Donahaye, E.J., Navarro, S., Bell, C., Jayas, D., Noyes, R., Phillips, T.W. [Eds.] (2007) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, Gold-Coast Australia. ***8-13th August 2004****. FTIC Ltd. Publishing, Israel. pp. 29-44*

LOW OXYGEN REQUIREMENTS FOR POPULATION CONTROL OF TWO MITE SPECIES OF STORED GRAIN

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ABSTRACT

The use of controlled atmospheres (CA) to disrupt the life cycle of mites is discussed. A different approach was required if the exposure time for CAs with low oxygen (O_2) in nitrogen (N_2) was to be reduced. Rather than attempting to kill all stages, the O_2 content of the CA to be used would not affect egg hatch but would kill the far more susceptible larvae. This would be technically easier to achieve and more economic to run, but would require treatment starting from the moment the crop was placed in storage. Different levels of O_2, 4, 5 and 6%, were used with N_2 as the main constituent as well as 5% O_2 and 7.5 % carbon dioxide (CO_2) in N_2 and the addition of 10 or 20% CO_2 to 6%O_2 in N_2. The two mite species tested were *Acarus siro* L. and *Tyrophagus longior* (Gervais), both of which, as eggs, show a high level of tolerance to CAs.

The laboratory tests were carried out at 20 and 25ºC on whole wheat with relative humidities (r.h.) of 75, 80 and 85%. The exposures were determined by the generation time of each species at each combination of temperature and r.h. Comparison of numbers at the start and end of the exposure allowed the reduction in population growth to be assessed.

The maximum O_2 content resulting in cessation of population growth was: 5% for *A. siro* at 25ºC, and 4% for *A. siro* at 20ºC and *T. longior* at 20 and 25ºC. With the addition of CO_2 these limits can be raised with cessation of population growth at: 6% O_2 and 20% CO_2 for *A. siro* at 20ºC and 6% O_2 and 10% CO_2 at 25ºC, and 6% O_2 and 10% CO_2 at 20 and 25ºC for *T. longior*.

INTRODUCTION

Bulk food storage provides a set of unique conditions and a particular number of invertebrates are well placed to take advantage of this environment. Certain astigmatid mites in particular are well adapted to take advantage of these conditions. They are able to complete their development more rapidly and produce more offspring at lower temperatures than insects and are rapidly becoming important

economic pests in temperate regions (Cunnington, 1976; White, 1995). Damage is caused by consumption of the grain germ and endosperm (Parkinson, 1990), contamination with fungal spores (Lacey, 1988) and the secretion of lipids, which taint the grain (White, 1995). Their allergens have been detected within the food chain and have been shown as a causative agent for asthma, rhinitis and eczema (Chambers et al., 1999). In cool, damp grain populations of up to 1000 mites/kg are often considered as unavoidable (Bell and Armitage, 1992).

Manipulation of the storage environmental conditions is an important part of the successful storage of grain and helps to maintain the grain quality and avoid conditions that would allow rapid pest development. This can be achieved by chilling, drying or altering the gaseous composition of the intra-granular atmosphere (Lacey, 1988). Ambient aeration is not able to cool the grain to prevent moderate infestations of mites, as *Acarus siro* L. may persist with grain temperatures as low as 4-9^{o}C (Armitage *et al*., 1994), and so the grain has to be dried to 12-13% moisture content (m.c.) (Burrell and Havers, 1976). However the surface grain will absorb moisture and thereby becomes favourable for mite population growth (Armitage and Cook, 1999).

Insecticides have been used in the past for mite control on grain with success, 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). Resistance to many compounds is becoming a worldwide problem (White, 1995) and in the UK in particular, pirimiphos-methyl, the main insecticide used for grain (Starzewski, 1991) now has limited efficacy against mites (Prickett, 1997).

Controlled atmospheres (CA) offer an alternative strategy for mite control especially with the mounting public concern about environmental contamination and a desire for pesticide-free food (Gibson, 1988). This technology involves the alteration of the natural ratio of the atmospheric gases, oxygen (O_2), nitrogen (N_2) and carbon dioxide (CO_2), to render the atmosphere in stores unfavourable to pests (Banks and Fields, 1995). Previous laboratory tests with CAs against mites have shown promising results (Hughes, 1943; Mitsura et al., 1973; Stepien, 1974; Lider *et al*., 1983; Navarro *et al*., 1985). The egg stage has been shown to be most tolerant to CAs (Li et al., 1998; Conyers and Bell, 2003) and to fumigants, including methyl bromide and phosphine (Bowley and Bell, 1981). White and Jayas (1991, 1993) have carried out successful field trials with CO_2 in small grain bins against mixed populations of several mite species in cool temperatures.

Previous work has shown that the most tolerant stage of astigmatid mites was the egg and to achieve complete control over *A. farris* (Oudemans), *A. siro, Lepidoglyphus destructor* (Schrank) and i (Gervais) required an exposure to 0.5% O_2 in N_2 for 22 days (Conyers and Bell, 2003). An increase in O_2 to 2% required an increase in exposure time to 38 days. A different approach was required if the exposure time was to be reduced. Rather than attempting to kill all stages, O_2 content of the CA to be used would allow egg hatch but would then kill the far more

susceptible larvae. This would be technically easier to achieve and have a lower running cost, but would require treatment starting from the moment the crop was placed in storage. If the use of CAs for mite population control is to realise its potential in the field, laboratory tests are needed to ascertain the O_2 levels required to achieve this aim, the exposure times required to prevent population growth and to show any differences in susceptibility of the various species.

Two astigmatid mite species were chosen for the tests. *A. siro* is the commonest species in UK grain stores throughout the year (Lynch *et al.,* 1991) and does the most damage to the grain (Parkinson, 1990). *T. longior* is another common inhabitant of grain stores though mainly a fungal feeder (Parkinson *et al.,* 1991) it does also reduce seed germination (Zdarkova, 1996) and has been shown to be the most tolerant of the species tested to CAs (Conyers and Bell, 2003).

MATERIALS AND METHODS

Two strains, *A. siro* strain A44 and *T. longior* strain T3 were used throughout the experiments. Each mite species was reared on food (yeast and wheat germ (3:1)) in 50 ml conical flasks. The cultures were sieved through a 180 μm mesh to remove the eggs. The mites were then exposed to an N_2-based CA on whole wheat at two temperatures, 20 and 25ºC, and three relative humidities (r.h.), 75, 80 and 85% rh, to assess the effect on their population growth. A parallel exposure under similar combinations of temperature and r.h. at the normal O_2 level (20.9%) was used as a comparison. The exposure length was derived from the generation time at each combination of temperature and r.h. (Table 2). Generation times for *A. siro* were determined by Cunnington (pers. com.) (Table 1). There was no information for *T. longior* so the results for *T. putrescentiae* (Schrank) were used but preliminary results showed that an increase of at least five days was needed.

Wheat Preparation

A preliminary assessment of the m.c. of the whole wheat to be used was made by drying a sample in a ventilated oven at 130ºC for 2 h (ISO 712). Three 1000 g batches of wheat were used for each species. These were then conditioned to the required m.c.s of 16, 17.5 and 18.5% (equivalent to r.h.s of 75, 80 and 85%) by dampening the grain with the appropriate quantity of distilled water. This was followed with shaking by hand for 5 min every hour for 4 h (Henderson, 1990). Then the samples were placed in ziploc bags at 5ºC and left for a week to allow uptake and even distribution of the moisture within the sample. Another assessment of each sample was carried out to ensure they had achieved the correct m.c. A further wetting and cool storage period was undertaken if the correct level had not been reached and this was followed by a further m.c. test.

Handling of Mites

A rounded spatula-full of mites was added to each glass jar (60 mm diameter x 60 mm height), which contained 50 g of whole wheat. An assessment was made of the mite numbers by counting the mites in five of these amounts, once each under a low powered binocular microscope. The sample was placed on to a zoned disc (Solomon, 1962) and evenly distributed with a small fine brush. The number of mobile mite stages on an eighth of the total disc area was counted and multiplied up. An average was then calculated for the five samples and rounded to the nearest 10. The jars were sealed with two 55 mm Whatman No. 1 filter papers (Whatman International Ltd., Maidstone, England) placed on top and secured with a metal screw lid with its centre removed. This was done 24 h before the start of the exposure to allow the mites to become acclimatised.

To assess the affect of the temperature r.h. combinations on the untreated samples, 5 jars were used for each r.h., with 15 in total for each of the two temperatures. The untreated samples were placed in glass containers (220 mm diameter x 250 mm height) over 500 ml of potassium hydroxide solution of correct specific gravity to produce the required r.h.s of 75, 80 and 85% (Solomon, 1951) and under the same combinations of temperature and r.h. as the treated samples. *A. siro* and *T. longior* were placed in separate glass containers so there was a set of three containers for each species at each temperature.

The treated replicates were placed on mesh grills in glass containers of similar dimensions. The 5 jars of both *A. siro* and *T. longior* were placed together so that there were three different sets of r.h. conditions at each temperature. Each CA was produced by a gas blender (Signal Instrument Co. Ltd., Camberley, Surrey). The gas stream was humidified by bubbling through an 80 ml column of glycerol/distilled water solution contained in a 100 ml measuring cylinder (Johnson, 1940), which under static conditions was designed to give a 5% higher r.h. than required to compensate for the observation in pilot tests that the moving gas stream did not fully equilibrate as it bubbled through the cylinder. It was essential that m.c. did not fall during the exposures and that the m.c. of the treated wheat remained at least as high as the controls so that the factor demonstrably affecting population growth was the CA alone rather than a drop in r.h. The solutions were topped up to the 80 ml level every three days. The CA was released under the samples at a rate of 90 ml/min. The output of the blender was set using O_2 (Model 570A, Servomex Ltd., Crowborough, Sussex) and CO_2 meters (Anagas CD 95, Environmental Instruments, Leamington Spa, Warwickshire) which were zeroed and calibrated before each assessment of each CA. CA readings were also taken throughout the exposures from the output of the treated glass containers with the same meters to ensure that the correct mixture was maintained.

After completion of an exposure, an assessment was made of the mite numbers. A similar method was used to that for the number of mites in one spatula. Each of the five replicate wheat samples for the control and the treatment was placed on a sieve (1.70 mm nominal aperture) and shaken for 30 seconds. The sample was passed through a further sieve (710 μm nominal aperture) before reaching the collection dish. Its contents were then placed on the disc. If the mite numbers were below 200 on the sample zones the mites on the whole of the disc were counted. An average population size was then calculated for the untreated and the treatment. A percentage reduction in population growth due to the CA was calculated by comparing the two population sizes. The five wheat samples for the control were combined as were those for the treatment and a final m.c. assessment was obtained for each.

RESULTS

Moisture content was used to assess the r.h. within the glass containers throughout the exposures. There was a drop in m.c. of the controls after exposure with an average of 0.3 % mc lost at both temperatures for *A. siro* and 0.4% mc lost at 20 and 25ºC respectively for *T. longior* (Table 3). For *T. longior*, the numbers in the controls at 75% r.h. for all the experiments were below the initial population level (Tables 4-9). In most cases, the comparable m.c. in CA-treated samples increased above the initial level and was therefore higher than its control value (Table 3). Therefore the moisture conditions for population growth were more favourable to mites in the treated samples than in the controls.

At 6% O_2 population suppression of *A. siro* occurred only at 75% r.h. for both temperatures when compared to the untreated, but there was no effective population reduction of *T. longior* (Table 4). When O_2 was reduced to 5%, populations of *A. siro* were reduced by at least 97% at all temperature/r.h. combinations (Table 5). For *T. longior* a 90% or higher level of population suppression was achieved only at 25ºC with 75 or 80% r.h. A further reduction to 4% O_2 gave population reduction of 99% or higher for *A. siro* and 94 % or higher for *T. longior* (Table 6).

The addition of 7.5% CO_2 to 5% O_2 made little difference to the population suppression of *A. siro* achieved by 5% O_2 alone (Tables 5 and 7). The interpretation of results for *T. longior* was hampered by very poor survival in controls, especially at 25ºC, although complete control was obtained at 25°C and 75% r.h.

TABLE 1
Mean developmental period from egg to adult in days of two mite species (Cunnington, pers. com.)

Species	Temperature (ºC)	r.h. (%) 75	80	85
A. siro	20	-	16	14
	25	14	12	11
T. putrescentiae	20	-	22	20
	25	16	14	12

TABLE 2.
Actual exposure lengths (Days) used

Species	Temperature (ºC)	r.h. (%) 75	80	85
A. siro	20	22	16	14
	25	16	14	12
T. longior	20	34	30	26
	25	26	20	18

However the addition of 10% CO_2 to 6% O_2 (Table 8) improved the population suppression of both species especially at the high r.h. levels when compared to 6% O_2 alone (Table 4). Below 85% r.h., at both temperatures, there was a reduction in *A. siro* similar to that using 5% O_2 alone (Table 5). At all r.h.s, the reductions in

TABLE 3
Initial and change in the moisture contents (m.c.) of whole wheat in the untreated jars and after exposure to 6% oxygen in nitrogen and to 6% oxygen and 20% carbon dioxide in nitrogen

			CA					
			6% O_2 in N_2			6% O_2 & 20% CO_2 in N_2		
			r.h. (%)					
Species	Temperature (°C)	m.c.	75	80	85	75	80	85
A. siro		Initial	16.3	17.8	18.6	15.7	17.5	18.5
	20	Post: Untreated	-0.2	-0.5	0.0	-0.3	-0.7	-0.3
		Treated	+0.3	+0.4	+0.3	+0.1	0.0	-0.1
	25	Post: Untreated	-0.1	-0.5	-0.2	-0.4	-0.5	-0.1
		Treated	+0.4	+0.4	+0.2	+0.2	+0.1	-0.4
T. longior		Initial	16.1	17.6	18.6	15.9	17.4	18.5
	20	Post: Untreated	-0.1	-0.5	-0.1	-0.4	-0.7	-0.4
		Treated	+0.8	+1.0	+0.6	+0.1	+0.6	+0.4
	25	Post: Untreated	-0.2	-0.6	-0.2	-0.6	-0.8	-0.2
		Treated	+0.7	+1.0	+0.5	+0.2	+0.9	+0.2

T. longior numbers were even greater, being equal to that achieved with 4% O_2 alone (Table 6). A further increase in CO_2 to 20% (Table 9) brought the levels of population suppression closer to those achieved with 5% O_2 at 85 r.h. for *A. siro* (Table 5) but not to the levels of 4% O_2 (Table 6).

TABLE 4
Average number of mites per jar before and after exposure to 6% oxygen in nitrogen and percentage reduction for the treated compared to the untreated

Species	r.h.	20ºC Time	20ºC Untreated	20ºC Treated	20ºC Reduction	25ºC Time	25ºC Untreated	25ºC Treated	25ºC Reduction
	%	Days			%	Days			%
A.siro		Initial	420				420		
	75	21	2730	270	90	18	3660	80	98
	80	17	3990	690	83	14	2790	470	83
	85	17	4270	1030	76	12	3630	600	84
T. longior		Initial	880				880		
	75	38	630	270	57	28	590	260	56
	80	35	1400	340	76	24	640	450	30
	85	31	1620	450	72	20	1260	830	34

DISCUSSION

Relative humidity is a very important factor controlling population growth in mites and if it falls to 65%, growth will cease for *T. longior* (Chmielewski, 1984) and at 60% *A. siro* also fails (Cunnington, 1965). The present tests covered some of the physical conditions that were the most favourable for oviposition, hatching and population increase for *A. siro* (20-25ºC and 80-90% r.h.) (Cunnington, 1985). Under the test conditions *A. siro* had a faster growth rate than *T. longior* and multiplied well in all the environmental combinations whereas T. longior showed higher population growth at 85% r.h, as suggested by Solomon (1946).

There was a notable difference in response to the CAs between the species as the only survivors in the treated *A. siro* cultures were adults. Although there was no

count made of the proportion of the different mobile stages it was noted especially on counts below 100, which occurred when O_2 content was at, or below 5% that there were no juveniles present, and there was no oviposition, even though the

TABLE 5
Average number of mites per jar after exposure to 5% oxygen in nitrogen and percentage reduction for the treated compared to the untreated

		20°C				25°C			
Species	rh	Time	Untreated	Treated	Reduction	Time	Untreated	Treated	Reduction
	%	Days			%	Days			%
A. siro		Initial	500				500		
	75	21	1640	20	99	20	1400	0	100
	80	20	4640	50	99	14	4750	30	99
	85	14	6060	140	98	14	8310	60	99
T. longior		Initial	480				480		
	75	35	170	50	71	27	90	10	90
	80	30	1310	300	77	22	870	50	95
	85	27	1850	490	73	17	1590	260	84

environmental conditions were favourable. Therefore an increase in exposure lengths would have ensured complete mortality of the population. With *T. longior* eggs had been laid but there was no evidence of hatch.

Previous work has shown that eggs of T. longior are tolerant of low O_2 atmospheres and egg hatch is delayed under CAs of 2% O_2 and lower (Conyers and Bell, 2003). The addition of CO_2 appeared to reduce the numbers of eggs present and therefore the combination of the two gases would be needed to suppress oviposition in this species most effectively. Such atmospheres can be provided by combustion of hydrocarbons. Burner gas with propane fuel yielding an O_2 level of 6.1% would also have 9.7% CO_2 present in the CA (Bell and Armitage, 1992). The presence of CO_2 as in burner gas should also be beneficial in the control of other mite species such as *A. siro*.

TABLE 6
Average number of mites per jar after exposure to 4% oxygen in nitrogen and percentage reduction for the treated compared to the untreated

Species	rh	20°C Time	20°C Untreated	20°C Treated	20°C Reduction	25°C Time	25°C Untreated	25°C Treated	25°C Reduction
	%	Days			%	Days			%
A. siro		Initial	970				970		
	75	21	3780	0	100	19	3020	0	100
	80	16	5680	20	99	13	3610	0	100
	85	16	9990	40	99	12	4300	10	99
T. longior		Initial	790				790		
	75	34	270	25	91	27	200	0	100
	80	30	1190	40	97	20	1380	20	99
	85	28	3440	130	96	20	2000	130	94

The results from this study are supported by other published work. The most tolerant stages of insects against low O_2 CA exposure are the egg and pupal stages, which have the lowest metabolic rate (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* (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 CAs.

The mobile stages of larvae, nymphs and adults are controlled very quickly. Hughes (1943) showed *A. siro* mobile stages were controlled in two and a half days

with a low O_2 level between 0.8-1.2% at 20°C and 100% humidity. Navarro et al. (1985) worked with adult *A. siro* at 15 and 26°C at 75% r.h. They found that 100%

TABLE 7
Average number of mites per jar after exposure to 5% oxygen and 7.5% carbon dioxide in nitrogen and percentage reduction for the treated compared to the untreated

		20°C				25°C			
Species	rh	Time	Untreated	Treated	Reduction	Time	Untreated	Treated	Reduction
	%	Days			%	Days			%
A. siro		Initial	420				420		
	75	27	620	10	98	14	620	20	97
	80	16	1680	50	97	12	1040	50	95
T. longior		Initial	520				520		
	75	33	120	30	75	20	110	0	100
	80	22	600	50	92	14	190	30	84

mortality was obtained when O_2 was 6% or less after a 5-day exposure at 15ºC whereas at 26ºC the time was reduced to 3 days. Pagani and Ciampitti (1991) used 100% N_2 against *A. farris* and *T. longior* on salami at 11 to 15°C. They achieved complete control of the mobile stages of both species after 6 days. In all these cases the O_2 levels were much less than the present study and there are few other studies with mites to support the present findings. Li *et al*. (1998) used 5% O_2, and 10 % CO_2 in N_2 at similar temperatures to see the effect on development and reproduction of *T. putrescentiae*. The results showed that the CA had an inhibition on both the factors studied and this increased with increasing temperature. The newly-hatched larva was most sensitive and the egg was the least. Exposure to the CA also reduced female longevity and fecundity.

Publications of work with CAs against mites are few and for evidence of the tolerance of eggs required work with another CA, CO_2. Stepien (1974) showed that egg stages were more tolerant than mobile stages of *T. putrescentiae* with 100% mortality in 99.5% CO_2 at 25°C and 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.

The present tests have demonstrated that these 4-5% O_2 CAs are able to control all stages of mite development in the environmental conditions found in a grain store. The advantage of these CAs is that they are more economic to generate than those

TABLE 8
Average number of mites per jar after exposure to 6% oxygen and 10% carbon dioxide in nitrogen and percentage reduction for the treated compared to the untreated

		20°C				25°C			
Species	rh	Time	Untreated	Treated	Reduction	Time	Untreated	Treated	Reduction
	%	Days			%	Days			%
A. siro		Initial	700				700		
	75	21	5640	40	99	18	5030	20	99
	80	19	5290	330	94	15	5170	80	98
	85	15	5010	450	91	12	5300	240	95
T. longior		Initial	1490				1490		
	75	35	520	0	100	32	770	0	100
	80	33	1200	60	96	22	1960	20	99
	85	32	3460	90	97	20	2380	80	96

with an O_2 content of 1% or less and require the same techniques for application based on a continuous flow of CA to the target bulk. They give control in a similar time to that of the mite life cycle. A CA with 6% O_2 and 10% CO_2 provided a good level of population suppression of both species which could be produced on-site from a propane-fuelled burner gas generator.

CONCLUSION

1) Nitrogen with low O_2:

a) Reduction in population levels was less at higher r.h. in most treatments.

b) 5% O_2: stopped population growth of *A. siro* at 25ºC, and at 75 and 80% r.h. at 20°C.

c) 4% O_2: stopped population growth of *A. siro* at 20°C and of *T. longior* at 20 and 25°C.

2) Addition of CO_2

a) Both species: 7.5% CO_2 with 5% O_2 content was no more effective than 5% O_2 alone.

b*) A. siro*: 6% O_2 and 10% CO_2: There was a reduction similar to that of 5% O_2 alone at both temperatures. 6% O_2 and 20% CO_2: Brought the levels of population suppression closer to those achieved with 5% O_2 but not to the levels of 4% O_2.

c) *T. longior*: 6% O_2 and 10% CO_2: The population suppression was equal to that with 4% O_2 alone.

TABLE 9
Average number of mites per jar after exposure to 6% oxygen and 20% carbon dioxide in nitrogen and percentage reduction for the treated compared to the untreated

		20°C			25°C			
Species	rh	Time	Untreated	Treated	Reduction	Time	Untreated	Treated	Reduction
	%	Days			%	Days			%
A. siro		Initial	470				470		
	75	24	1730	70	96	18	2170	20	99
	80	17	3310	100	97	14	3990	92	98
	85	14	4010	100	98	12	3760	50	99
T. longior		Initial	1100				1100		
	75	38	430	0	100	33	430	0	100
	80	33	1330	0	100	32	2100	0	100
	85	32	2820	0	100	20	1840	20	99

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