Mortality of snails, *Cernuella virgata* and *Cochlicella acuta*, exposed to fumigants, controlled atmospheres or heat disinfestation.

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Abstract

Introduced snails from the Mediterranean region have become a major problem for the grain industry in southern Australia in recent years. Apart from the losses and inconvenience caused by their feeding and effects on harvesting machinery, some snails may become mixed with harvested grain or legumes. This causes downgrading of the commodity or the requirement for further handling to remove the snails. The snails also present a quarantine problem with some export countries. A range of fumigants, controlled atmospheres and heat disinfestation were assessed as methods for killing *Cernuella virgata*, the Mediterranean white snail, and *Cochlicella acuta*, the small conical snail, in stored grain. The snails were exposed while inactive or aestivating, the form in which they are found contaminating harvested grain.

Except for low oxygen atmospheres, snails require a higher dosage rate of all fumigants tested than those needed to eliminate all insect stages in grain. A 100% mortality was obtained by exposures of 5.4 g/m^3 phosphine for 10 days, 30 g/m^3 methyl bromide for 24 hours, greater than 150 g/m^3 carbon disulphide for 24 hours and 10 g/m^3 hydrogen cyanide for 24 hours. Controlled atmospheres of 80% carbon dioxide for 10 days of low oxygen atmospheres of 1% at ≥25°C for 10 days were required for 100% mortality. Heat disinfestation at peak temperatures of 67°C killed all *Cernuella virgata* while only 57°C was required to kill *Cochlicella acuta*. Disinfestation using low oxygen atmospheres or heat appears the most practical for use with a wide range of stored grains. Phosphine is notably ineffective, requiring excessive dosages for extended periods. The snails became more sensitive to fumigants and other treatments with increasing time in aestivation.

Introduction

Introduced snails from the Mediterranean area have become a major agricultural problem in southern and Western Australia (Baker 1989).The Mediterranean white snail, *Cernuella virgata* (da Costa), and the small conical snail, *Cochlicella acuta* (Müller), were first recorded in South Australia (SA) in 1920 at Millicent and 1953 at Minlaton, respectively (Baker 1986, and references therein). In recent seasons these snails have extended their range from their former infestation focus on the Eyre and Yorke Peninsulas of SA into Victoria and New South Wales. In the 1992–93 season, with a dry mid winter and an above average rainfall in spring and early summer, snail numbers have increased to plague proportions.

White snails cause extensive damage to crops through their feeding. The behaviour of the snails increases the chance that they are harvested with the grain. To avoid dehydration and temperature extremes near the ground in summer the snails climb up the stalks of the crop. They attach themselves to the plant and go into a period of dormant activity called aestivation (Pomeroy 1968). They may then be harvested with the crop causing it to be downgraded or rejected. The snails also make harvesting difficult as they clog machinery. In heavy infestations a third or more of harvested grain or legume may be composed of snails, requiring extensive grain cleaning to give an acceptable grade of commodity. These species of snails are subject to quarantine in many countries and so present a problem in certifying the grain free of pests at the export terminal. Previous work by Richardson and Roth (1965) had shown that aestivating *Cochlicella barbara* (Linnaeus) were very resistant to methyl bromide, hydrogen cyanide, phosphine and other fumigants. It has therefore become critically important to determine what treatments will kill the snails and to provide a range of alternatives to cover a variety of commodities, including those sensitive to particular treatments, such as malting barley.

The response of *C. virgata* and *C. acuta* to fumigants, controlled atmospheres (CA) and heat disinfestation was assessed. Phosphine (PH_3), methyl bromide (MeBr) carbon disulphide (CS_2) and hydrogen cyanide (HCN) were used as fumigants. Carbon dioxide (CO_2) and low oxygen (O_2) controlled atmospheres were tested.

Materials and methods

Snails

In January of 1985 and 1986 approximately 10000 *C. virgata* were collected from Bulgowan, 12 km west of Weetulta, SA. They were collected from fenceposts, stems and stalks of plants and placed for transportation in dry pesticide-free barley. All snails were aestivating at the time of collection.

The *C. virgata* obtained in February 1993 were shipped to Canberra mixed with barley in sacks. The snails were from the Wallaroo and Kadina area, SA and approximately 30000 snails were received. A large proportion of the snails broke aestivation in transit, though became quiescent again in Canberra. *C. acuta* was also available in large numbers in 1993. Approximately 25000 snails from Port Giles, SA were received in February 1993.

On arrival in Canberra, the snails were sorted into classes based on their approximate size and stored in either pesticide-free barley (9% moisture content) in 1985–86, or wheat (10% moisture content) in 1993, at 25°C and ambient relative humidity. The snails were assessed as being alive or dead by appearance and by apparent density, determined by rolling the snail around in the palm of the hand. Dead snails are light and roll differently from live snails. The reliability of this method for determining live snails was approximately 97% for *C. virgata* and 85% for *C. acuta*, as determined by dissection of a sample.

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For fumigation tests, a sample size of 27 was used consisting of a ratio for *C. virgata* of 3 small:12 small to medium:9 medium to large:3 large. This ratio reflects the size distribution of the snails obtained in the initial 1985 sample. For *C. acuta* the size mixture was 18 small : 8 medium : 1 large. Snail samples were weighed and placed in 70 × 50 mm crystallising dishes containing 100 g of 60% r.h. barley or wheat. Each fumigation was conducted at 25°C in a 2.5 L desiccator with a stirrer in the base and a Quickfit cone screwthread adaptor top with a rubber septum. Two randomly selected samples were placed in each desiccator (both of *C. virgata* in 1985–86 experiments and one each of *C. virgata* and *C. acuta* in 1993). Controls were conducted for every fumigation time period. The concentration of the fumigant in each desiccator was measured within an hour of dosing and again just before opening the desiccator at the end of the exposure period.

After all treatments the snails were left overnight to air and removed from the grain the next day. The number of living snails remaining after treatment was determined by wetting the snails lightly with water. This stimulated most of the snails to emerge from their shells within an hour. Emergence of the snails was observed over a 4-hour period and after 24 hours. The snails were stored on petri dishes, with meshed ventilation holes in their lids, at 25°C and ambient relative humidity, typically around 30%. After 14 days the mortality was reassessed using the wetting technique. Those which did not emerge were dissected. Shells with dry and decomposed contents were scored as dead. A very few snails (<15%) were apparently alive, but did not emerge. These were scored as alive for subsequent analysis.

Fumigants

PH_3 was obtained from a concentrated source generated from an aluminium phosphide pellet. The concentration of the source was determined with a Tracor MT–150 gas chromatograph (1.8 m, 80/100 mesh Porapak Q column) using a Gow Mac gas density detector . The dosage rates were 0.8, 1.5, 2.5 and 5.0 g/m^3 PH_3 for each exposure time of 4, 6, 8 or 10 days. The PH_3 concentration in the desiccators was determined using a Tracor MT-220 gas chromatograph (0.82 m 120/150 mesh Porapak Q column) with a flame photometric detector.

The MeBr dosage rates were 6, 12, 24 and 32 g/m^3 for 24 hours and 3, 6, 12 and 16 g/m^3 for 48 hours. The MeBr dose was obtained from the headspace above a liquid source in a sealed vial with a Mininert valve top. The MeBr concentration in the desiccators was measured using a Shimadzu 6AM series gas chromatograph (2 m, 20% OV101 80/100 mesh GasChromQ column) with a flame ionisation detector.

The dosage rates for CS_2 were 25, 50, 100 and 150 g/m^3 for 24 hours. CS_2 was injected as a liquid into the desiccator after a volume of air, equivalent to the volume of the resulting gas, had been removed. The CS_2 concentration in the desiccators was determined using the flame ionisation detector.

The dosage rates for HCN were 5, 10, 20 and 40 g/m^3 for 24 hours and 2.5, 5, 10 and 20 g/m^3 for 48 hours. The HCN was created in the desiccators by the reaction of sodium cyanide with 4M sulphuric acid. A volume of air, equivalent to the volume of the resulting gas, was removed before the sulphuric acid was injected into the sodium cyanide in the desiccator. The HCN concentration in the desiccators was determined, in the 1993 experiments, using a Varian Model 3300 gas chromatograph (15 m, Megabore DB-WAX capillary column) with a thermal conductivity detector.

Controlled atmospheres

Dosage rates for low O_2 treatments were 0.5 and 1.0% O_2 with the balance nitrogen for a fumigation period of 5 and 10 days. These concentrations were obtained by mixing air and nitrogen flows using massflow controllers, Brooks 5850 TR Series with Model 5876 and 5872.6 control units. The gas was passed through a glycerol–water mixture to humidify the air to 60% r.h. and through the desiccators at a flow rate of 100 mL/minute. The experiments were conducted at temperatures of 15, 20, 25 and 30°C.

Dosage rates for CO_2 were 30, 40, 60 and 80% for each fumigation time of 2, 5, 7 and 10 days. The desiccators were dosed by injecting 700 mL CO_2 or larger volumes into the desiccator with the screw cap off to allow displaced air to escape. Experiments were also conducted at temperatures of 15, 20, 25 and 30°C. Low O_2 and CO_2 concentrations were measured using a Fisher Gas Partitioner Model 1200 gas chromatograph (1.8 m, 80/100 mesh Porapak Q, and a 3.3 m, 60/80 mesh Molecular Sieve 13X columns) with a thermal conductivity detector.

Heat disinfestation

For heat disinfestation trials in 1986 and 1993 a sample size of 25 was used for *C. virgata*, with the size ratio of 1 small : 7 small to medium : 10 medium to large : 7 large. In 1993, a sample size of 25 was also used for *C. acuta* with a size ratio of 18 small : 6 medium : 1 large. The snails were counted out, weighed and then placed in 100 g of 60% r.h. barley or wheat overnight. The snails were added to 900 g of barley or wheat before treatment to make a 1000 g sample. The 1000 g samples were heated in a laboratory-scale fluidised bed to 50, 55, 60, 65 or 70°C and then cooled quickly to 35°C (Dermott and Evans 1978). Controls for the experiment consisted of fluidised and non-fluidised samples. Each treatment was conducted in triplicate.

Results

Probit analysis was conducted on the PH_3 and CO_2 results giving LD_{99} for mean concentration × time (CT) dosage as shown in Tables 1 and 2. The results were corrected for control mortality before probit analysis using Abbot's correction (Finney 1971). The relationship between LD_{50} and the length of time in storage of the snails is given Figures 1 and 2. The effect of temperature on the response of the snails to CO_2 is given in Figure 3. This experiment was conducted 120 days after the collection of the snails.

The response of the snails to MeBr is shown in Figure 4. Results for *C. acuta* were suitable for probit analysis, giving an LD_{99} mean CT dosage rate of 16.8 g day/m^3 with 5% upper and lower fiducial limits of 26.6 g day/m^3 and 13.7 g day/m^3 respectively.

The dosage rates of CS_2 below 100 g day/m^3 were unsuccessful in killing all the snails, as shown in Figure 5. The 1985 *C. virgata* results were suitable for probit analysis giving a LD_{99} mean CT dosage rate of 110.5 g day/m^3 with 5% upper fiducial limits of 143.7 g day/m^3 and lower limits of 95.7 g day/m^3.

The effect of HCN on the snails is shown in Figure 6. There was some survival of *C. virgata*, recently collected from the field, even at the high initial dose of 20 g/m^3 HCN (mean concentration of 8 g/m^3) for a fumigation time of 2 days. Sorption of HCN in these experiments was high, with less than 50% of the initial dose remaining in the headspace at the end of the treatment.

Mortality of 100% was obtained at low O_2 concentrations of 0.5 and 1.0% for 5 and 10 days at temperatures 25°C. At 20°C, 4% of the *C. acuta* survived at 1% O_2 while at 15°C survival at 1% O_2 occurred for both snails. *C. acuta* also survived at the

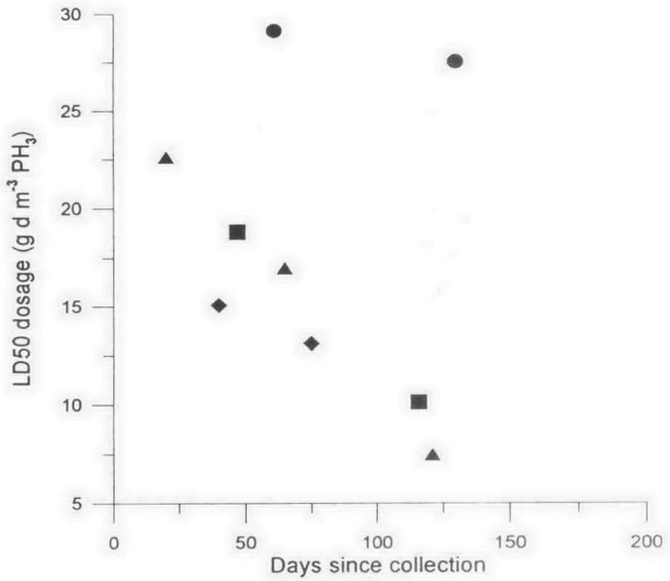

Fig. 1. The relationship between phosphine LD_{50} dosages at 25°C and the length of time in storage of *Cernuella virgata* and *Cochlicella acuta*. *C. virgata* ◆1985, ▲1986, ■1993, *C. acuta* ●1993.

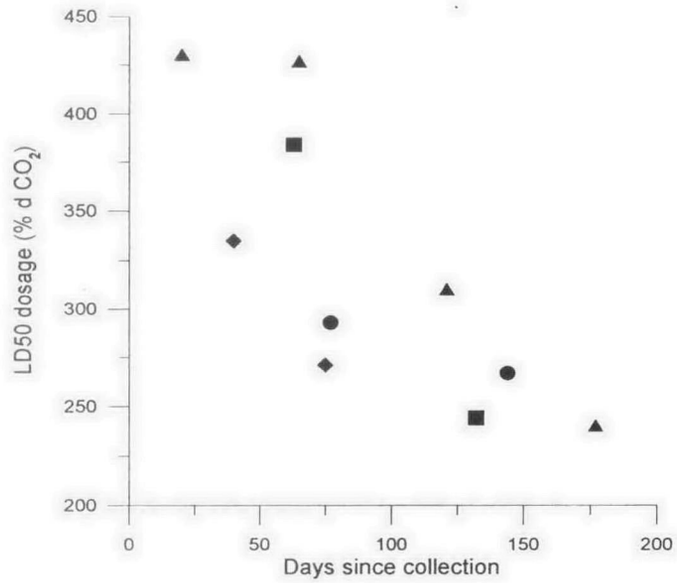

Fig. 2. The relationship between carbon dioxide LD_{50} dosages at 25°C and the length of time in storage of *Cernuella virgata* and *Cochlicella acuta*. *C. virgata* ◆1985, ▲1986, ■1993, *C. acuta* ●1993.

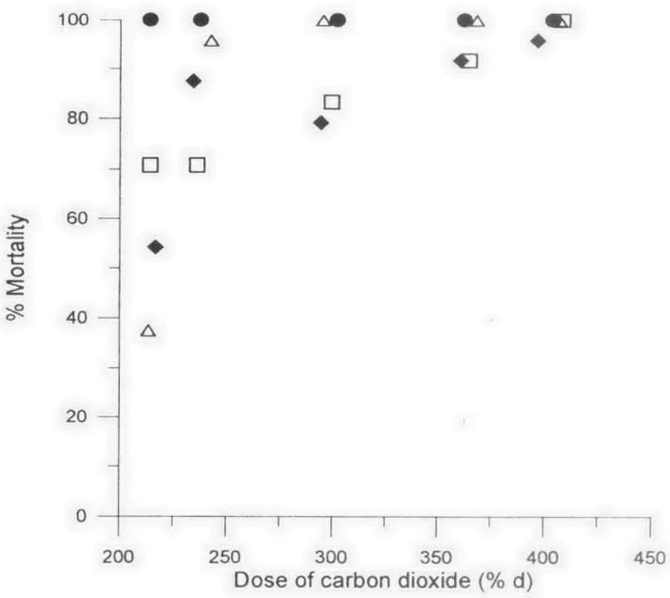

Fig. 3. The effect of temperature on response of *Cernuella virgata* to carbon dioxide, □15°C,◆ 20°C, △25°C, ●30°C.

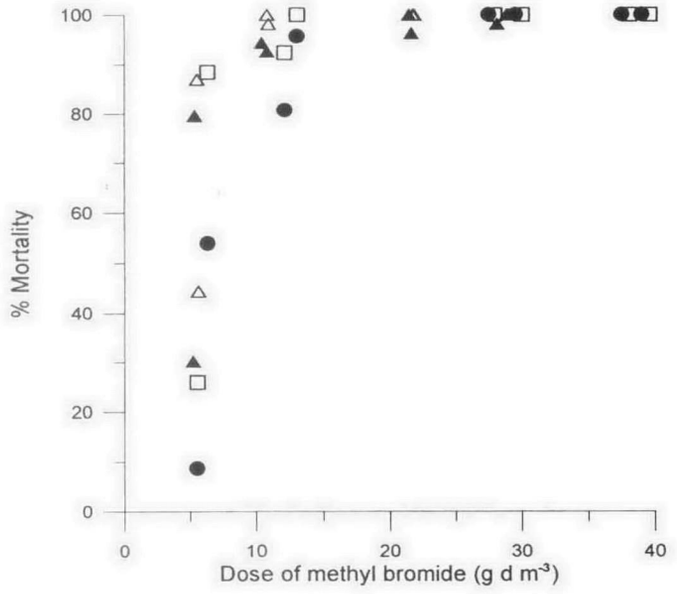

Fig. 4. Response of *Cernuella virgata* and *Cochlicella acuta* to methyl bromide at 25°C. *C. virgata* ▲34 days stored 1986, △76 days stored 1986, □18 days stored 1993, *C. acuta* ● 32 days stored 1993.

0.5% O_2 level. This work was conducted after the snails had been in storage for more than 90 days.

Probit analysis of the heat disinfestation results gave LD_{99} temperatures as shown in Table 3. No additional mortality was incurred by the fluidising action of the method.

Discussion

C. virgata were the most difficult to kill with PH_3 in the 1986 experiment conducted 20 days after collection of the snails. On this occasion a mean concentration of 4.4 g/m^3 PH_3 for 10 days would have been required to kill all *C. virgata*. This value is higher than the dosage rate of 0.26 g/m^3 PH_3 for 10 days required to kill all stages of insect grain pests (C. J. Waterford, personal communication 1993). *C. acuta* exhibited an even greater tolerance to PH_3 than *C. virgata*. A higher dosage rate of 5.4 g/m^3 PH_3 for 10 days would be required for 100% kill of these snails. Maintenance of such a high PH_3 concentration in a store for 10 days is likely to be impractical on technical, cost and environmental grounds, ruling out phosphine as a possible treatment.

C. acuta and *C. virgata* exhibited similar responses to CO_2. A mean concentration of 80% CO_2 for 10 days or a more easily obtainable level of 60% CO_2 for 15 days would be required to kill all snails, whereas a dosage rate of 70% initially with 35% CO_2 for 15 days will kill all insect stages (Annis 1987). The CO_2 levels would require continuous

Table 1. Exposure of *Cernuella virgata* and *Cochlicella acuta* to phosphine, showing dosages required for 99% mortality and its change with length of time since collection of the snails.

Snail	Year	Days since collection	LD_{99}		
			Dosage CT (g.day/m^3)	upper 5% fiducial limit	lower 5% fiducial limit
C. virgata	1985	40	24.7	27.9	22.6
C. virgata	1985	75	22.8	26.1	20.6
C. virgata	1986	20	43.4	55.8	36.7
C. virgata	1986	65	31.6	40.4	26.9
C. virgata	1986	121	15.7	19.8	13.7
C. virgata	1993	47	42.0	51.4	36.4
C. virgata	1993	116	30.1	41.3	24.7
C. acuta	1993	61	53.4	67.2	45.7
C. acuta	1993	130	50.8	63.7	43.8

Table 2. Exposure of *Cernuella virgata* and *Cochlicella acuta* to carbon dioxide, showing dosages required for 99% mortality and its change with length of time since collection of the snails.

Snail	Year	Days since collection	LD_{99}		
			Dosage CT (% d)	upper 5% fiducial limit	lower 5% fiducial limit
C. virgata	1985	40	735.0	1198.9	576.0
C. virgata	1985	75	632.9	988.5	501.6
C. virgata	1986	20	800.2	907.6	725.2
C. virgata	1986	65	786.0	927.2	698.2
C. virgata	1986	121	647.5	794.8	565.8
C. virgata	1986	177	731.8	1411.5	553.5
C. virgata	1993	63	796.0	917.5	715.1
C. virgata	1993	132	610.1	745.6	531.6
C. acuta	1993	77	574.2	667.0	515.0
C. acuta	1993	144	556.5	702.0	480.2

Table 3. Peak heat disinfestation temperatures required for 99% mortality of *Cernuella virgata* and *Cochlicella acuta*.

Snail	Year	Days since collection	LD_{99}		
			Peak grain temperature (°C)	upper 5% fiducial limit	lower 5% fiducial limit
C. virgata	1986	55	66.7	67.9	65.7
C. virgata	1993	74	62.7	64.6	61.9
C. acuta	1993	89	57.0	60.0	55.4

Table 4. Summary of treatment options for obtaining 100% kill of *Cernuella virgata* and *Cochlicella acuta* in a stored product environment.

Treatments	Dosage
Rapid treatments	
Methyl bromide	CT = 720 g.hour/m^3 or 30 g/m^3 for 1 day (average concentration)
Hydrogen cyanide	CT = 240 g.hour/m^3 or 10 g/m^3 for 1 day (average concentration)
Heat disinfestation	67°C (peak temperature)
Slower treatments	
Carbon dioxide	60% for 15 days (constant)
Low oxygen	1% at 25°C for 10 days
Impractical treatments	
Phosphine	CT = 1296 g.hour/ m^3 or 5.4 g/m^3 for 10 days (average concentration)
Carbon disulphide	CT = 3600 g.hour/m^3 or 150 g/m^3 for 1 day (average concentration)

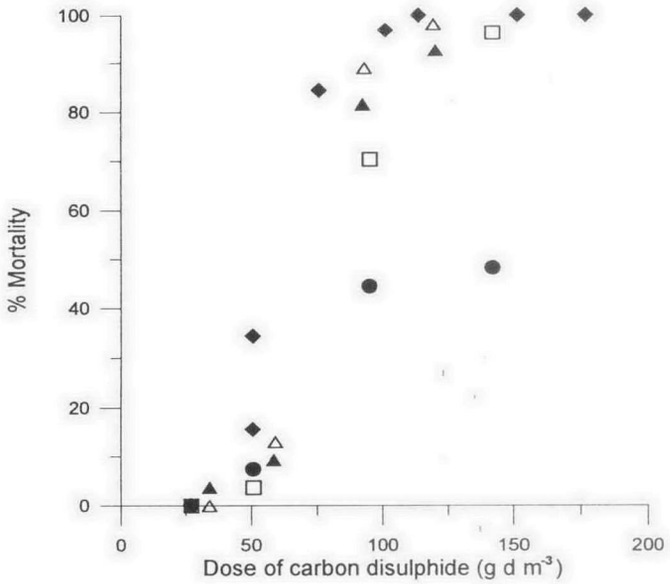

Fig. 5. Response of *Cernuella virgata* and *Cochlicella acuta* to carbon disulphide at 25°C. *C. virgata* ◆ 95 days stored 1985, ▲ 34 days stored 1986, △ 76 days stored 1986, □ 18 days stored 1993, *C. acuta* ● 32 days stored 1993.

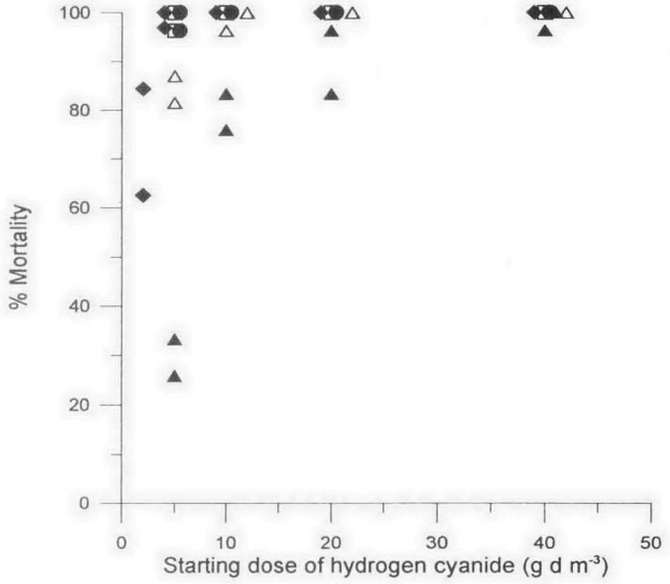

Fig. 6. Response of *Cernuella virgata* and *Cochlicella acuta* to hydrogen cyanide at 25°C. *C. virgata* ◆ 102 days stored 1985, ▲ 34 days stored 1986, △ 76 days stored 1986, □ 95 days stored 1993, *C. acuta* ●109 days stored 1993.

topping up to compensate for leakage at present Australian standards of sealing of CA stores but are technically feasible.

The increase in sensitivity of the snails to PH_3 and CO_2 with the length of time that they are held in storage was found in each year's collection of snails. The LD_{50} dosage rates (Figs 1 and 2) gave the best representation of the mortality trends in the different experiments. This response presents an option for fumigating the grain later in the storage period when the snails would be more sensitive to the treatment. The scattering of the LD_{50} values with different years reflects the condition of the snails before collection.

The MeBr, CS_2 and HCN experimental results with a smaller number of dosage rates were not suitable for probit analysis. A reasonable estimate of the dosage rates required to kill all snails can be determined from the graphs. MeBr requires a mean dosage rate of 30 g/m^3 for 1 day (*CT* product of >720 g.hours/m^3). This level is about 4 times higher than required to eliminate common insect grain pests and is likely to give unacceptable germination loss from malting barley (Strong and Lindgren 1959). The highest dosage rate of CS_2 used obtained a 100% kill for only the longest stored *C. virgata*. *C. acuta* were also very tolerant to CS_2 suggesting that concentrations well in excess of a mean 150 g/m^3 for 1 day would necessary to obtain 100% kill. HCN would require, as shown in Figure 6, an initial dose of at least 40 g/m^3 for fumigation period of 1 day (mean dosage rate of 10 g.days/m^3 after accounting for sorption losses on the grain). This dosage rate would be difficult to obtain in the field due to the high rate of sorption of HCN (Lubatti and Harrison 1944). The snails exhibited increased sensitivity to these fumigants with time in storage, a response also observed by Richardson and Roth (1965), with *C. barbara*. The dosage rates required are also higher than those used for insect pests of grain. The snails' greater tolerance to fumigants may be due to the adaptive mechanisms that they use to prevent dehydration. These mechanisms, the epiphragm that they secrete across the mouth of the shell (Barnhart 1983) and the reduction of respiration rate and metabolism with aestivation (Godan 1983), may aid in reducing fumigant uptake.

Heat disinfestation will successfully kill all *C. virgata* at a temperature of 67°C. This temperature is slightly higher than the 65°C required to kill insects (Evans and Dermott 1981). The smaller species *C. acuta* was killed at a lower temperature.

At lower temperatures, the snails, like insects (Banks and Annis 1990), are less susceptible to CO_2 and low O_2. This is a factor that would have to be taken into account when determining the duration of fumigation. Oxygen concentrations of 1% will kill all snails at temperatures 25°C. Given that the time required to kill all insect stages is 4 weeks at a grain temperature of 23°C and an O_2 of 1.2% (Banks and Annis 1990) this procedure would give 100% snail mortality.

Conclusion

The results of this study suggest a range of treatment options for *C. virgata* and *C. acuta*. Selection from within these options will be based on the availability of fumigants, industrial safety, level of sealing, cost of treatment, time available and commodity being fumigated. The treatments are summarised in Table 4.

Acknowledgments

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