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Fumigation with phosphine — still an important tool for successful stored product protection in the future?

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ABSTRACT

The most important active ingredient worldwide used for controlling stored-product pests is phosphine gas. Through its positive properties with regard to eco-toxicity as well as its good penetration properties and the associated excellent effectiveness against stored-product pests, phosphine has become indispensable for successful stored-product protection over decades.

Nevertheless, recent data indicate that some major stored-product insect species, in some parts of the world, have developed increased tolerance to this agent. In this regard, questions are raised whether and to what extent phosphine can still be used successfully in stored product protection in the future. To resolve this issue, the goal of the described tests was to determine data on sufficient dosage and exposure time which would show the conditions under which successful fumigation can be performed and how further development of insect resistance can be counteracted.

Hence laboratory experiments were conducted to determine the limiting concentrations in insect strains comprising grain weevil [*Sitophilus granarius* (L.)] and flour beetle [*Tribolium castaneum* (Herbst)] which result in the complete control of all insect life stages. In subsequent fumigation tests under practical conditions, it was shown that insufficient concentrations and short exposure times can frequently be regarded as the main reason for unsuccessful fumigation and therefore contribute to the risk of the development of reduced mortality caused by phosphine. A successful fumigation depends on various parameters such as properties of the product or object to be fumigated, sufficient sealing, temperature and moisture conditions, insect species and its tolerance status and insect life stage. As a consequence, both dosage/exposure time and monitoring of gas concentration play a significant role in fumigation to reduce all kinds of uncertainties caused by these parameters. Recommendations for better fumigation practices in regard to gas monitoring and minimum dosages/exposure times are given in this work. Moreover, in order to get an impression about the status of insect tolerance to phosphine in Europe, a program which already started in Greece, has been extended to several European countries. The program consists of sampling of insect strains and its screening regarding their sensitivity towards phosphine.

Key words: Exposure time, Gas monitoring, Limiting gas concentration, Phosphine, Phosphine tolerance in Europe, *Sitophilus granarius*, *Tribolium castaneum*

There are various factors which must be taken into consideration in order to successfully exterminate insects using phosphine.

It is generally known that various species of insects exhibit very different degrees of sensitivity to phosphine with regard to its efficacy. Even among the same species, their sensitivity varies, depending on the developmental state they are in. It has been proven that larger quantities of PH_3 or a longer exposure time are necessary to kill insects in the resting stages, such as pupae and eggs, than larvae or adults (Mueller, 1998).

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In addition, it is important to keep in mind that in practice, fumigation can generally not be performed under ideal temperature and moisture conditions. This is especially true for the temperature under practical conditions, as it is often significantly below 20°C and varies between day and night. However, low temperatures often cause the metabolism of insects to slow down significantly and since this reduces the effectiveness of the phosphine as a result, higher concentrations are needed for extermination in practice than under laboratory conditions.

In light of the increased tolerance against phosphine gas among insect strains, which has been frequently observed in recent years and described in depth in scientific literature, dosage levels must be high enough to prevent the development of additional resistant insect strains (Opit et al., 2012).

With respect to the risk of insufficient concentrations and the resulting decreased efficiency, it is also difficult to assess the effects of factors such as the varying permeability of goods or gas loss due to leakage in fumigated objects.

The experiments described here demonstrate the differences in the sensitivity of two selected storage pests to phosphine under standardized laboratory conditions. These experiments could also confirm the differing sensitivity to gas of these pests at various stages of development. Finally, an experiment set up under practical conditions using quantitative measurements could demonstrate the importance of adequate dosage and appropriate exposure time for successful fumigation.

The experiments under practical conditions also demonstrated the incredible importance of functioning gas monitoring for the success and effectiveness of fumigation.

In order to obtain an overview of the possible tolerances of insects in Europe to phosphine, a screening project previously conducted in Greece was started in several other European countries. The procedure and objectives of this project are described briefly.

MATERIALS AND METHODS

The laboratory experiments described here are to be used as a basis for determining the concentrations required to definitely kill all developmental stages of the selected insect species with normal sensitivity after a predefined exposure time. For this purpose, the animals were tested in a concentration series in the laboratory regarding their sensitivity to phosphine. By defining a 100% mortality rate, the ‘lethal limiting gas concentration’ could be determined under the previously described conditions.

The tests were carried out in 0.5 m³ gas-tight chambers at a temperature of 20°C and a r.h. of 65%. The results of each experiment were assured to be comparable based on the defined laboratory conditions. The development of gas over the fumigation period could be continuously monitored using gas concentration measurements at specified intervals.

The tests were carried out using two storage pests that generally cause significant problems, namely the grain weevil [*Sitophilus granarius* (L.)] and the red flour beetle [*Tribolium castaneum* (Herbst)].

The goal of the efficiency test was to control all developmental stages. Therefore, a breed mix (growing medium with egg, larvae, pupae and adult stages) was used. Since the number of imagines in these breed mixes can sometimes not be sufficient, a certain number of adult insects were to be planted as well. This facilitated meaningful results for all developmental stages.

A bag product (Detia bag) was used as a fumigation product in the experiment. In order to allow the experiments to be comparable, a consistent exposure time of 4 days (96 h) was chosen. In order to approach the gas concentration limit for surviving imagines of the two insect species, the exposure time could be shortened to 1–2 days (24–48 h) as needed (Table 1).

The insects were inserted in permeable containers and placed in the chambers along with the fumigation product in the calculated quantity. After the predetermined periods of time, the gas concentration

Table 1 Phosphine concentrations tested at 20°C and 65% r.h.

PH_3 concentration (ppm)	Exposure time (hours)	Developmental stage of insects
50	24	Imagines
50	48	Imagines
50	96	Imagines, pupae, larvae, eggs
100	96	Imagines, pupae, larvae, eggs
200	96	Imagines, pupae, larvae, eggs
400	96	Imagines, pupae, larvae, eggs
600	96	Imagines, pupae, larvae, eggs
800	96	Imagines, pupae, larvae, eggs
1000	96	Imagines, pupae, larvae, eggs

was measured in the chambers. Following the pre-specified exposure times, the chambers were ventilated, the containers with the insects were removed, and the mortality was measured based on the number of dead insects. Breed mixes not exposed to gas served as a control group. All breed mixes were subsequently kept at breeding conditions (25–27°C; 50% r.h.) and examined weekly for the hatching of new insects.

RESULTS AND DISCUSSION

The results of the experiments using only imagines illustrate the fact that a phosphine concentration of 50 ppm over an exposure time of 24 h is sufficient to reach 100% mortality of *T. castaneum* imagines. However, surviving *S. granarius* beetles could be observed under these conditions. However, the majority of these were irreversibly damaged.

After an exposure time of 48 h at 50 ppm, there were also no surviving insects observed among *S. granarius* imagines. This result is presented schematically in the following Table 2 without showing the individual analyses.

The results of the experiments following treatment of breed mixes revealed that hatching *T. castaneum* beetles could still be observed at a phosphine concentration of 50 ppm over a period of 96 h based on the hatch rate after incubation. However, there was no more hatching after treatment with 100 ppm for 96 h, meaning 100% mortality was achieved in this situation.

Surviving variants of *S. granarius* were observed up to a tested gas concentration of 800 ppm. Though the number of hatching beetles was low compared to the control groups, one can still not assume a 100% success rate for this concentration. All developmental stages of this pest were not completely killed until a gas concentration of 1000 ppm.

The hatching time points of both *T. castaneum* and *S. granarius* beetles after incubation revealed that most surviving animals were in the pupal or egg stage. This confirms the conclusion that these stages are the most tolerant to phosphine.

Table 3 shows the results for both pests in schematic form.

Table 2 Exposure time at which imagines from different beetle species survived at a concentration of 50 ppm at 20°C and 65%.r.h.

Exposure time (h)	*Tribolium castaneum*	*Sitophilus granarius*
24	All imagines dead	Surviving imagines
48	All imagines dead	All imagines dead

Table 3 Phosphine concentrations at which 100% killing of all developmental stages of various beetle species is achieved (exposure time: 96 hours)at 20°C and 65% r.h.

PH_3 concentration (ppm)	*Tribolium castaneum*	*Sitophilus granarius*
50	Surviving stages	Surviving stages
100	Completely killed	Surviving stages
200	nt	Surviving stages
400	nt	Surviving stages
600	nt	Surviving stages
800	nt	Surviving stages
1000	nt	Completely killed

nt, Not tested

These experiments clearly show that there is a great difference in the sensitivity to phosphine of individual species and of the development stages within the species. While for *T. castaneum*, it appeared possible to destroy 100% of all developmental stages with relatively low concentrations of gas, this goal could not be reached for *S. granarius*, which is much less sensitive, even with concentrations of 1000 ppm and exposure times of four days.

Imagines of both species can be fully destroyed even with rather low doses of 50 and 100 ppm, while some of the other developmental stages required much higher quantities to reach the extermination goal. This is particularly apparent for *S. granarius*: While complete extermination of imagines could be achieved at a concentration of 50 ppm and an exposure time of 48 h, 1000 ppm over an exposure time of 96 h is required in extreme cases for the other developmental stages (Table 3).

Though only few insects from the individual developmental stages survive, these of course form the source of a new infestation. Furthermore, they represent a great risk for developing resistance to the active substance. If the least-resistant insects are continuously selected and can reproduce, increasing doses and exposure times will be needed in the future to successfully exterminate these insects using phosphine.

The experiments here were carried out using insect strains with normal sensitivity to phosphine. Temperature, relative humidity, and gas concentrations can be optimally set to fumigation conditions under laboratory conditions. Considering that gas concentrations of as much as 1000 pm are required in these circumstances to exterminate 100% of pests, it becomes clear that much higher doses must be used in practice to achieve complete killing of all storage pests and their developmental stages.

This assumption was confirmed by a practical experiment in containers under the suboptimal fumigation conditions which frequently occur in practice. At the same time, breed mixes of *T. castaneum* and *S. granarius* were tested in containers filled with bagged goods (different grain flours) at a dose of 5 g PH_3/m^3 over an exposure time of 5 days.

It could be observed that the mathematically theoretical gas concentration of 3500 ppm was not achieved despite apparently adequate sealing. At the time of the maximum gas concentration, the measured values were only in the range of 2000 ppm. This clearly demonstrates the importance of measuring gas concentrations in practice. This is very frequently neglected, since it is assumed that the dosage always provides a sufficiently high gas concentration. However, as is obvious, gas losses caused by leaks must always be expected in fumigation in practice despite good sealing measures. The absorption by products or slow penetration caused by the boundaries of the objects being fumigated are examples of sources of gas loss which always cause the actual gas concentration to be lower than the theoretically possible concentration.

The results of this experiment show that complete killing of all developmental stages of *T. castaneum* was achieved with an exposure time of 5 days at the specified dosage. Although it was possible to exterminate the active stages of *S. granarius* with no problems, isolated hatched beetles were again observed after the breed mixes were incubated, and thus 100% killing of all developmental stages of this insect species could not be achieved under the prevailing conditions. Nonetheless, the low hatch rate compared to the control variant indicates that phosphine is still effective, and that the selected dosage of 5 g PH_3/m^3 at an exposure time of 5 days should be adequate for successful fumigation under more favourable conditions. However, the dosage and exposure time are in a borderline area which may not completely meet the required degree of effectiveness in case of unfavourable fumigation conditions. In this experiment, two issues were likely the primary factors responsible for the inadequate effectiveness against *S. granarius*. First, the increased permeability of the fumigated containers. As described above, only gas concentrations of approx. 2000 ppm could be achieved, though 3500 ppm should be mathematically possible. Second, the temperature was only between 10º and 15°C at times during the fumigation phase. These relatively low temperatures also influenced the decreased sensitivity of the insects tested. However, because the specified experimental conditions are certain to occur in fumigation in practice, it is clear that experimental parameters for the size of the dose and the length of the exposure time used in this experiment are absolutely necessary under such conditions in order to ensure that successful fumigation can be carried out.

CONCLUSION

It can be concluded that various parameters important for fumigation with phosphine in practice, such as properties of the product to be fumigated; sufficient sealing; temperature and moisture conditions; insect species and its resistance status; insect development state, are frequently not taken into adequate consideration. For this reason, dosages which are too low and exposure times which are too short are frequently chosen, which may lead to the selection of tolerant strains among the surviving insects in the worst-case scenario.

Tolerance and resistance are often discussed in cases of failed fumigation with surviving insects, though it can frequently be assumed that inadequate consideration of the factors listed above is also responsible for inadequate effectiveness. Because appropriate gas monitoring is also frequently neglected, it is often impossible to determine why fumigation was not successful.

Taking the experiments described here into consideration, it is not surprising that adequate effectiveness is not always guaranteed at the frequently-used doses ranging from 1 to 2 g PH_3 per m³. It is easy to speak of increased tolerance and resistance in case of failed fumigation, although other parameters could be responsible for the lower effectiveness.

In order to now obtain a factual overview of the general status of insect tolerance to phosphine in Europe, a programme initiated in Greece was expanded to several other European countries. The first step of this project, which is planned for three years, is to collect and subsequently test different storage pests for their tolerance to phosphine.

For this purpose, insect samples were gathered in organised form from fumigation companies in Germany, France, Italy, Spain, Hungary, Great Britain, Poland, Czech Republic, Bulgaria, and Turkey. These insect strains shall be bred at the University of Thessaly in Greece, and their tolerance status to phosphine compared with strains with normal sensitivity shall be tested using various testing methods.

The different testing methods shall be verified in a subsequent step. This is to provide the option of better classifying the factor of tolerance to phosphine in the future.

REFERENCES

Mueller DK (1998) Stored Product Protection. A Period of Transition. Insects Limited, Inc., Indianapolis, Indiana 46280 USA, pp 98–99.

Opit GP, Phillips TW, Aikins MJ, Hasan MM (2012) Phosphine resistance in *Tribolium castaneum* and *Rhyzoperta dominica* from stored wheat in Oklahoma. Journal of Economic Entomology **105**(4): 1107–1114.