the bottom with wood glue. For each species, a culture was selected and was passed separately

through a 180 μm sieve into a pan. This removed all the mobile stages except some larvae and protonymphs and left the eggs and some food. The contents of the pan were then discarded. The next day the culture was sieved again but this time the content of the pan was used as a source of eggs. This meant that the eggs were 0–1 day old. Under a microscope the eggs were removed singly with a single-haired paint brush (pure sable, size 00). Approximately ten eggs were placed in each sub-cell with some food. The sub-cells were covered with microscope slides and held in place with clips. The cells were placed in 5 L glass desiccators over a potassium hydroxide solution which gave 80% r.h. There was one desiccator for each species. A week later a further batch of 0–1 days old eggs were set up for each species, with the only exception being *T. longior*. With this species a high density of eggs in the food for the second test allowed the use of a small scoop to place eggs and food into the sub-cells. This meant that the egg count per cell was much higher.

The hatch from the control eggs was monitored by viewing under a microscope every day for 3 weeks. The egg hatch data was then compiled and grouped for the four sub-cells within each unit. An ANOVA oneway test was then run with Tukey pairwise comparison to see if the results from each of the three units could be combined (Minitab Release 13.30, Minitab Inc., State College P.A., USA). A further test on the data from the two separate experiments for *A. siro* and *L. destructor* was then carried out to see if they could be combined for an overall mean hatch time.

The exposures to MAs were carried out in perspex cells (70 $\times$ 70 $\times$ 3 mm) with a circular hole of 43 mm diameter. On one side of the cell a white Whatman filter paper No. 1 (Whatman International Ltd., Maidstone, UK) was attached using wood glue. The filter paper allowed gas to diffuse into the cell but prevented the mites from escaping. For each species, a culture was selected and was passed separately through 180 μm sieve. Approximately 0.2 g of this mixture which contained mixed-age eggs was placed within each cell, and a square cover glass of the same size as the cell and was held firmly over the top by four clips. The food contained around 30 eggs for *A. farris*, 50 for *A. siro* and *L. destructor* and 100 for *T. longior*.

The MAs (Table 1) were produced using a three channel gas blender (Signal Instrument Co. Ltd., Camberley, Surrey) provided with high purity compressed gases from cylinders. For the high CO_2 atmospheres a mixture of this gas and O_2 was used with the latter replaced with compressed air for the 60% CO_2 mixture. For the high N_2 atmospheres this gas was combined with O_2 , and finally for an atmosphere similar to that produced by burning propane in an exothermic gas generator, N_2 , CO_2 and O_2 were used. After the gases were mixed to the required proportions, the gas stream was split into eight with each gas flow limited to 100 mL/min by means of flow control valves. The eight streams were then humidified to 80% r.h. by passing the gas over solutions of potassium chloride (Winston and Bates 1960). Once humidified the gas stream went through the top of the lid and, via a tube, into the bottom of the exposure chamber which consisted of a 5 L desiccator. The perspex cells were placed on a plastic mesh layer supported above the bottom of the desiccator.

A vent in the top of the desiccator lid created a flow-through system and the gas mixture was able to flow out to the atmosphere. This set-up ensured a constant gas

Table 1. Modified atmospheres tested.

Modified atmosphere		
N ₂	Simulated burner gas	CO ₂
0.5% O ₂ , 99.5% N ₂	0.5% O ₂ , 10% CO ₂ , 89.5% N ₂	0.5% O ₂ , 99.5% CO ₂
2.0% O ₂ , 98% N ₂	2.0% O ₂ , 10% CO ₂ , 88% N ₂	2.0% O ₂ , 98% CO ₂
		60% CO ₂ , 40% Air (8.0% O ₂)

mixture within the exposure chamber with no loss through leakage or respiration. The apparatus was kept in a controlled environment room at 15 °C. Oxygen, CO₂ and humidity levels were monitored every day using a Model 570A paramagnetic O₂ analyser (Servomex Ltd., Crowborough, Sussex, UK), a Model PA 404 infrared CO₂ analyser (Servomex Ltd.) and a Protimeter DP680 (Protimeter Ltd., Marlow, Bucks, UK) respectively, and the apparatus were adjusted if required.

For each exposure period three cells were prepared, with ten exposure periods increasing in steps of two days for each test, plus three cells to act as controls throughout the experiment. The exposure periods were selected to find the time interval required to prevent egg hatch. Treated cells were removed from the chamber and placed in a desiccator separate to the controls at 15 °C and 80% r.h. They were checked every week until nymphs were detected or three months had elapsed. If no egg hatch had occurred after three months the cells were discarded. Hatch in any of the three replicates was used as a positive indication of tolerance for that period of exposure. After this an assessment of the MA exposure time required to give 100% mortality could be given. A comparison of the responses of *A. farris*, *A. siro*, *L. destructor* and *T. longior* to exposure to the MAs was then made through regression analysis. Only data sets with 5 or more points were used for the analysis. The data for 'Time' and 'Number of days to egg hatch after the end of MA exposure' were transformed by $\log_{10}(x + 1)$ after a selection process for possible transformations using the largest and smallest values for each treatment (Kirk 1968). Regression analysis was done for each MA that was used for each species (Minitab Release 13.30, Minitab Inc., State College, P.A., USA). A test for equality of slopes for several regression lines was then done for each species (Sokal and Rohlf 1995). If there was no difference then a general regression line was produced. If there was a significant F_s then a detailed analysis of the differences between regression coefficients of the MAs was tested with paired F-tests (Sokal and Rohlf 1995).

Results

The results of the control egg hatch for the four species are shown in Table 2. There was no difference between the replicates for *A. farris* (ANOVA, F 0.86 (DF 108), $p=0.425$). The overall mean for this species was the shortest hatch time of all the species. For *A. siro* this was the case for the first test (ANOVA, F 0.33 (DF 107)

$p=0.722$) but for the second one replicate was discarded (ANOVA, $F\ 6.35$ (DF 128), $p<0.002$). The two means were then combined (ANOVA, $F\ 6.64$ (DF 199), $p=0.011$) for an overall mean hatch time which was next in order together with *L. destructor*. For *L. destructor* the first experiment showed no difference between means (ANOVA, $F\ 0.84$ (DF 137), $p=0.432$). One replicate of the second experiment was rejected (ANOVA, $F\ 3.73$ (DF 107), $p<0.027$). The means for the two experiments were combined (ANOVA, $F\ 0.03$ (DF 209), $p=0.854$) for the overall result. The longest mean time for hatch was *T. longior*. There was no difference between the replicates (ANOVA, $F\ 0.10$ (DF 506), $p=0.909$).

When the range of days over which eggs hatch was considered, it was noticeable that the *Acarus* species had more delay after the mean, *L. destructor* had an equal balance before as after the mean and *T. longior* showed a longer period where hatch occurred prior to the mean.

The minimum exposures to the MAs which were observed to prevent hatch of mite eggs are shown in Table 3. *Tyrophagus longior* was always the most tolerant species surviving the longest exposure times for all the MAs. This pattern was more defined in the gas mixtures with 0.5% O_2 levels and in all CO_2 mixtures. *Acarus farris* and *A. siro* followed in the hierarchy of survival for the remaining species in each of the MAs, and then finally *L. destructor*. There was little difference in the efficacy of burner gas and N_2 . Therefore the addition of 10% CO_2 to the nitrogen had no effect on the egg hatch of these mites. With an increase in the O_2 content in the two N_2 -based mixtures, there was a rapid loss of the efficacy. However with CO_2 the exposure periods required to prevent egg hatch showed only a slight increase with the change to 98%, and at 60%, even though the O_2 content of the mixture increased from 0.4 to 8%, CO_2 was almost as effective and would be more economical to use at this O_2 level.

Figures 1, 2, 3 and 4 show the effect that exposure to MAs had on the time taken for the eggs to hatch. Exposure to the MAs delayed egg hatch by at least the time under gas in all the species. A further delay in hatch occurred once the exposure exceeded a certain time for each species, irrespective of the MA used. There was a hierarchy with *A. farris* at 4 days, *L. destructor* at 6 days, *A. siro* at 8 days, and *T. longior* at 12 days. If the mean number of days required for hatch is used as a reference for each species, and egg development stops under gas, then hatch should be taking place this number of days after the eggs were removed from the MA. This was not the case even with the MA which caused the least delay in hatch. For *A. farris* there was a further 20 days before hatch, the same for *A. siro* and *L. destructor*, and for *T. longior* a further 10 days. The differences in the number of days to egg hatch after different MA exposure times did not have any pattern with regard to the different gaseous constituents and may be due to the range of egg hatch for each species that was seen in Table 2.

Regression analysis was carried out on the egg hatch data in Figures 1, 2, 3 and 4 to see if it was possible to detect any difference in the delay in hatch between the different MAs for each species (Table 4). This was not so for *A. farris* and *L. destructor*. A general equation was produced for these 2 species. For *A. siro* ($F_s=5.825$, $p<0.01$) only 3 comparisons were possible with the constraint of 5 data

Table 2. Mean number of days ($\pm$ S.E.) and range for egg hatch for four species of mites at 15 °C and 80% r.h.

Species	Replicate	Number of eggs	Mean ($\pm$ S.E.)	St deviation	95% Confidence limits	Range
<i>A. farris</i>	A	40	7.98a (0.34)	2.14	7.29–8.66	4–15
	B	35	7.80a (0.35)	2.08	7.08–8.52	4–13
	C	34	8.59a (0.60)	3.49	7.37–9.81	4–16
	Combined	109	8.11 (0.25)	2.62	7.61–8.61	4–16
<i>A. siro</i>	1 A	35	8.29b (0.32)	2.62	7.63–8.95	4–15
	1 B	36	8.67b (0.49)	2.97	7.66–9.67	5–21
	1 C	37	8.70b (0.36)	2.21	7.97–9.44	5–16
	1 Total	108	8.56d (0.23)	2.39	8.10–9.01	4–21
	2 A	43	10.02c* (0.27)	1.77	9.48–10.57	6–14
	2 B	49	8.78c (0.27)	1.92	8.23–9.33	5–12
	2 C	37	10.08c (0.37)	2.25	9.33–10.83	7–19
	2 Total (B&C)	92	9.36d (0.20)	1.94	8.96–9.76	5–19
	1 & 2 Combined	200	8.93 (0.16)	2.23	8.61–9.24	4–21
	Combined					
<i>L. destructor</i>	1 A	39	9.08a (0.45)	2.81	8.17–9.99	4–13
	1 B	46	9.22a (0.35)	2.37	8.52–9.92	4–13
	1 C	53	8.55a (0.40)	2.91	7.75–9.35	4–15
	1 Total	138	8.92c (0.23)	2.71	8.47–9.38	4–15
	2 A	47	8.62b (0.52)	3.53	7.58–9.65	4–15
	2 B	36	10.61b* (0.52)	3.12	9.56–11.67	4–15
	2 C	25	9.72b (0.64)	3.18	8.41–11.03	4–14
	2 Total	72	9.00c (0.40)	3.43	8.19–9.81	4–15
	1 & 2 Combined	210	8.95 (0.21)	2.97	8.54–9.35	4–15
	Combined					
<i>T. longior</i>	A	38	11.42d (0.48)	2.95	10.45–12.39	6–19
	B	231	11.26d (0.18)	2.71	10.90–11.61	4–20
	C	238	11.32d (0.14)	2.08	11.06–11.59	7–20
	Total	507	11.30 (0.11)	2.45	11.09–11.51	4–20

a, b, c, d: Tukey's pairwise comparisons; *separation of means $p < 0.05$.

points though there was one representative from each of the MAs. 2% O₂ in 98% N₂ was significantly different from the other 2 MAs (Table 4). This was unusual as a difference between the N₂-based MAs is unexpected. The flatter slope for 2% O₂ in 98% N₂ suggests that this MA has less effect on egg hatch after MA exposure.

Table 3. The minimum exposures (Days) to modified atmospheres observed to prevent egg hatch in four species of mite at 15 °C and 80% r.h.

Species	Modified atmospheres						
	Simulated burner gas		N ₂		CO ₂		
	10% CO ₂ , 89.5% N ₂	10% CO ₂ , 88% N ₂	99.5%	98%	99%	98%	60%
	0.5% O ₂	2% O ₂	0.5% O ₂	2% O ₂	0.5% O ₂	2% O ₂	40% Air (8% O ₂)
<i>A. farris</i>	16	24	8	30	6	20	16
<i>A. siro</i>	6	36	8	32	12	14	20
<i>L. destructor</i>	2	12	8	12	8	10	10
<i>T. longior</i>	16	36	22	38	20	28	30

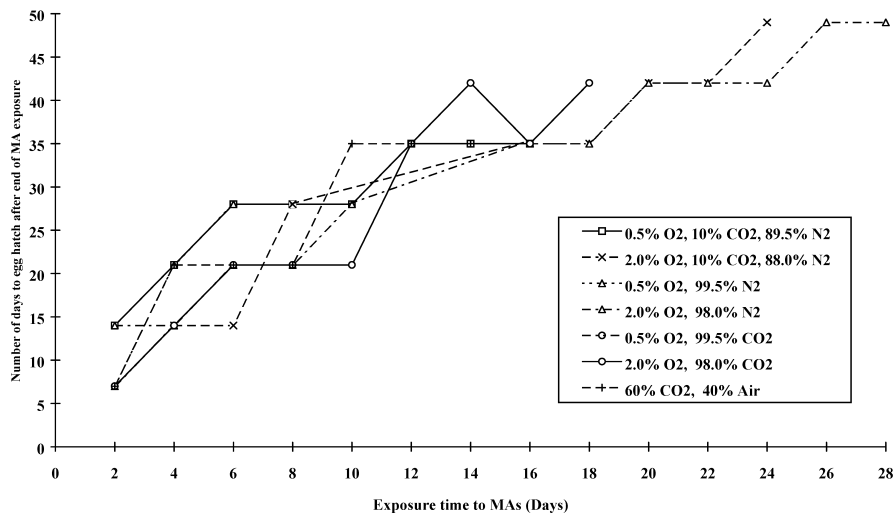


Figure 1. The delaying effect of sublethal MA exposure on the hatch of mixed-age eggs of *Acarus farris*.

The difference between the N₂-based MAs was also seen in *T. longior* ($F_s=4.731$, $p<0.01$), particularly between 2% O₂ simulated burner gas and the N₂-based MAs (Table 5). However for this species 2% O₂ simulated burner gas had the shallower slope. There was a pattern for a decrease in slope and therefore a decrease in the delay of hatch due to MA exposure with an increase in O₂ level for the 2 N₂-based MAs but this was only significant for simulated burner gas. This pattern was reversed for the CO₂-based MAs but this was not a significant difference. There were only two significant differences between the N₂-based MAs and the CO₂-based MAs. These were not the same N₂-based MA so there was an indication that there

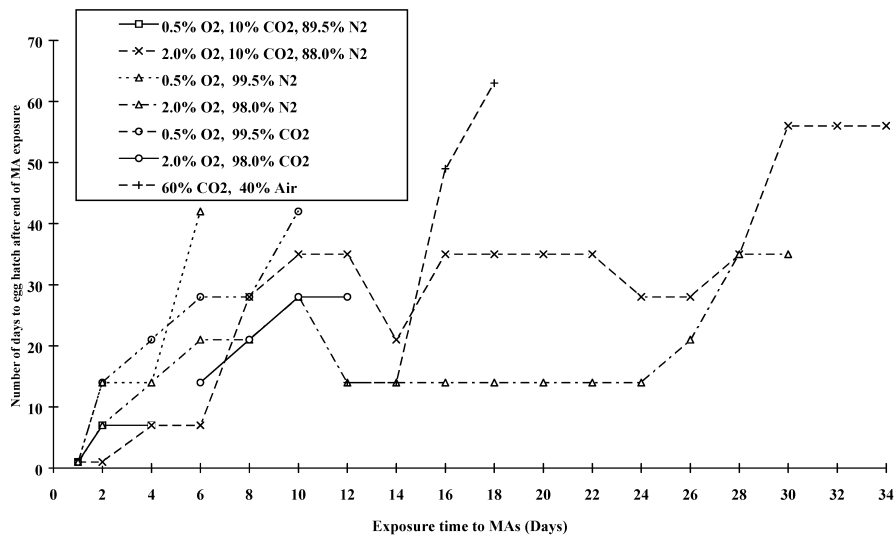


Figure 2. The delaying effect of sublethal MA exposure on the hatch of mixed-age eggs of *Acarus siro*.

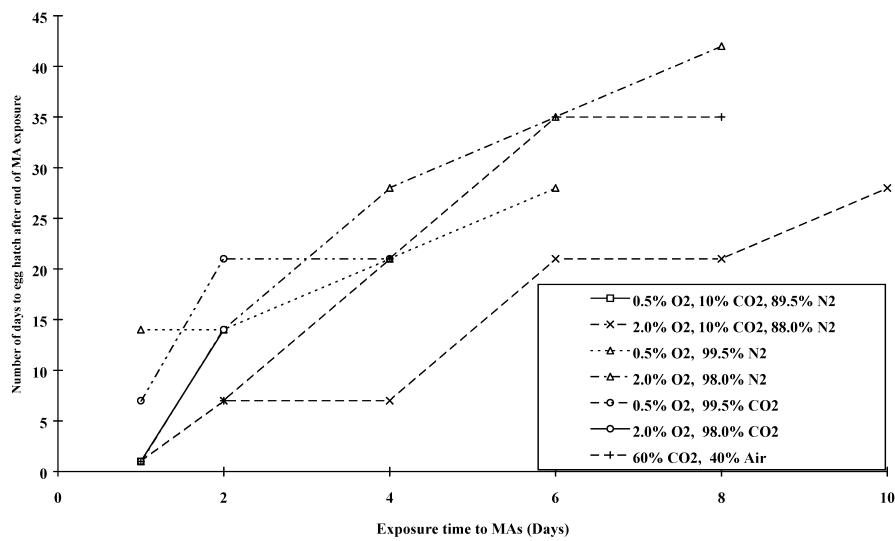


Figure 3. The delaying effect of sublethal MA exposure on the hatch of mixed-age eggs of *Lepidoglyphus destructor*.

may be a difference but the actual scale of the figures show that there is no significance to this pattern.

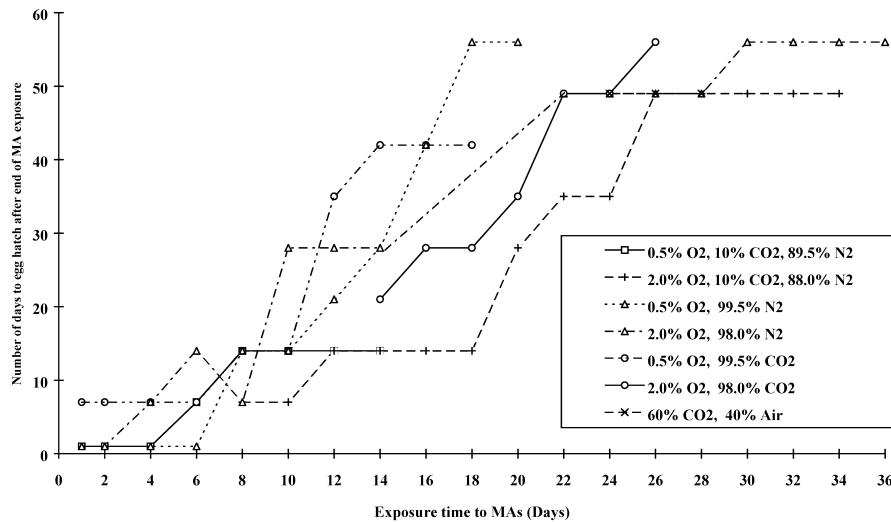


Figure 4. The delaying effect of sublethal MA exposure on the hatch of mixed-age eggs of *Tyrophagus longior*.

Discussion

Published results for the mite species gave average length of time for egg hatch as 12.7–19.7 days (20 °C and 85% r.h.) for *A. farris* (Chmielewski 1984), 7.6 days (15 °C and 80% r.h.) for *A. siro* (Cunnington 1985), 11.7 days (14 °C and 75% r.h.) for *L. destructor* (Barker 1983) and 15.8 days (20 °C and 85% r.h.) for *T. longior* (Chmielewski 1984). The results for *A. farris* and *T. longior* are at a higher temperature so egg hatch time should be much longer with the decrease to 15 °C as this temperature is near the lower end of the temperature range for oviposition of these species. However in both cases the hatch time was much shorter in the present study than the previous studies at 20 °C. There was better agreement for *L. destructor* where the use of a lower temperature and r.h. in Barker (1983) would mean a slightly shorter hatch time at the conditions used in the present study. The difference in the results for *A. siro* under the same conditions were small. Genetic variation in populations may account for this.

The most tolerant stages against low O₂ MA exposure are those with the lowest metabolic rate. For insects these are the egg and pupal stages (Reichmuth 1987). There is no pupal stage in mite development but there is an inert hypopus which may be formed between the protonymph and tritonymph in *Acarus* and *Lepidoglyphus* (Hughes 1976). Its expression is determined by adverse environmental conditions allowing for dispersal and/or dormancy (Knulle 1991). This stage has shown increased tolerance to fumigants over all other developmental stages (Cunnington and Heuser 1953; Barker 1982). This tolerance may extend to MAs as well.

The mobile stages of larvae, nymphs and adults are controlled very quickly as the few published results for the use of MAs against mites demonstrate. Hughes

Table 4. Regression analysis for each mite species.

Species	Modified atmosphere	Number	$\log_{10} (\text{time} + 1)$ ($\pm$ S.E.)	$\log_{10}$ (constant + 1)	R^2
<i>A. farris</i>	General	44	0.541 (0.029)	0.235	0.283
<i>A. siro</i>	2% O ₂ , 10% CO ₂ , 88% N ₂	18	1.137 (0.117)	0.045	0.854
	2% O ₂ , 98% N ₂	16*	0.583 (0.152)	0.560	0.512
	0.5% O ₂ , 99.5% CO ₂	6	1.500 (0.375)	0.150	0.800
<i>L. destructor</i>	General	16	0.937 (0.092)	-0.377	0.117
<i>T. longior</i>	0.5% O ₂ , 10% CO ₂ , 89.5% N ₂	8	1.276 (0.227)	-0.240	0.840
	2% O ₂ , 10% CO ₂ , 88% N ₂	14	0.821 (0.077)	0.541	0.905
	0.5% O ₂ , 99.5% N ₂	11	1.697 (0.245)	-0.564	0.842
	2% O ₂ , 98% N ₂	15	1.243 (0.093)	-0.058	0.932
	0.5% O ₂ , 99.5% CO ₂	10	0.893 (0.178)	0.428	0.760
	2.0% O ₂ , 98.0% CO ₂	7	1.646 (0.164)	-0.594	0.953

* Significant difference $p < 0.05$.Table 5. Separation of regression coefficients for *Tyrophagus longior*.

Modified atmosphere	0.5% O ₂ , 10% CO ₂ , 89.5% N ₂	2% O ₂ , 10% CO ₂ , 88% N ₂	0.5% O ₂ , 99.5% N ₂	2% O ₂ , 98% N ₂	0.5% O ₂ , 99.5% CO ₂	2.0% O ₂ , 98.0% CO ₂
0.5% O ₂ , 10% CO ₂ , 89.5% N ₂	—	*	NS	NS	NS	NS
2% O ₂ , 10% CO ₂ , 88% N ₂		—	**	**	NS	*
0.5% O ₂ , 99.5% N ₂			—	NS	*	NS
2% O ₂ , 98% N ₂				—	NS	NS
0.5% O ₂ , 99.5% CO ₂					—	NS
2.0% O ₂ , 98.0% CO ₂						—

NS Not significantly different $p > 0.05$; *Significantly different $p < 0.05$ level; **Significantly different $p < 0.01$ level.

(1943) showed *A. siro* mobile stages were controlled in two and a half days with a low O₂ level between 0.8–1.2% at 20 °C and 100% humidity. The results for CO₂ showed that at least 30% was needed to produce instantaneous anaesthesia while

100% gave complete mortality after 4 days, the same result was achieved by 100% N₂. The higher temperature used in this work would have increased the effectiveness of low O₂ atmospheres. Navarro et al. (1985) worked with *A. siro* in a direct comparison between 15 and 26 °C at 75% r.h. 2% O₂ in N₂ produced 100% mortality after 2 days with the latter temperature and after 6 days with the former temperature, although with 40% CO₂ and 21% O₂, both temperatures gave complete mortality after 4 days. Pagani and Ciampitti (1991) used 100% CO₂ and N₂ against *A. farris* and *T. longior* on salami at 11 to 15 °C. They achieved complete control for both species after 6 days for both gases. However they were only actively monitoring mobile stages and their final check was made only 8 days after the end of exposure. Therefore they would have missed eggs hatching after this period.

Newton (1991, 1993) used mixed cultures of *A. siro* and *Tyrophagus putrescentiae* (Schränk) in a fumigation 'bubble' with 60% CO₂ in tests at a range of temperatures. The former species was controlled by a 14-day exposure at 15 °C and above while the latter survived at 15 °C but succumbed to 20 °C. This is a shorter time for control of *A. siro* than obtained in the present study but, although the efficacy would be increased at the higher temperature and the delay in hatch would probably be reduced as well, the cultures from 15 °C may not have been kept long enough to ensure complete hatch.

Stepien (1974) used 25 °C for tests with *T. putrescentiae*. The higher temperature and different species make comparisons with the present tests difficult. However Stepien (1974) did show that egg stages were more tolerant than mobile stages with 100% mortality in 99.5% CO₂ at 85% r.h. after 6 days exposure of eggs whilst the mobile stages were all killed in 1 day. It was also noted that 1 day-old eggs were more tolerant than older eggs. It is thus the egg stage that is the target for control of mite species.

To minimise the exposure time, correct selection of atmosphere will be important both for the species concerned and for the gas-tightness of the storage area. Where a good seal is not possible and O₂ levels cannot be brought down to 0.5% with burner gas or N₂, then CO₂ will be the most effective MA. In general, results from trials with MAs have indicated that CO₂ atmospheres are usually more toxic than O₂-deficient ones (Bell et al. 1980; Navarro and Donahaye 1990). Both CO₂ and anoxia force the spiracles open and rates of water loss will be up to seven times as high as when the spiracles are closed (Mellanby 1934). In addition CO₂ causes acidification of the body fluids, due to the formation of carbonic acid. The presence of this acid means that less lactate and therefore a lower energy yield, is required for inhibition of glycolysis than with anoxia and this energy deficit may be the reason for the more rapid lethal action of CO₂ (Adler 1994).

Navarro et al. (1985) have shown that an atmosphere as low as 30% CO₂ was capable of controlling *A. siro* adults in 4 days, at 15 °C and 75% r.h. This is supported by field-scale trials of White and Jayas (1991) who controlled all mites in bins after 42 days where the CO₂ rarely exceeded 40% and the grain temperatures were between 12 and 15 °C. These gas concentrations are suitable for controlling some beetle species such as *Cryptolestes ferrugineus* (Stephens) and *Tribolium castaneum* (Herbst) (White and Jayas 1993). However more tolerant species such

as *Sitophilus granarius* (L.) require higher concentrations and the exposure times required to kill all stages, as shown by Reichmuth (1990), are longer than those for *T. longior* and *A. siro*.

These tests have demonstrated that MAs are able to control all stages of mite development in the environmental conditions found in a grain or cheese store providing the MA can be maintained for the required exposure time. The eggs are far more tolerant than the mobile stages. For an accurate assessment of the efficacy of a treatment it is this stage that must be monitored and an adequate time must be given to allow for the delay in egg hatch due to MA exposure.

There was a range of responses by the different species to the gases and therefore correct identification of the mites present is important or alternatively the use of the exposure period required for the most tolerant species, *T. longior*. The most important aspect for control is to insure that the exposure continues until all eggs would be killed. Dosage schedules for MA use must allow for the mite or insect to be controlled during its most tolerant phase (Reichmuth 1987). Alternatively it may be possible for a short exposure killing all the mobile stages to be followed by an interlude allowing the eggs to hatch, and then a further exposure to remove the population completely. A similar strategy was used very effectively by Bowley and Bell (1981) in laboratory experiments with phosphine and methyl bromide against mites. The combined time for this two-treatment strategy may be less than the times needed for a single MA exposure. This method would also combine well with the usual practices for grain storage of drying followed by ambient cooling (Armitage et al. 1994). The MA would insure the removal of mites from the grain bulk at the beginning of the grain storage season and may limit any surface infestations developing later in the storage season which can occur during cool, moist weather after aeration.

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