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Rapid bioassay for determining the phosphine tolerance

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Abstract

The resistance testing kit is an assay to receive very quickly detailed information about the behaviour of adult insects in a phosphine atmosphere. The used concentration of 3,000 ppm has a narcotic effect on non-resistant insects within a few minutes. Insects with a low phosphine susceptibility are still active after this time period. Using the resistance testing kit we succeed to distinguish between the tolerance of different insect species, the tolerance of one species at different concentrations and of different temperature regimes. The evaluation of different concentrations of phosphine, of their effectiveness against resistant strains of *Lasioderma serricornis* shows that at usual fumigation conditions resistant beetles can not be controlled with phosphine whereas at higher concentration 100 %-mortality of all development stages is achieved. Furthermore, longer exposure times have also a positive effect on the mortality of all development stages. The results confirm that the resistance testing kit is an effective device to recognize the resistance in an early stage, to fight against the development of resistance and to fight against the spreading of resistance. In future the resistance testing kit should be part of an integrated pest management system and a requirement for a good fumigation practice.

Key words: resistance testing kit, resistance,

phosphine, magnesium phosphide, stored-product pests.

Introduction

The losses of food in stored foodstuffs caused by insects can be avoided by application of existing and approved pest control methods e.g. fumigation with phosphine. But the continuous improper use of phosphine for the control of stored-product pest insects, e.g. inadequate sealing and barely adequate exposure time, has led to the development of resistant strains in many countries. In the case of a worldwide spread of phosphine resistance, food which is sufficient to feed a lot of people will be destroyed or damaged by resistant insects. In order to prevent further development of resistance and to combat it where it has developed, the resistance needs to be measured and monitored over time. But all methods need too much time to detect the resistance and to allow the fumigators to respond with an appropriate fumigation strategy where the fumigation is being carried out (Hori and Kasaishi, 2005; Bell et al., 1994; Reichmuth, 1992).

Winks (1985) examined the LD₉₉ for adults of a susceptible strain of *Tribolium castaneum* (Herbst) and found at high concentrations an increased time required to achieve a high mortality. This was the basis for claims that narcosis confers protection against phosphine.

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Years later (Reichmuth, 1991; Winks and Waterford, 1996) the correlation between phosphine resistance and narcotic response in *Tribolium castaneum* was described. Now, the first rapid-test, based on the time to knockdown adult insects has been developed for the most common stored-product pest insects. Within 30 minutes it is possible to establish if a population contains resistant beetles or not. Hence, the fumigators are able to increase the dosage or the exposure time before the fumigation and therefore they can be sure to conduct a successful fumigation.

Material and methods

Resistance test kit

The Resistance-Test-Kit consists of a 100 ml syringe, a 5 l flexible plastic canister, a cannula with a rubber hose and a special magnesium phosphide pellet. For generating a phosphine containing atmosphere in the plastic bag, 50 ml of water are put into the canister, then two pellets are added, the canister is closed immediately with the lid and is shaken carefully. The pellets are completely decomposed after 5 minutes.

Usually two pellets cause a concentration between 4,000 to 6,000 ppm. With regard to a phosphine concentration of 3,000 ppm ($=c_0$) in the syringe the atmosphere generated in the canister is diluted with air. With respect to that the cannula with the rubber hose is connected through the lid with the plastic canister and the concentration ($=c_1$) is measured with a Dräger tube. Then the amount ($=v_{\text{bag}}$) which has to be taken from the plastic canister is calculated with formula 1. The difference to 100 is the amount of air ($=v_{\text{air}}$) which is needed to adjust 3,000 ppm in the syringe (formula 2).

$$\frac{c_0 [\text{ppm}] \times 100 \text{ ml}}{c_1 [\text{ppm}]} = v_{\text{bag}} [\text{ml}] \quad (1)$$

$$100 \text{ ml} - v_{\text{bag}} = v_{\text{air}} \quad (2)$$

For instance, if the concentration in the plastic bag is 4,000 ppm than you must dilute 75 ml from the plastic bag with 25 ml air.

To conduct the resistance test remove the butt of the syringe, add 20 insects into the syringe, push the butt carefully into the syringe and adjust the air volume ($=v_{\text{air}}$) in the syringe. Next, connect the syringe with the canister (cannula and adapter), fill the syringe up to 100 ml and observe the behaviour and the activity of the insects. Every minute note the number of the knocked down beetles and generate a time/knocked down beetles diagram.

Insects

Four strains of *Lasioderma serricorne* (LS₀, LS₁, LS₂, LS₃), two strains of *Sitophilus granarius* (SG₀, SG₁) and one strain of *Tribolium castaneum* (TC) and *Oryzaephilus surinamensis* (OS) were used for the test.

LS₀, both strains of *Sitophilus granarius*, TC and OS originated from laboratory cultures reared in our laboratory.

The strains LS₁, LS₂ and LS₃ were multiplied in our laboratory from samples received from Central Science Laboratory, UK. LS₂ and LS₃ had a known resistance against phosphine.

Strain SG₁ contained susceptible and resistance beetles.

Selection of resistant beetles

Hundred beetles of strain SG₁ were put in the syringe of the resistance test kit and treated with 3,000 ppm of phosphine as described before. The resistant test was interrupted after 12 minutes and the active beetles were selected from the non-active beetles. The active ones were enabled to recover for 24 h and then tested once again. The resistance test was finished until all beetles were inactive.

Fumigations

The fumigations were conducted in a 500 l gas-tight chamber at a temperature of 20 °C and

a r.h. of 60 %. Due to the fast degassing properties pure magnesium phosphide was used as fumigant and the humidity was adjusted with a saturated ammonium nitrate solution.

The insect samples used for the fumigations consisted of all development stages of the species and were put in transparent plastic cages to whose bottom and lid a metal screen was fixed. The samples were exposed to phosphine under the conditions described in the Coresta standard (CS_{20°C}: 6 days / 300 ppm; CS_{>20°C} 4 days / 200 ppm).

The concentration was measured with Dräger tubes.

After the exposure, the beetles were bred for 84 days and the hatch was documented weekly.

Results

The probit lines in Figure 1 received from the resistance test kit trials with four strains of different species (LS₀, TC, SG₀ and OS) are well separated from each other. They show that the test is able to distinguish between their susceptibility against phosphine. The most susceptible strain in the test was *Lasioderma serricorne* where all beetles were narcotized after 5 minutes. *Tribolium castaneum* followed with a knock-down time of 6 minutes. *Sitophilus granarius* and *Oryzaephilus surinamensis* needed 8 respectively 9 minutes for the narcosis of all adult beetles (Figure 1).

The probit line in Figure 2 was received from test kit trials with a strain of *Sitophilus granarius* (SG₁) which contained normal as well as resistant beetles. The slope of this line is decreasing after 6 min which is similar with a decrease in the susceptibility against phosphine. According to the description in section 2 the resistant beetles were selected, enabled to recover for 24 h and tested once again. They showed the same behaviour in a phosphine atmosphere of 3,000 ppm as the day before. This declared the low susceptibility of these beetles against phosphine.

The different slope of the three probit lines in Figure 3 illustrate the possibility to distinguish between three strains of *Lasioderma serricorne*

(LS₁, LS₂ and LS₃) with different susceptibility against Phosphine. LS₁ has the steepest slope and therefore the highest susceptibility whereas LS₃ has the lowest slope and the lowest susceptibility.

Mortality tests were conducted with mixtures of all development stages of LS₁ and LS₂ under Coresta standard (CS_{20°} and CS_{>20°C}). Already after 7 days the first hatch was observed for the resistant strain LS₂ while strain LS₁ was treated with a 100 %-mortality and the control sample showed a normal development. In contrast, at higher concentration (400 respectively 600 ppm) LS₁ as well as LS₂ did not survive the fumigation.

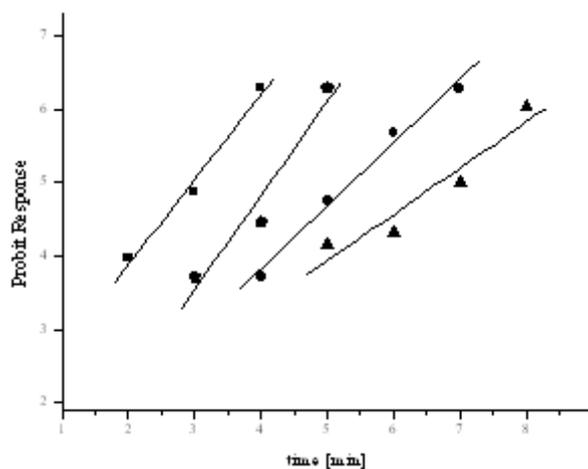


Figure 1. Probit regression lines obtained by the resistance test of the strains LS₀ (rectangle), TC (pentagon), SG₀ (circle) and OS (triangle).

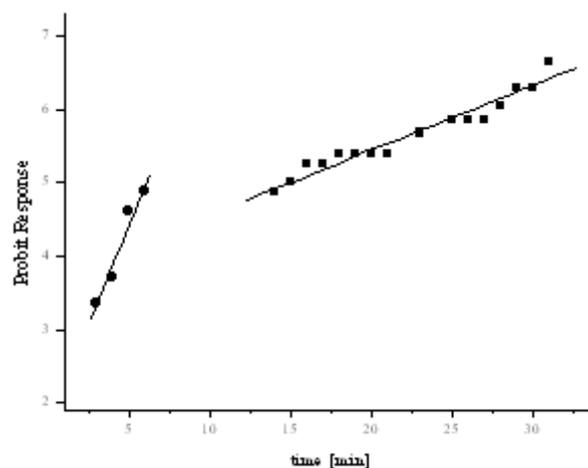


Figure 2. Probit regression lines obtained by the resistance test of strain SG₁ (circle: non-resistant; rectangle: resistant).

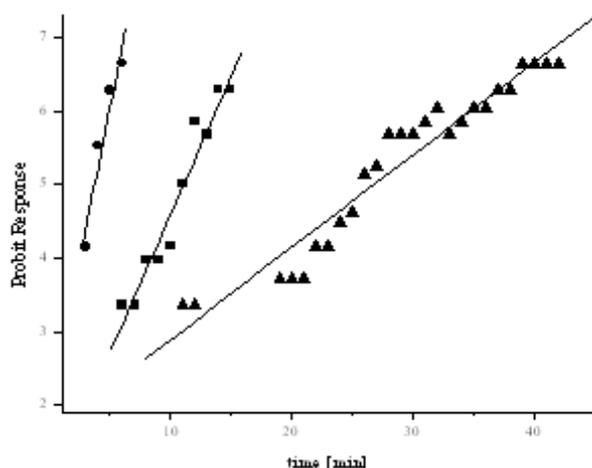


Figure 3. Probit regression lines obtained by the resistance test of the strains LS₁ (circle), LS₂ (rectangle) and LS₃ (triangle).

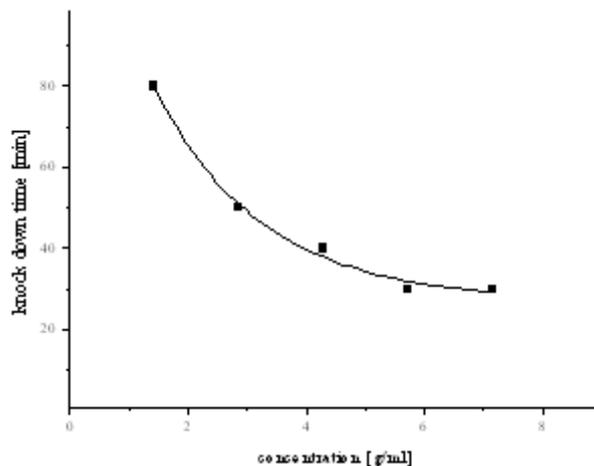


Figure 4. Relationship between the knock down time of the resistant beetles of strain SG₁ at different concentrations.

Table 1. Hatch of LS₁ and LS₂ after the phosphine treatment. Upper Table: CS_{20°C}, Lower Table CS_{>20°C}.

Time [d]	7	14	21	28	35	42	49	56	63	70	77	84
LC	6	10	11	11	12	24	56	65	69	72	72	72
LS ₁	0	0	0	0	0	0	0	0	0	0	0	0
LS ₂	10	11	11	11	11	11	11	11	11	11	11	11
Time [d]	7	14	21	28	35	42	49	56	63	70	77	84
LC	6	10	11	11	12	24	56	65	69	72	72	72
LS ₁	0	0	0	0	0	0	0	0	0	0	0	0
LS ₂	6	6	6	7	9	12	12	12	12	12	12	12

According to the method “Selection of resistant beetles” the resistant beetles from strain SG₁ were separated and the resistance test was carried out at 1,000, 2,000, 3,000 and 4,000 ppm. The concentration/knock-down-diagram yield a non-linear relationship (Figure 4).

Trials concerning the temperature dependency of NT₉₉ of LD₀, SG₀, TC and OS are summarized in Figure 5. In all cases NT₉₉ increase with decreasing temperature.

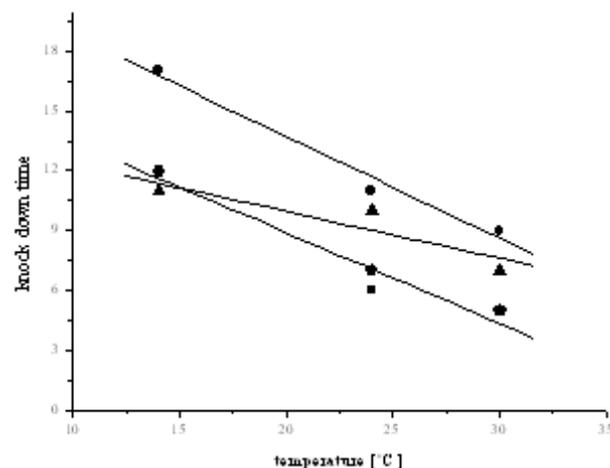


Figure 5. Relationship between the knock down time of the strains LS₀ (rectangle), SG₀ (circle), TC (triangle) and OS (pentagon) at different temperature levels.

Discussion

As shown in Figure 1 the susceptibility against Phosphine decreases with the following sequence: *Lasioderma serricorne*, *Tribolium castaneum*, *Sitophilus granarius* and *Oryzaephilus surinamensis*. This sequence agrees with mortality tests whereupon *Lasioderma*

serricornes is the most susceptible and *Oryzaephilus surinamensis* the most resistant species.

Figure 2 demonstrates the possibility to separate resistant beetles in a sample which contain both type of beetles (resistant and susceptible). In practice, the user can not decide if this result is caused by one strain which contains beetles with different susceptibilities or if the sample is a mixture of two strains who differ in their susceptibility. Nevertheless, it is a signal for the presence of resistant beetles and the user can prevent their development and distribution.

The main application of the resistance test kit is the decision if a strain is resistant or not (Figure 3). Usually, fumigators verify the structure, the sealing and other circumstances but they do not "TEST THE PEST" prior the fumigation although this can be very helpful to choose the right dosage respectively the right exposure time. On the other hand, if there are living insects after the fumigation they can test them and will see if the insects are really resistant or if the fumigation was carried out incorrectly.

The mortality tests demonstrate that it is not always possible to control resistant strains of insects with usual fumigation conditions. Therefore, all experts (producer, user, supplier etc) should support the proper use of phosphine especially concerning the dosage, the exposure time and the application at low temperature.

As expected, the temperature as well as the test concentration affects the results of the test. This can be due to a higher uptake of phosphine at higher temperatures respectively at higher concentrations. For reliable results it is important that the conditions during the test do not differ from those who are recommended.

The results show that the phosphine resistant test is a suitable bioassay to determine the phosphine susceptibility of stored product insects. It is possible to distinguish between different strains of one species or between different resistant beetles of one strain. The information about the susceptibility are obtained within a few minutes. Therefore, pest controllers are able to test the pest before the application of the

fumigant. Hence, they can vary the phosphine concentration respectively the exposure time so that also resistant beetles are treated successfully with phosphine. This possibility is a big step to prevent the worldwide development and the intercontinental spread of phosphine resistance.

The mortality test illustrates that phosphine resistant strains of *Lasioderma serricornes* can be killed with phosphine, within reasonable time periods and with achievable concentrations. Therefore, higher registered phosphine concentrations and longer exposure times should be considered.

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