Crop & Food Research Confidential Report No. 744

Elevated carbon dioxide fumigation of thrips (Thrips tabaci Lind.) in export onions

BBC Page, D Brash, C W van Epenhuijsen & M Bendall

October 2002

A report prepared for
New Zealand Onion Exporters Association

Copy 1 of 5

New Zealand Institute for Crop & Food Research Limited
Private Bag 11 600, Palmerston North, New Zealand

Contents

1 Executive summary

Onion thrips (Thrips tabaci) continue to be a major problem in export onions. Controlled atmospheres using elevated carbon dioxide gas have shown promise in controlling New Zealand flower thrips and other export insect pests.

We tested five CO_2 concentrations (15, 30, 45, 60, and 100%) over three periods (24, 48 and 72 hours) on adult thrips in stored onions. Onions were treated and stored at 20°C to reflect ambient onion storage conditions within New Zealand. Egg mortality and onion quality were assessed 4 weeks after treatment.

- The level of thrips mortality in the controls was high and similar to earlier work, even though the onions were handled carefully to avoid killing thrips through physical damage.
- The effectiveness of the CO_2 treatments was also similar to earlier work: Total thrips mortality was achieved after 48 hours at 30% CO_2, but differences between the CO_2 treatments and the control were not large.
- Nymph mortality increased with the concentration of CO_2, but was unchanged by the duration of exposure. However, adult thrips and nymphs were found in all treatments, indicating that the treatments would not meet quarantine standards for export onions.
- There was no evidence of any egg mortality.
- Informal observations on onion quality indicated that firmness, skin colour and presence of rots were not affected by any of the CO_2 concentration/durations tested.

2 Introduction

Quarantine regulations for horticultural crops require produce to be free from all live insects. Pesticides are usually used for insect control on export crops; however, more consumer friendly pest control methods are being sought.

An insect pest that is a major problem for the export onion industry is onion thrips (*Thrips tabaci* Lind). Only a small number of fumigants are available to control this pest. Therefore alternative control methods are being sought by the onion industry. Elevated CO_2 control atmospheres are being tested as these have been shown to be effective against several other insect pests (Stevenson & Hurst 1995; Carpenter et al. 1996).

The current work is a follow-up to previous work carried out during March 2001. The results of this previous work, in which thrips were exposed to various concentrations of CO_2 gas, showed that a higher level of thrips mortality occurred in the CO_2 treatments than in the control treatments (air supplied by a compressor). However, the control treatments also had a high level of thrips mortality, which increased over time ($P<0.05$). Differences between the control treatment and the CO_2 treatments were not large. This current work was performed to see if we could obtain more reliable data on the relationship between thrips mortality and CO_2 time/concentrations. Excessive handling of the onions was thought to be the main reason for the high thrips mortality in the control and we wanted to find out if more careful handling of the onions would reduce the mortality level in the controls. To avoid killing thrips by crushing them, onions were never handled around the middle and were instead moved by grasping the neck leaf material.

The previous work did not investigate the extent to which elevated CO_2 gas could be used to control the hatching of eggs, nor did it include an evaluation of onion quality after exposure to high levels of CO_2. The second trial reported here looked at these issues.

3 *Methods and materials*

Export grade Pukekohe Long Keeper onions (cv. Dominator, Pukekohe grown), which were naturally infested with onion thrips, were used in the trial.

3.1 *Experimental design*

Twenty-four plastic bags, each containing 60 randomly selected onions, were constantly supplied with gas via pressurised cylinders (BOC Gases, Palmerston North). Six different CO_2 concentrations, 0, 15, 30, 45, 60 and 100%, were applied to the onion-filled bags and each treatment was replicated four times. A control treatment consisted of the supply of air only. The bags were treated at 20°C to reflect the ambient temperature that the onions would normally be stored at. The concentrations of CO_2 were regularly measured throughout the experiment using a CO_2 gas sampler (Model TYP: Analytical Development Co., England). Twenty randomly selected onions were removed from each treatment and assessed for thrips at intervals of 24, 48 and 72 h after the bags were filled with gas. The bags were then resealed.

3.2 *Adult mortality*

Thrips assessment on onions was performed by cutting the bulb in half (from top to bottom) and removing the first two layers of skin. If any thrips were present, one or two layers of the white fleshy bulb scales were also removed. The area near the roots was also peeled and checked, and if thrips were present, the bulb and scales were separated as well. Counts were recorded of both live and dead thrips in each bulb.

To accurately assess adult mortality, treated infested onions were stored at 15°C for 24 hours before assessment. This allowed a recovery period for thrips that were in a moribund state

3.3 Eggs and mortality

A similar method to that described above was used to assess the effects of the CO_2 treatments on egg hatchability and nymph mortality in onions. Once treated, onions were stored at 20°C in air for 4 weeks before counts were made of adult thrips.

3.4 Onion quality assessment

Post treatment onions (12 from each treatment) were set aside for 4 weeks at 20°C in normal air before being assessed visually for their quality (firmness, flesh colour, and rots).

3.5 Statistical analysis

The effectiveness of the CO_2 treatments (concentration and duration) did not differ between onions that were in the same bag (this was assessed using exact binomial confidence intervals). Therefore, 'onion' was not included as a random effect in any of the statistical analyses.

Treatments with a 100% kill rate were not included in the statistical analysis. Since they had no variation, including them would result in a downward bias in the standard errors appropriate for the treatments without a 100% kill.

Where the treatments that could be included in the analysis were balanced, analysis of variance was used to analyse the number of live insects immediately after treatment. Otherwise, residual maximum likelihood was used (Patterson & Thompson 1971).

A generalised linear mixed model (using the Poisson distribution with a log link function) was used to analyse the number of adults 4 weeks after treatment (Schall 1991). The curves fitted to the number of live adults 4 weeks after treatment were rectangular hyperbolae, fitted using a modified Newton method of maximising the likelihood (Ross 1990). The sign test (Siegel 1956) was used to evaluate the negligible but consistent increase in thrips where numbers were too small to apply parametric methods.

Analysis of variance was used to analyse the number of nymphs 4 weeks after treatment, and the data required a log transformation to stabilise the variance. The data were very skewed, so the backtransformed means are shown (the raw means are more heavily influenced by unusually high values).

4 Results

4.1 Analysis of live thrips

Counts of live and dead thrips on each onion allowed us to assess the percentage of onions with thrips present. The percentage of onions that had thrips on them before the experiment began did not vary between the treatments (P=0.7848 for CO_2 concentration; P=0.8937 for duration of exposure; and P=0.659 for the interaction between concentration and duration).

There was a progressive decrease in the number of thrips alive as the concentration of CO_2 increased (P<0.001, Table 1). Treatments with 100% kill could not be included in the statistical analysis.

Table 1 Number of live thrips per bag in each treatment (predicted). Figures in brackets are confidence intervals for thrips numbers.

		CO_2 concentration %					
		0%	**15%**	**30%**	**45%**	**60%**	**100%**
0 wks	24 h	8.8	4.9	0.078	0.5	0	0
SEM:1.6	48 h	7.1	2.1	0	0	0	0
	72 h	8.2	3.5	0	0	0	0
4 wks	24 h	6.8 (3.4, 13)	4 (1.6, 9.7)	3 (1.1, 8.4)	2 (0.57, 7)	0.25 (0.0073, 8.6)	0.25 (0.0073, 8.6)
	48 h	4.8 (2.1, 11)	5.3 (2.4, 11)	1 (0.17, 5.8)	0.75 (0.097, 5.8)	2.8 (0.94, 8)	1 (0.17, 5.9)
	72 h	2 (0.57, 7)	3.5 (1.4, 9)	0.5 (0.041, 6.2)	0.5 (0.041, 6.2)	2 (0.57, 7)	2.3 (0.69, 7.3)

4.2 Adults alive after 4 weeks

When differences between individual treatments were examined, no effect of concentration and exposure to CO_2 on the number of thrips could be detected (Table 1&2). However, there was a detectable trend in the expected direction, and when curves were fitted to this trend the decrease in the number of thrips with increasing CO_2 concentration was statistically significant (P<0.001). However, there was no clear effect of concentration evident for the exposure time of 72 hours (P=0.061). There appears to be only one point that does not fit with the relationship at 100% CO_2 concentration for the 72 h exposure time, but there is no statistically significant relationship even when this point is removed from the analysis. There was a lot of variation around the curves that were fitted, as can be seen in Figure 1. The statistical model only accounted for 18% of the variance.

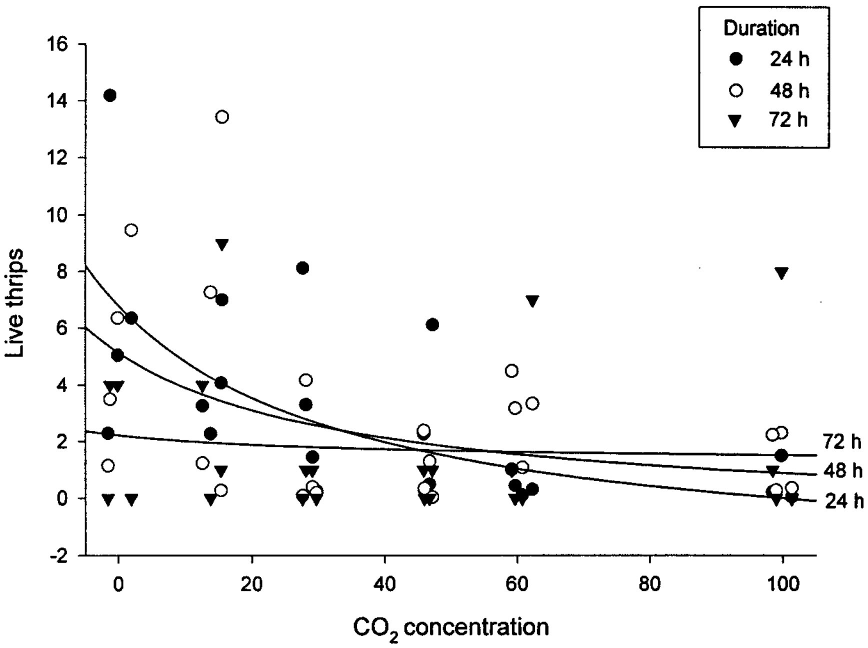

Figure 1. Numbers of live thrips four weeks after treatments with CO_2 gas. Points have been 'jittered' so that overlaid points are visible.

The changes in the numbers of live thrips between the initial treatment date and after the 4 week storage period were analysed. Treatments for which no live thrips were recorded at any of the assessment times could not be included in a statistical analysis of both times of assessment. Therefore, onions dosed with a concentration higher than 15% were excluded from this statistical analysis, except for treatments with a 24 hour duration of exposure at concentrations lower than 60%.

When this subset of treatments was analysed, no overall change was detected in the number of live thrips 4 weeks after treatment (P=0.192), nor were there changes in just a few treatments (P=0.938).

The treatments that could not be included in the statistical analysis also need to be considered. On the whole, in the excluded treatments, there is evidence of an increase in the number of thrips after 4 weeks, as this happened consistently across all the treatments (P<0.001) it indicates that despite good adult kill rates from treatments of 30% plus CO_2 eggs were surviving and thrips hatching from these. However, the consistent increase across all treatments is the only basis for claiming that there was an increase. The actual increase was only 2 or 3 thrips.

4.3 Nymphs alive after 4 weeks

There was a decrease in the number of nymphs alive as the concentration of CO_2 increased (P=0.044). Duration of exposure to the gas revealed no evidence of any effect (P=0.977), nor did any interaction occur between duration of exposure and concentration of CO_2 (P=0.474) (Table 2).

Table 2: Effect of CO_2 concentration on onion thrips nymph population four weeks after treatment (back transformed means after log transformation).

	CO_2 Concentration %					
	0	15	30	45	60	100
Back-transformed means	1.0	1.5	1.6	0.4	0.8	0.1
Log of nymphs	0.01	0.44	0.5	-0.73	-0.16	-1.99
LSD (for use with logged data):	1.6					

4.4 Onion quality assessment

Subjective assessment of variables such as the firmness of the onions, flesh colour, and the presence of mould or rots after 4 weeks post treatment showed that high CO_2 treatments did not cause any reduction in the visual quality of the onions, regardless of CO_2 concentration or treatment duration, within the 4 week holding period.

5 Discussion

As indicated in our previous work, total adult thrips mortality in onions was achieved at 30% CO_2 concentration after 24 to 48 hours of exposure. The effect of treatment duration followed a similar pattern seen in New Zealand flower thrips where 100% mortality was seen at 40% CO_2 concentration (at 24°C) after 36 hours (Carpenter et al. 1996). Higher CO_2 concentrations appear to result in increased thrips mortality, as seen in the increase in New Zealand flower thrips mortality when thrips were exposed to 18% CO_2 compared to 9% CO_2 at 20°C (Potter et al. 1994).

Elevated carbon dioxide levels reduce adult thrips populations in onions, and if the incidental mortality is similar in an industry situation, total mortality can be achieved. However, we were testing CO_2 concentration/durations specifically aimed at the export onion industry where there is a need to control thrips populations over the period of shipping and transport. The life cycle of onion thrips is reported to range from 9 to 23 days, depending on temperature (Harris et al. 1936; Harrison & Jacks 1952; Rahman & Batra 1945). We held the onions for 28 days at 20°C, which allowed ample time for eggs to hatch and a re-infestation to occur. Elevated CO_2 concentrations have been found to kill eggs and larvae in some insects (Benschoter 1987). There was an effect of CO_2 concentration on the number of live nymphs, but nymphs and adult thrips were still found in all treatments, 4 weeks after treatment. It is therefore not possible to guarantee that all thrips would be dead at the time of any quarantine inspection. The treatments would thus fail to meet the probit 9 level required by quarantine regulations.

6 Recommendations

- While total adult thrips mortality can be successfully achieved at 30% CO_2 after 24-48 duration, the treatment is ineffective on unhatched eggs and therefore not suitable for export onions under the conditions in which the trial was carried out. It therefore cannot be recommended for control on export onions.
- Informal observations on onion quality indicated that the CO_2 had no adverse effects on onion quality after 4 weeks. A high CO_2 treatment could therefore be used for adult thrips disinfestation.
- There may be factors other than the CO_2 that may be limiting factors that influence the efficiency of the CO_2 treatment on thrips eggs and their ability to hatch. Temperature is one of the most obvious factors. The mortality of New Zealand flower thrips increased with high temperatures and high CO_2 level (Potter et al. 1994). Using higher temperatures may be a key to control of unhatched eggs. This needs further testing.

7 References

Benschoter C.A.1987: Effects of modified atmospheres and refrigeration temperatures on survival of eggs and larvae of Caribbean fruit fly (Diptera: Tephritidae) in laboratory diet. Journal of Economic Entomology 80: 1223-1225.

Carpenter, A., Wright, S., Lash, P. 1996: Response of adult New Zealand flower thrips, *Thrips obscuratus,* to high carbon dioxide and low oxygen atmospheres at various temperatures. Bulletin of Entomological Research 86: 217-221.

Harris, H.M., Drake, C.K. and Tate H.D. 1936 Observations on the onion thrips. Iowa Collegiate Journal of Science 10 pp 155-172.

Harrison, R.A. and Jacks, H. 1952: Control of onion thrips at Pukekohe. New Zealand Journal of Science and Technology, Vol 34 pp 335-338.

Stevenson, D.G.; Hurst, P.L. 1995: Mortality of black field crickets (*Teleogryllus commodus*) in carbon dioxide atmospheres. New Zealand Journal of Zoology. 22: 381-385.

Patterson, H.D. & Thompson, R. 1971: Recovery of inter-block information when block sizes are unequal. Biometrika 58, 545-554.

Potter, M.A., Carpenter, A., Stocker, A, and Wright, S. 1994: Controlled atmospheres for the post harvest disinfestations of *Thrips obscuratus* (Thysanoptera: Thripidae). Journal of Economic Entomology 87: 1251-1255.

Rahman, K.A. and Batra, A.L. 1945: The onion thrips (*Thrips tabaci* Lindeman, Thripidae, terebrantia: Thysanoptera) India Journal of Agriculture, 14 308.

Ross, G. J. S. 1990: Nonlinear estimation. Springer-Verlag. New York.

Schall, R. 1991: Estimation in generalized linear models with random effects. Biometrika, 78, 719-727.

Siegel, S. 1956: Nonparametric Statistics for the Behavioural Sciences. McGraw-Hill, New York.