Correlation between phosphine resistance and narcotic response in *Tribolium castaneum* (Herbst)

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Abstract

Phosphine, at high concentrations, induces a narcotic response in insects similar to 'knockdown' from which they can recover if exposure is not excessive. For most species of stored-product pest investigated, this response has been associated with dramatic changes in tolerance of phosphine at high concentrations (up to 50 times). This study reports changes in resistance to phosphine over 30 generations of selection of a susceptible strain of *Tribolium castaneum*. The selection, based on narcotic response to brief exposures at high concentrations of phosphine, provided a method of identifying the target beetles without killing them. In addition, as the resistance level changed so did the narcotic response. The correlation between the changes in time to narcosis and time to death is discussed. The narcotic response to phosphine or 'knockdown' at high concentrations has been used as the basis of a quick method for indicating the presence of resistant field strains that exhibit this form of phosphine resistance.

Introduction

Phosphine is one of the few remaining fumigants available for the disinfestation of stored products now that methyl bromide is likely to be restricted in use because of its ozone depleting nature. However, increased tolerance of stored-product pests to phosphine in recent times (Tyler et al. 1983) has made the effective use of phosphine more difficult. Studies of phosphine resistance in *Rhyzopertha dominica* (F.) (Price 1984) show that one resistance mechanism is active exclusion where the insect actively keeps the fumigant away from susceptible sites. At high concentrations, phosphine also induces a narcotic effect from which insects can recover if the exposure is not excessive (Winks 1984). The response is similar to the knockdown response when insects are exposed to insecticides. The high concentration region above about 0.5 mg/L, where the narcotic response is most pronounced, is associated with significant changes in tolerance of phosphine of up to 50 times in *Tribolium castaneum* (Herbst) (Winks 1984). In addition, it has been shown that resistant strains of *Rhyzopertha dominica* (F.) take longer to succumb to the narcotic effect than do susceptible strains. On the basis of this behavioural response, a quick test to indicate the presence of resistance has been proposed (Reichmuth 1991). It was thought that narcosis was a form of protective mechanism, in that the insects when narcotised did not take up as much phosphine. However, preliminary work for this investigation showed that it was the insects that resisted narcosis or remained active which survived. Those that succumbed quickly were the first to die. This observation is consistent with a mechanism of active exclusion. This study examines the relationship between, and changes in, narcotic response times at higher concentrations and resistance levels at a lower concentration in selections of a susceptible strain of *T. castaneum*.

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Materials and Methods

Origin and maintenance of insect material

Test insects used were a susceptible strain of *T. castaneum* (CTC_4) held in laboratory culture since collection in 1965 from a produce merchant's store in Brisbane, Australia, and selections cultured from this strain. Culturing and general handling techniques follow those described in Winks (1982).

Fumigation chambers

The phosphine exposure and selection were carried out in a purpose-built chamber (Fig. 1) in which the insects could be placed and the air conditioned. The chamber top could then be sealed and the enclosed space dosed with an appropriate volume of phosphine through the septum. The insects were observed through the glass top. At the end of exposure, phosphine was rapidly removed and the insects sorted for selection. Narcotic response times were determined in small plastic cell-culture flasks with optically clear sides. Response lines for strains were determined in a multi-chamber apparatus described in Winks and Waterford (1983).

Production and measurement of phosphine

Phosphine used to dose chambers was produced by hydrolysis of pellets of aluminium phosphide (Phostoxin®) according to a published method (Anon. 1975) and introduced into the chosen chamber using gastight syringes. The concentration of the phosphine source was determined by gas chromatography using the response of a Gowmac® gas density detector. The volume injected was calculated from the source concentration and the volume of the fumigation chamber.

Selection methods

All dosing and handling were carried out in a laboratory that was maintained at 25°C. One hundred adults were used for each selection. They where placed in the selection chamber fitted with a quick-release lid and a septum through which gas could be injected (Fig. 1). The insects were starved and conditioned in an incubator at 25°C, 57% r.h. overnight. The lid was placed on top and clamped shut sealing the chamber. Phosphine source, approximately 18 µL, sufficient to produce a narcotic concentration of 2 mg/L was injected through the septum. The gas was immediately stirred by repeated removal and injection of a quantity of the atmosphere within the chamber, using a 10 mL syringe. The insects were then

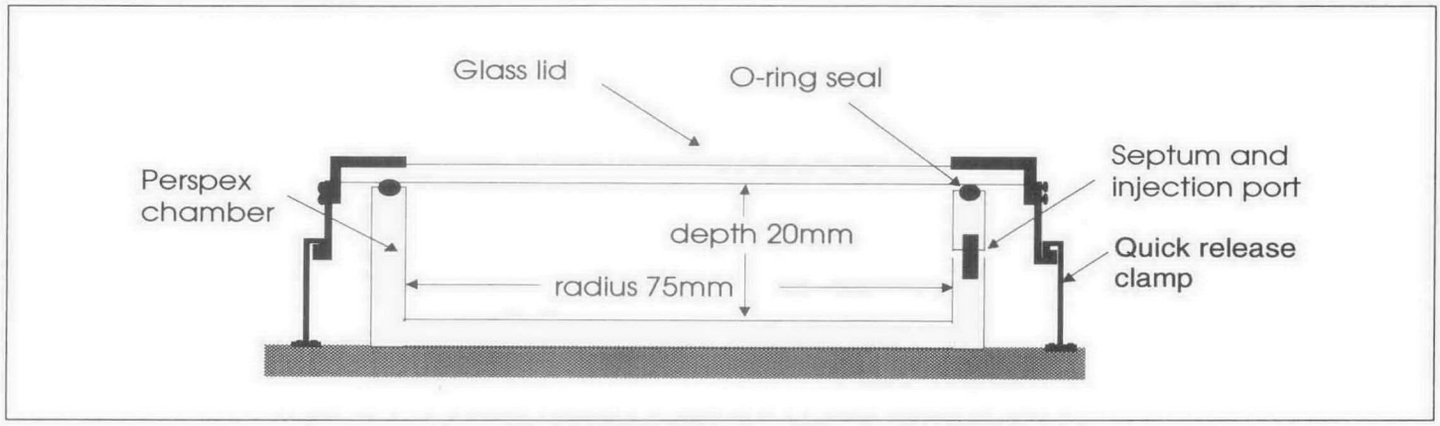

Fig. 1. Diagram of apparatus used to dose and select on the basis of narcotic response to phosphine.

observed through the glass top. Two selections were made based on the behavioural response of the insects. The first, in which the insects were observed until more than 50% had become immobile or narcotised, was termed narcotic tolerance. The top was removed and the phosphine fanned off. The 30 least narcotised or most active beetles were then removed into one recovery dish, using soft forceps, the remainder into another dish. The reverse was done for the narcotic-susceptible selection. The insects were observed until about 20% had succumbed or ceased to move. The top of the chamber was removed and the 30 most deeply narcotised removed into a recovery dish with the soft forceps. The remainder (active beetles) were placed into another dish. Time of exposure was recorded in both cases. The mortality was assessed at 7, 14 and 21 days to ensure end-point mortality was reached. The survivors of the 30 least narcotised were set up as parents of a strain designated CTC_4NR_1 where NR means narcotic resistant. The survivors of the 30 most narcotised were set up as parents of CTC_4NS_1 where NS means narcotic susceptible. The progeny of these two strains became the test insects for the next selection. The narcotic-resistant progeny were selected for 30 generations. The narcotic-susceptible progeny were selected for 10 generations.

Narcotic and mortality response assessments

The parent strain (CTC_4), the 7th, 10th and 30th generation for narcotic resistance (CTC_4NR_7, CTC_4NR_{10} and CTC_4NR_{30}) and narcotic susceptibility (CTC_4NS_{10}) were assessed to determine times to narcotic response following a modified method described in Winks (1984). Instead of groups of 10 being assessed periodically for the number responding, the time to narcosis was determined for a number of individual insects. This was done by placing individual insects into small flasks and injecting sufficient volume of phosphine to provide an atmosphere of 2 mg/L. The insects were observed and the time to narcosis was recorded. Narcotic response time was estimated from linear regression analysis of the cumulative response of a number of individuals. Three strains (CTC_4, CTC_4NS_{10}, CTC_4NR_{10}) and the 20th generation for narcotic tolerance (CTC_4NR_{20}) were also assessed for mortality response when exposed to a fixed concentration of 0.1 mg/L phosphine for a range of exposure times in a multi-chamber apparatus described in Winks and Waterford (1983). Groups of 200 adults were used at each exposure time. End-point mortality response was determined from successive observations using the method recommended by Winks (1982). Results were analysed using the method of Finney (1971).

Results

Dosage estimates and parameters of probit regression equations fitted to end-point mortalities of adults of the tested selections are given in Table 1. Probit mortality lines are shown in Figure 2. These show a change in resistance to phosphine in opposite directions from the parent strain CTC_4 depending on the method of selection. Table 2 shows the estimates of times to narcosis for various selections and parameters of regressions of time to narcosis on exposure to 2 mg/L phosphine. The regression lines for times to narcosis (Fig. 3) show similar response changes in both directions from the parent strain, depending on the method of selection.

Discussion

The selection on the basis of narcotic tolerance led to a steady and significant change in both narcotic tolerance and phosphine resistance and the selection based on narcotic susceptibility led to a small decrease in both narcotic tolerance and resistance to phosphine. There was no evidence of a rapid

Table 1. Dosage estimates and parameters of regression of probit mortality on log dosage for adults of *Tribolium castaneum* exposed to 0.1 mg/L phosphine for various exposure times at 25°C, 57% r.h

Strain	LD_{50} mg.hour/L	LD_{99} mg.hour/L	Slope ±SE	Mean probit response (Y)	Heterogeneity	
					$\chi 2$	*d.f*
CTC_4NS_{10}	0.229	0.378	10.7 ± 0.54	5.33	2.63	6
CTC_4	0.249	0.425	10.0 ± 0.48	5.26	2.83	7
CTC_4NR_{10}	0.965	2.59	5.43 ± 0.50	5.23	28.13	6
CTC_4NR_{20}	1.84	3.653	7.8 ± 0.41	5.01	1.18	4

Table 2. Times to 50 and 99% narcosis (NT_{50} and NT_{99}), and parameters of the regression of probit transformed times to narcosis for adults of *Tribolium castaneum* when exposed to 2 mg/L phosphine at 25°C, 57% r.h.

Strain	NT_{50} (minutes)	NT_{99} (minutes)	r^2	No. used (n)	Intercept (a)	Slope b ± SE
CTC_4NS_{10}	5.2	6.6	0.98	96	−11.19	22.5 ± 0.37
CTC_4	7.1	10.9	0.96	88	−5.56	12.4 ± 0.27
CTC_4NR_7	7.8	14.2	.93	96	−3.0	8.9 ± 0.24
CTC_4NR_{10}	25.8	86.7	0.91	94	−1.2	4.42 ± 0.14
CTC_4NR_{30}	263	420	0.79	19	−2.33	11.43 ± 1.4

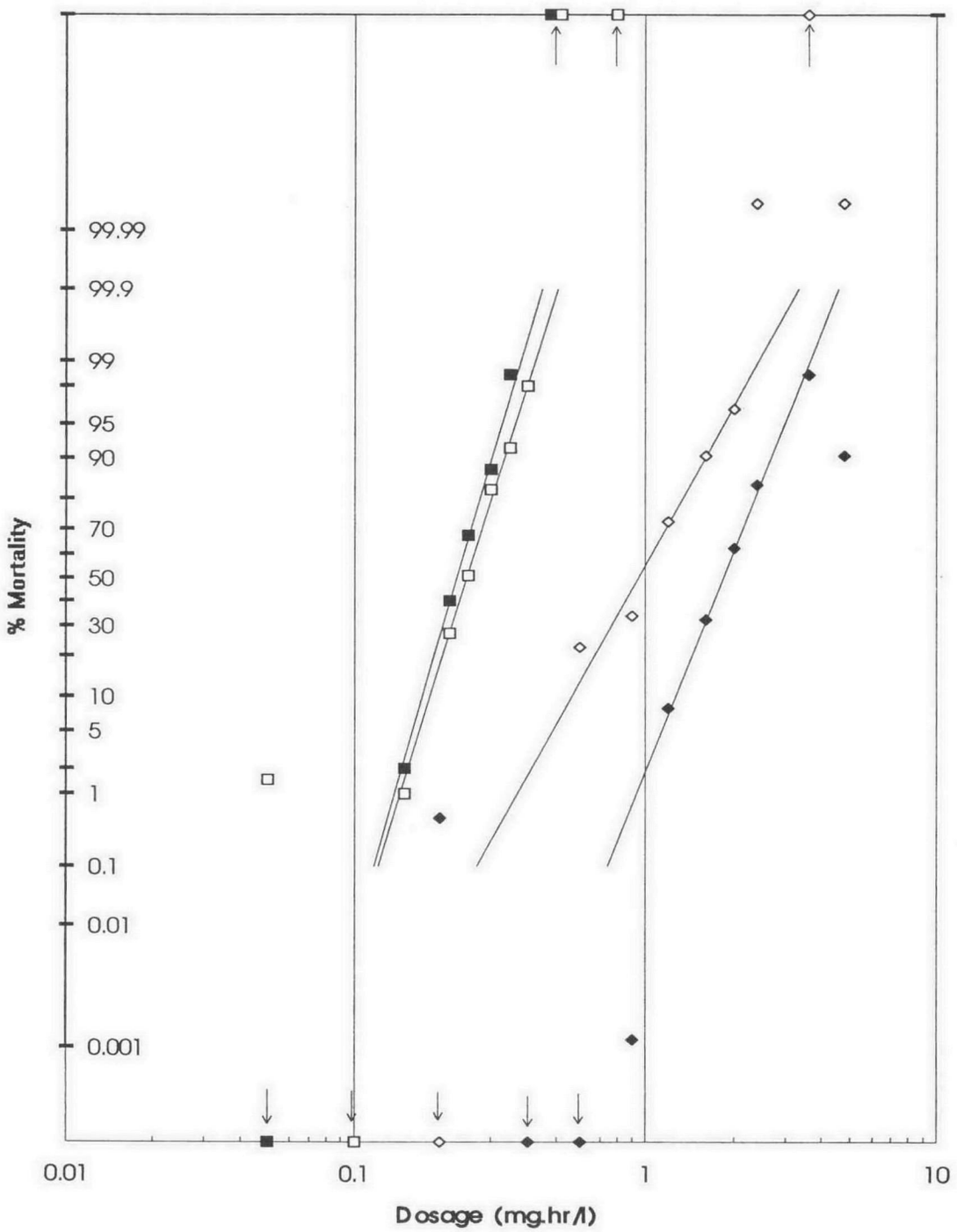

Fig. 2. Probit lines fitted to end-point mortalities for narcotic tolerant strains CTC_4NR_{10} (◇—◇) and CTC_4NR_{20} (◆—◆), a narcotic-susceptible strain, CTC_4NS_{10} (■—■) and the parent strain CTC_4 (□—□) after exposure to 0.1 mg/L phosphine for a range of exposure times.

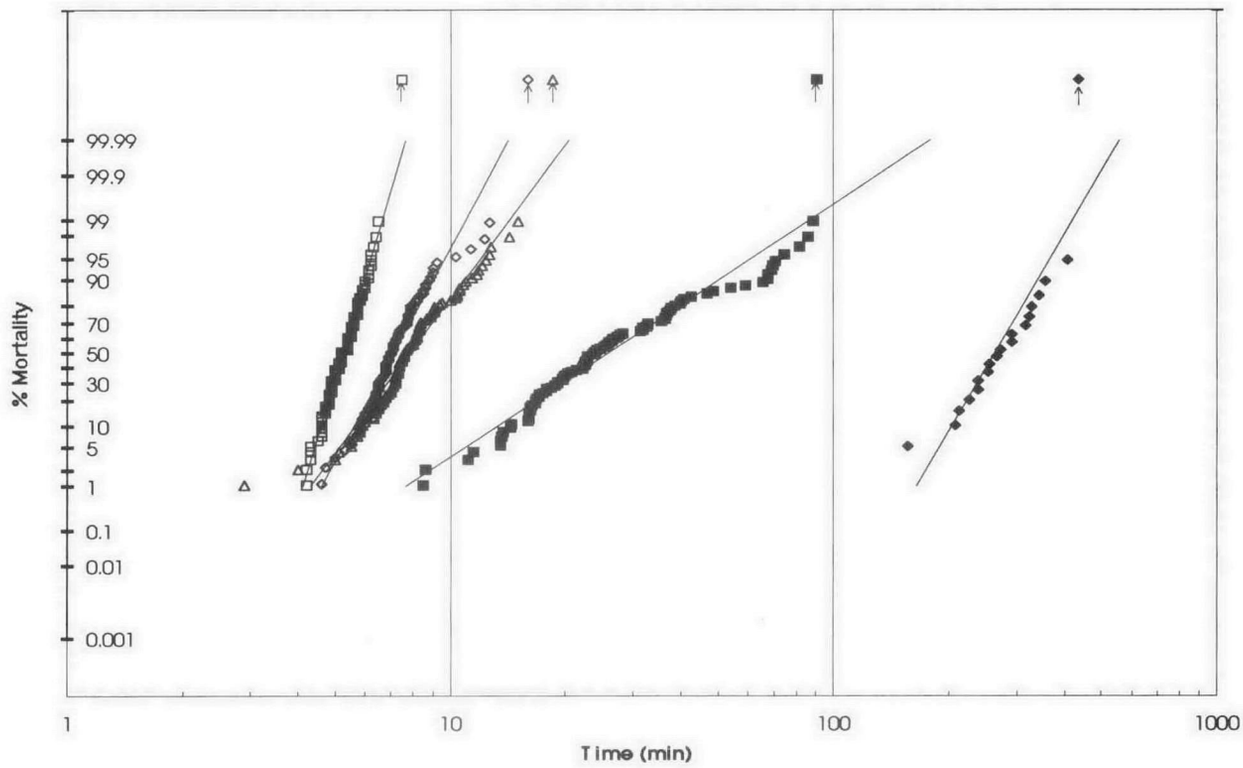

Fig. 3. Response of individual insects showing time to narcosis for the selected strains: narcotic-resistant CTC_4NR_7 (Δ—Δ), CTC_4NR_{10} (■—■) and CTC_4NR_{30} (◆—◆), narcotic-susceptible CTC_4NS_{10} (□—□) and the parent CTC_4 (◇—◇). The lines are regressions through the probit-transformed cumulative responses of each strain.

shift in resistance or narcotic tolerance, just a gradual change in the slopes of the response lines, first a decrease in slope and then an increase. When the exposure times for narcotic tolerance selections are graphed against generation number (Fig. 4) the rate of change seemed slow at first, then seemed to increase sharply after about the 10th generation with some suggestion of slowing down after the fifteenth.

The times to death when exposed to 0.1 mg/L phosphine, of the parent and selected strains of *T. castaneum*, compared with times to narcosis when exposed to 2 mg/L phosphine fall along a straight line when the time to mortality is log transformed (Fig. 5).

A question could be posed: is this a specific correlation or could it be a general one for all species? Equivalent points for

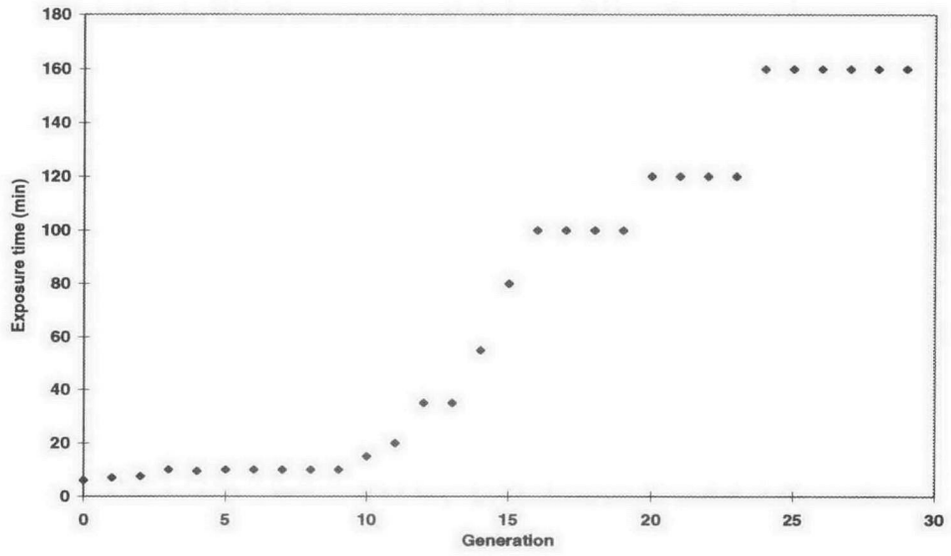

Fig. 4. Exposure times used to achieve approximately > 50% narcosis for the purpose of selecting the 30% least narcotised for 30 generations of selection.

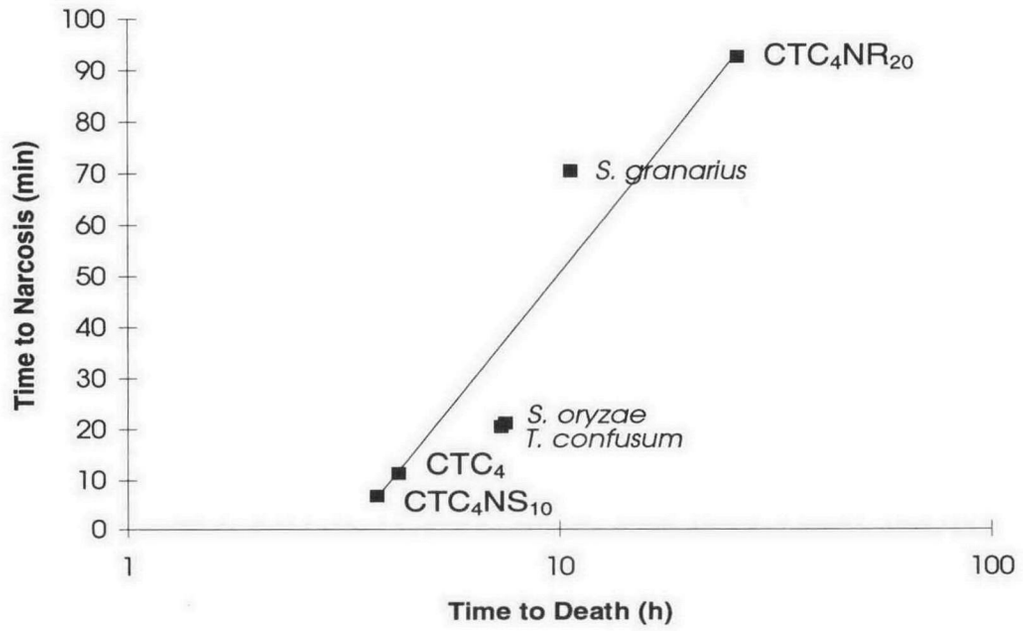

Fig. 5. Time to death (log scale) at 0.1 mg/L phosphine versus times to narcosis at 2 mg/L for narcotic-tolerant (CTC_4NR_{10}) and narcotic-susceptible (CTC_4NS_{10}) strains with the parent strain (CTC_4) □—□ 99% response levels. Equivalent points for three other stored-product species are added for comparison.

other species are plotted and they do fall generally along the same line. The position of these points is not inconsistent with a general correlation between narcotic response and lethal response. However, more research is needed.

If active exclusion is the general resistance mechanism for phosphine, and increased narcotic tolerance is a means of quickly determining the presence and efficiency of the mechanism, then techniques based on assessing the narcotic response, as proposed by Reichmuth (1991), may be a robust means of rapidly detecting strains with this form of resistance before a fumigation. With not much more effort the level of resistance could be determined in less than a day. This information could be useful in deciding what treatment should be applied to an infested commodity. Techniques based on the narcotic response could also be a useful tool for rapid screening of field strains possessing this type of resistance, without killing the parents — thus allowing a more detailed assessment of particularly resistant strains.

However, there may be other mechanisms of resistance possible that would not be detected by this response. The only way to assess these is by determining the full lethal response of the strain from graded response lines. In addition, the observation is in adults and says nothing about the relative response of other stages which may be more important in determining the significance of resistance to phosphine in the field.

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