Efficacy of phosphine fumigation of apples for codling moth (*Cydia pomonella*) disinfestation

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Abstract Codling moth, although rare on New Zealand export pipfruit, is a quarantine actionable pest that limits access to countries where it is not present and requires specific control measures during production. Fumigation with phosphine gas is a disinfestation technology that has recently been extended to fresh produce. Apples infested with codling moth fifth instar larvae and eggs were fumigated for two durations at concentrations from 500 to 3500 ppm phosphine at two temperatures. Larval mortality assessed 3 days post fumigation at 0.5°C increased with increasing dose, with little difference between 48- and 72-h treatments. In contrast, mortality after fumigation of larvae at 12°C did not increase with dose and resulted in overall higher mortality than 0.5°C. Codling moth eggs were more susceptible to fumigation at 0.5°C than at 12°C; fumigation at 12°C had minimal effect at all doses. The implications for improved market access using phosphine fumigation are discussed.

Keywords: codling moth, phosphine fumigation, apples.

INTRODUCTION

Codling moth (*Cydia pomonella* (L.)), although rare on New Zealand export pipfruit, is a quarantine actionable pest that limits access to countries where it is not present and requires specific control measures during production (Waddell & Petry 1996; Walker et al. 1998). New Zealand apples have been able to be exported to Japan since 1993 after first being fumigated with methyl bromide followed by cool-storage as a mandatory postharvest treatment (Anonymous 1993). Methyl bromide use is being limited worldwide because of its toxic and ozone-depleting properties (Anonymous 2001), leading to a search for alternatives.

Phosphine has a long history as a product for disinfesting grain (Chaudhry 1997) and recently has been used as a pest mitigation treatment at cool-storage temperatures for a wide variety of fresh produce in Chile (Horn et al. 2010) and for kiwifruit in New Zealand (Jamieson et al. 2012). An efficacious fumigant against codling moth could replace methyl bromide for the Japanese

market and facilitate access for New Zealand apples to other countries where codling moth is not present. In consultation with the pipfruit sector, this project sought to determine the efficacy of phosphine fumigation against two key life-stages (young eggs and fifth instar larvae) of codling moth. Any new treatment would need to achieve complete mortality of a large number of codling moth (>10,000) to be a feasible alternative to methyl bromide as a mandatory treatment to satisfy an importing country's statutory requirements. The aim of the present study was to find a fumigation regime that would not compromise apple quality or packhouse logistics, e.g. requiring excessive time.

MATERIALS AND METHODS

Fruit and insects

Codling moths were obtained from the Plant & Food Research insect rearing facility (Mt Albert Research Centre, Auckland) and reared on modified Brinton's artificial diet (Brinton et al. 1969) (Ashby et al. 1985). Briefly, insects were reared in individual polystyrene tubes at 25°C and fifth instar larvae were programmed for diapause by exposing them to a photoperiod of 12:12 h light:dark. 'Granny Smith' apples (110 count size, equivalent to approximately 75 mm diameter) were infested with codling moth eggs and fifth instar larvae as described by Dentener et al. (1998). These life-stages were chosen because they were the hardest to kill with the toxic fumigant methyl bromide (Waddell & Petry 1996). Actively feeding larvae were carefully extracted from the tubes and placed within holes punched in apples (three evenly spaced holes per apple) using a compressed-air driven punch. The larvae were sealed within the holes using an agar gel (2% agar in water) that was in turn held in place with a piece of masking tape. Infested fruit were placed on fruit packing trays and contained within a securely fastened gas permeable nylon mesh bag.

Freshly emerged laboratory-reared codling moths were allowed to mate for 48 h at 25°C before the introduction of 'Granny Smith' apples on which the moths were able to oviposit for 24 h. Two apples with 1-day-old eggs were similarly placed on cut sections from fruit packing trays and put in gas permeable nylon mesh bags (Dentener et al. 1998) (mean ± SEM of 93 ± 8 eggs per replicate). All infested apples were stored at the appropriate fumigation temperature overnight before starting the fumigation the next morning.

Fumigation

Phosphine fumigations were carried out between November 2011 and February 2012 at the Plant & Food Research fumigation facility in Palmerston North, New Zealand. For each combination of temperature (nominal 0.5 or 12°C) and duration (48 or 72 h), four nominal concentrations were examined for each run: 500, 1000, 2000 or 3500 ppm (vol/vol). Each run constituted a replicate and was started on a different day. There were three replicates for each temperature, duration and concentration combination (16 treatments). Larval and egg controls were exposed to similar handling but remained at 25°C. The measured phosphine concentrations and fumigation temperatures are given in Table 1.

For each run, 30 apples infested with codling moth (28 apples each with three fifth instar larvae and two infested with 1-day-old eggs) were placed into each of four 27-litre chambers (55300-00 modified vacuum desiccators, Labconco Kansas City USA). These chambers were evacuated to approximately vacuum 40 kPa using a vacuum pump, and a predetermined dose of 1.39% phosphine gas in nitrogen was injected through a non-return valve using a 1-litre Perspex syringe. Residual vacuum was then released by opening then closing a shut-off valve, and the phosphine was then mixed thoroughly with a fan fitted with magnets and controlled by an external motor placed on the horizontal glass door. After mixing, the chambers were transferred to a temperature-controlled shipping container for the duration of the run.

Concentrations in the chambers were monitored twice daily for the duration of the run. Monitoring consisted of withdrawing duplicate 3-ml samples of gas from the chambers

Table 1 Range of measured phosphine concentrations (lowest-highest, mean of the duplicate samples, ppm vol/vol) within the fumigation chamber and apple fruit flesh temperatures (mean ± SEM) for each replicate fumigation run, treated for 48 or 72 h at a nominal temperature and concentration.

Rep.	Duration (h)	Temp. (°C)	Concentration (ppm)			
			500	1000	2000	3500
		0.5				
1	48	1.17 ± 0.06	437-556	860-1011	1720-2049	3154-3784
	72	0.99 ± 0.04	407-562	850-1042	1678-2033	2878-4004
2	48	1.33 ± 0.07	494-602	1102-1249	2075-2298	4059-4263
	72	1.09 ± 0.05	481-615	1017-1204	2057-2386	3764-4312
3	48	1.83 ± 0.08	417-683	927-999	1703-1936	32083597
	72	1.49 ± 0.06	392-518	882-1035	1701-2002	3100-3459
		12				
1	48	13.1 ± 0.06	454-566	794-1040	1666-1864	3347-3561
	72	12.8 ± 0.05	461-577	722-1070	1794-2049	3415-3643
2	48	13.1 ± 0.02	444-552	886-1045	1869-1945	3544-3699
	72	13.0 ± 0.02	379-543	636-1046	1755-2022	3476-3832
3	48	12.3 ± 0.03	426-557	885-1070	1598-2012	2771-3077
	72	12.2 ± 0.03	421-564	729-1039	1819-2052	2780-3558

through an airtight septum, and injecting the samples into an SRI 8610C gas chromatograph equipped with a Flame Ionisation Detector (FID) and a J&W GS-Q 30 m × 0.53 mm column. Chromatography conditions were: oven temperature isothermal at 100°C, carrier gas helium at 45 psi, FID at 300°C and injector temperature at 250°C. Fruit flesh temperatures in the chambers were monitored throughout the fumigations using SAPAC dataloggers. Oxygen and carbon dioxide concentrations were measured within the fumigation chambers near the completion of one representative run at 0.5 and 12°C for both the 48 and 72 h treatments. Analysis was completed using a Checkpoint portable headspace gas analyser (PBI Dansensor, Ringsted Denmark).

After the completion of the treatment, each chamber was evacuated by opening the doors under a fume hood and aerating for 10 min. Apples and eggs were then transferred to 25°C prior to mortality assessment.

Mortality assessment

The larvae and eggs were examined 3 and 10 days, respectively, after treatment, and their mortality status was recorded (Dentener et al. 1998). Larvae were removed from the apples and examined using a magnified lamp; any larvae that moved after being gently prodded were deemed alive. Eggs were examined using a binocular microscope and those that had hatched were recorded as live, while unhatched eggs were counted as dead.

Statistical analysis

Data displayed in graphs were corrected for mortality in the untreated controls using Abbott's correction formula (Abbott 1925). Minitab (Minitab® 15.1.0.0. ©Minitab 2006 Inc.) was used to calculate means and standard errors. Negative binomial Generalized Linear Models (GLMs) were used to analyse the effects of concentration, treatment duration and temperature on mortality. The Modern Applied Statistics with S package (Venables & Ripley 2002) provides R (Anonymous 2013) functionality to estimate the over-dispersion that is commonly found in these types of data. Analysing data for the two temperatures separately for each life-stage, the control data were examined to check the stability of the replicates and the effect of

duration. Since both effects were insignificant, it was assumed that the effect of concentration on the measured mortalities of treated individuals was unaffected by handling and the differences observed could be attributed to the concentration and its duration only.

RESULTS

No combination of phosphine concentration, duration or temperature tested caused 100% mortality of fifth instar codling moth larvae (Figure 1). At 0.5°C, the concentration of phosphine had a significant effect on the mortality of fifth instar codling moth larvae ($P<0.001$), but at 12°C concentration had minimal effect. At neither lower nor higher temperature did the duration of treatment significantly influence codling moth larvae exposed to phosphine fumigation. The duration of fumigation had a small but non-significant effect on larval mortality at both temperatures tested. Larvae fumigated at 12°C had the highest (98.0–99.6%) observed mortality (Figure 1).

In contrast, for the eggs the effect of duration was shown to be significant ($P<0.05$) for the lower and higher temperatures. The separate concentrations were also significantly different ($P<0.05$) but no linear relationship can be drawn between them, as shown by the decreased effect at the highest concentration (Figure 2).

Oxygen and carbon dioxide concentrations were respectively decreased and increased within the fumigation chambers at both temperatures compared to ambient concentrations (21% oxygen and 0.04% carbon dioxide) (Table 2). Unexpectedly there was greater change at 0.5°C than at 12°C.

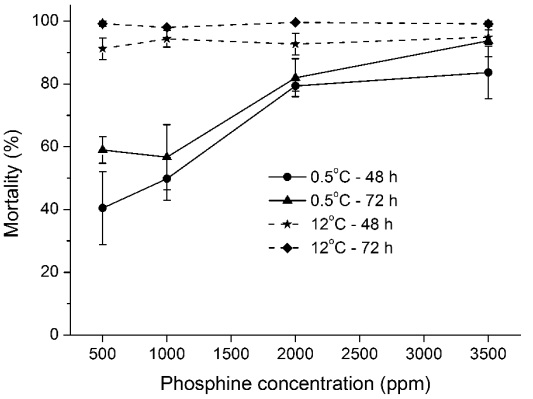

Figure 1 Mortality (mean ± SEM corrected for control mortality) of fifth instar codling moth larvae within apples exposed to different phosphine concentrations for 48 or 72 h at 0.5 or 12°C.

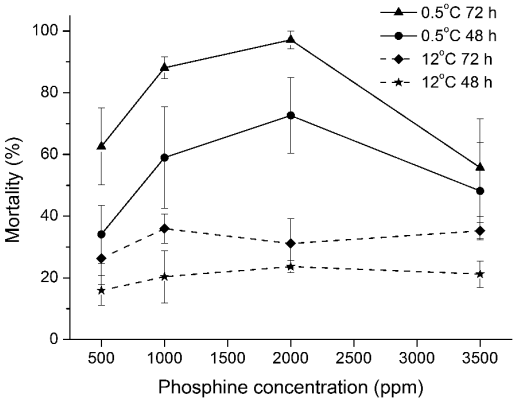

Figure 2 Mortality (mean ± SEM corrected for control mortality) of 1-day-old codling moth eggs on apples exposed to different phosphine concentrations for 48 or 72 h at 0.5 or 12°C.

DISCUSSION

None of the treatment combinations examined in this study provided 100% mortality of the codling moth larvae or eggs, which would be the first step in developing a mandatory postharvest treatment. Furthermore, lower phosphine fumigation temperatures were more efficacious for 1-day-old eggs, but higher temperatures were better for controlling fifth instar larvae. Many of the codling moth larvae categorised as live after exposure to higher concentrations and/or higher temperature could be classified as moribund. These larvae were pink, not flaccid, but only capable of slight movements and when these barely alive larvae were examined 10 days after treatment, most remained moribund. While appearing to be near dead, as discussed by Jamieson et al. (2012), a phytosanitary inspector will justifiably determine moribund larvae to

Table 2 Mean (± SEM) measured oxygen and carbon dioxide concentrations (%) within the fumigation chambers after 48 or 72 h.

	Temperature			
	0.5°C		12°C	
	48 h	72 h	48 h	72 h
Oxygen	17.8 ± 0.7	17.9 ± 0.4	19.6 ± 0.1	20.6 ± 0.1
Carbon dioxide	0.68 ± 0.05	1.19 ± 0.07	1.34 ± 0.06	1.01 ± 0.10

be alive in the absence of an extensive scientific study to demonstrate otherwise.

Bo et al. (2010) measured the toxicity of phosphine at low temperature to *Carposina niponensis*, the peach fruit moth, a fruit-boring species with similar habits to codling moth. For this species, they found that narcosis occurred when the phosphine concentration was >1.67 mg/litre (1120 ppm) at 0°C. Narcosis meant that when the insects were exposed to high concentrations of phosphine, they were paralysed, respiration was reduced and therefore absorption of phosphine was also lowered. Narcosis may explain why at 12°C the mortality of fumigated larvae in the present study did not increase with increasing concentration, in contrast to 0.5°C. The 1-day-old eggs in the present study may also have been affected by narcosis, as shown by increasing mortality at lower concentrations at 0.5°C followed by lower mortality at higher concentrations. Phosphine toxicity typically increases with temperature and has been shown to be related to the insects' metabolic rate (Chaudhry 1997; Chaudhry et al. 2004). The present study showed that fumigation of codling moth eggs at 0.5°C was more efficacious than 12°C. However, it is more likely to be the temperature component of the treatment at 0.5°C that caused high mortality than the phosphine. Codling moth eggs are susceptible to low temperatures, as described by Dentener et al. (1998), where cool-storage is an important component of the postharvest treatment for Japan access for New Zealand apples. This study calculated the median lethal time to 99% mortality as 27 days at 0.5°C (Dentener et al. 1998).

Bo et al. (2010) concluded that phosphine fumigation at 0°C could kill 100% of peach fruit moth fifth instar larvae (the most tolerant life-stage) at 0.83 mg/litre (544 ppm) for 10 days or at 1.39 mg/litre (928 ppm) for 8 days. These are very long exposure times that would not be well accepted by the New Zealand pipfruit sector. Furthermore, this study was undertaken using naked insects and it is probable that larvae within the fruit flesh would be harder to kill because less of the fumigant may reach the insect and also because the larva would be exposed to a lower oxygen atmosphere.

Oxygen is important for the toxicity of phosphine against insects (Chaudhry 1997; Liu 2011). Studies on Indian meal moth eggs demonstrated that phosphine fumigation at 15 and 10% oxygen resulted in significantly greater survivorship than normoxic (20.7%) conditions and super-atmospheric oxygen concentrations greatly increased phosphine toxicity to a wide variety of insects (Liu 2011). Oxygen concentrations within fumigation chambers during this trial were not reduced greatly by fruit and insect respiration at both temperatures. However, oxygen concentrations within the fruit flesh surrounding the codling moth larvae may have been much lower because of fruit and insect respiration. Liu (2012) suggested that highly oxygenated atmospheres may be important for reducing the long fumigation times associated with phosphine fumigation to achieve complete mortality. For perishable commodities this is particularly important so that products are not delayed in reaching their market, and so that chamber efficiency is high, with low costs.

The results of the present study suggest that 72-h exposure to phosphine at 12°C is close to providing complete control of codling moth larvae but at this temperature, phosphine had minimal effect on 1-day-old eggs. Furthermore,

12°C for 72 h may have adverse effects on fruit quality (Johnston et al. 2005). Based upon the results of the present study, the pipfruit sector has chosen not to pursue the development of a mandatory phosphine postharvest quarantine treatment at this time. Phosphine could potentially have a role as a mitigation treatment to improve market access by reducing the risk of detecting live pests, similar to the way it has been used on kiwifruit (Jamieson et al. 2012). Using phosphine in this way would depend on its activity against other pests and its effects on fruit quality, and would require a careful comparison of costs and benefits. Jamieson et al. (2012) highlighted the need for the commercialisation of phosphine for perishables to be based upon cohesive research, which has not always happened internationally.

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