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The efficiency of phosphine against eggs of lesser grain borer Rhyzopertha dominica (Fab.) and larger grain borer Prostephanus truncatus (Horn) (Coleoptera: Bostrichidae)

Die Wirkung von Phosphin auf Eier des Getreidekapuziners *Rhizopertha dominica* (Fab.) und des Großen Kornbohrers *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae)

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Abstract

Fumigation tests were conducted at 20 ± 1 °C and 70 ± 5 % r.h. to investigate the tolerance of eggs of *Rhyzopertha dominica* and *Prostephanus truncatus* to fumigation with phosphine. The eggs of *P. truncatus* were found to be more tolerant than the eggs of *R. dominica* over a wide range of exposure periods and concentrations. Increase of exposure length was more effective than increase of gas concentration to achieve complete control.

The concentrations for a 50 % and 95 % mortality of the eggs of *P. truncatus* were higher than the corresponding concentrations of the eggs of *R. dominica*.

Zusammenfassung

Eier von *Rhizopertha dominica* und von *Prostephanus truncatus* wurden bei 20 °C und 70 % r. F. mit Phosphorwasserstoff begast. Die Eier von *P. truncatus*, die in der vorliegenden Arbeit erstmalig mit Phosphin behandelt wurden, erwiesen sich bei diversen getesteten Konzen-

trationen und Einwirkzeiten als widerstandsfähiger gegenüber denen von *R. dominica*.

Die Phosphin-Konzentration für die LC 50 und die LC 95 war jeweils ca. doppelt so hoch für den Großen Kornbohrer wie für den Getreidekapuziner.

1. Introduction

Prostephanus truncatus, a known pest of maize in America, is now recorded as a serious pest of stored maize in East and West Africa and is a very destructive insect pest of maize in tropical climates (HODGES 1982). There have been few studies on the control of *P. truncatus* especially by fumigation. The experiments described here were carried out to investigate the lethal effect of phosphine against the eggs of *P. truncatus* at 20 ± 1 °C and 70 ± 5 % r.h. in comparison with the eggs of *R. dominica*.

2. Materials and Methods

2.1. Rearing of Insects

Both species were cultured at 30 ± 1 °C and 70 ± 5 % r.h. A laboratory strain of *R. dominica* cultured on wheat and a susceptible strain of *P. truncatus* from Tanzania cultured on maize were used. Both strains are kept in culture at the Institute of Stored Products Protection, Federal Biological Research Centre, Berlin (West), FRG.

2.2. Providing Eggs for Fumigation Tests

2.2.1. *R. dominica*

About 500 adults from the stock culture were placed on 50 g wheat flour and held under the previously described conditions. Two days later, the adults were removed and transferred to a new quantity of wheat flour. The eggs were found in groups in the flour and were difficult to count. To overcome this difficulty, the flour containing the eggs was thoroughly mixed and divided into samples of 2 g. Each sample was introduced in a glass tube (4.5 cm long and 1.5 cm in diameter) which was closed at both ends with fine muslin.

2.2.2. *P. truncatus*

Most of *P. truncatus* adults stay inside the maize kernels and therefore the majority of the eggs are laid internally. After preliminary trials, the following method was used to obtain the eggs of this insect:

1. 500 adults from the stock culture were placed on 300 g maize in a 2-litre glass jar.
2. Two days later, the adults were removed by sieving, leaving most of them inside the kernels.
3. The sieve with maize kernels was exposed in an oven to 75 °C dry heat for 3 min. Under these conditions, many adults emerged from the grain. During the process of emerging, most of the frass containing many of the eggs came out of the kernels.
4. Eggs collected in this way and sieved out were carefully transferred (using a soft brush) to small dishes and examined under a binocular microscope. Damaged eggs were discarded.
5. For fumigation tests, batches of 25 eggs, each introduced in a glass tube (1.5 cm long and 1.0 cm in diameter), closed at both ends with fine muslin were used.

The effect of exposure to 75 °C for 3 min on the incubation period and % hatch is shown in Table 1.

The results in this table do not indicate any harmful effects on the viability of the eggs.

2.3. Fumigation Procedure

All fumigations were performed at 20 °C and 70 ± 5 % r.h. The equipment used comprised Dreshel flasks, with ground-glass joints (NOACK et al., 1983, DERMARCHELIER, 1984). The flasks with a volume of 0.55 l were connected to each other with PVC tubing. The joints were greased.

Phosphine is conveniently obtained for laboratory dosing purposes from pellets of Phostoxin, a proprietary formulation of aluminium phosphide. The 0.6 g pellet releases 0.2 g phosphine by reaction with diluted sulphuric acid (ANONYMOUS, 1975). The concentration of phosphine was determined by gas chromatography on a Carlo Erba 4100 gas chromatograph. A thermionic detector, in the phosphorous mode, was used. Operating conditions were: detector N-P; detector temperature 160 °C; column 10 % OV on Chromosorb W, HP-80-100 mesh; column temperature 160 °C and flow rate (nitrogen) 60 ml/min; injector temperature 120 °C and oven temperature 70 °C.

2.4. Post Fumigation Procedure

After exposure, the egg batches of *R. dominica* were transferred to a glass petri dish with 5 g wheat grain and those of *P. truncatus* to a small petri dish without diet and kept at 25 °C for a few hours after which they were moved to 30 °C (BELL et al., 1984). HOLE et al. (1976) found in tests at 15 °C that the effect of transferring developing stages of *Tribolium castaneum*, *Sitophilus granarius*, *Lasioderma serricorne* and *Trogoderma granarium* to higher temperatures immediately after fumigation, was to reduce the level of kill.

The criterion of death used for the eggs of *R. dominica* was failure to develop to adults, and therefore the results were assessed as reduction in progeny compared with the mean of three untreated controls (DESMARCHELIER, 1984). The emerging adults were counted daily until no more adults emerged. In case of *P. truncatus* eggs, a hatch count provided the assessment of kill. The control hatch was made for each experiment unit, and percentage hatch was corrected for the control response according to ABBOTT's formula (1925).

Percentages mortality in relation to concentrations at fixed exposure periods and different exposure at fixed concentrations, were determined by probit analysis (FINNEY, 1971), using the program of NOACK and REICHMUTH (1978).

3. Results

The data obtained are tabulated in Tables 2, 3 and 4 and represented graphically in Figs. 1, 2 and 3.

Table 1. The effect of exposure to 75 °C for 3 min on the incubation period and % hatch.

Exp. no.	Ave. inc. period at 30 °C (days) (control)	Ave. inc. period after exp. to 75 °C (days)	Ave. % hatch at 30 °C (control)	Ave. % hatch after exp. to 75 °C
1	5.5	5.0	91.0	88.8
2	6.0	5.0	96.0	89.4
3	5.5	5.5	85.6	92.0

Table 2. Parameters of probit regression equations and response estimates for eggs of *Rhyzopertha dominica* and *Prostephanus truncatus* exposed to different concentrations of phosphine for exposure periods ranging from 20 to 72 hours at 20 °C and 70 ± 5 % r.h.

Species	Exposure period (hr)	LC_{50} mg/l	LC_{95} mg/l	Parameters of line a	b ± S.E.	d.f.	r	Confidence limits at 95 % LC_{50} (mg/l) lower	upper	LC_{95} (mg/l) lower	upper
R. dominica	20	0.40	9.73	1.91	1.18 ± 0.18	4	0.96	0.30	0.54	4.03	23.50
	24	0.26	2.26	0.84	1.73 ± 0.24	3	0.84	0.19	0.35	1.31	3.90
	48	0.15	1.26	1.12	1.78 ± 0.07	3	0.99	0.12	0.20	0.67	2.38
	72	0.05	0.53	2.15	1.65 ± 0.42	5	0.96	0.04	0.07	0.35	0.82
P. truncatus	20	2.68	143.45	1.75	0.95 ± 0.06	5	0.99	1.92	3.75	66.15	311.08
	24	1.40	103.54	2.25	0.88 ± 0.11	5	0.89	0.98	1.99	39.95	268.35
	48	0.59	7.11	0.81	1.51 ± 0.01	4	0.81	0.46	0.75	4.71	12.43
	72	0.27	3.35	1.38	1.49 ± 0.20	3	0.95	0.20	0.36	1.46	7.68

Table 3. Means of percentages mortality of 0–2 days old eggs of *R. dominica* and *P. truncatus* exposed to phosphine at 20 °C and 70 ± 5 % r.h.

Species	Average concentration mg PH_3/l	% Mort. at each exposure period (hrs) 24	48	72	96	120	144	168
R. dominica	0.13 ± 0.0	25.0	50.3	71.7	93.0	100	100	100
	0.32 ± 0.0	55.3	69.3	95.7	100	100	100	100
	0.54 ± 0.1	64.0	85.0	100	100	100	100	100
R. truncatus	0.12 ± 0.0	20.0	27.3	42.0	59.3	81.0	93.0	100
	0.29 ± 0.0	18.7	42.3	63.0	79.7	89.0	100	100
	0.60 ± 0.0	41,7	51.0	71.0	92.0	100	100	100

3.1. Different Concentration at Fixed Exposure Periods

The mortalities resulting from each of the exposures of 20, 24 and 48 hrs increased slightly beyond 80–90 % for *R. dominica* and 70–80 % for *P. truncatus* at the higher tested concentrations (Fig. 1). At 72 hr exposure, however, the mortality increase beyond 80 % occurred at higher concentration for *P. truncatus* (Figs. 1 and 2). It is obvious that LC 50 and LC 95 values for eggs of *P. truncatus* are higher at all exposure times than those of *R. dominica*, indicating that the eggs of *P. truncatus* are more tolerant to phosphine than the eggs of *R. dominica*. Even in the range of low concentrations between 0.01 and 0.1 mg PH_3/l still some mortality occurred (Figs. 1 and 2). The lines representing the changes of log concentration against log time of exposure at the LC 50 and LC 95 levels (Fig. 3) over the range of exposure periods tested for eggs of *R. dominica* and *P. truncatus* were approximately parallel. At all exposures, the LC 95 values increased very sharply as the period of exposure decreased from 72 to 20 hr especially for eggs of *P. truncatus* (Table 2).

3.2. Different Exposure Periods at Fixed Concentrations

Regression lines of probit mortality against time of exposure varied considerably in slope over the concentration range tested (Table 3). Eggs of *R. dominica* were less tolerant than

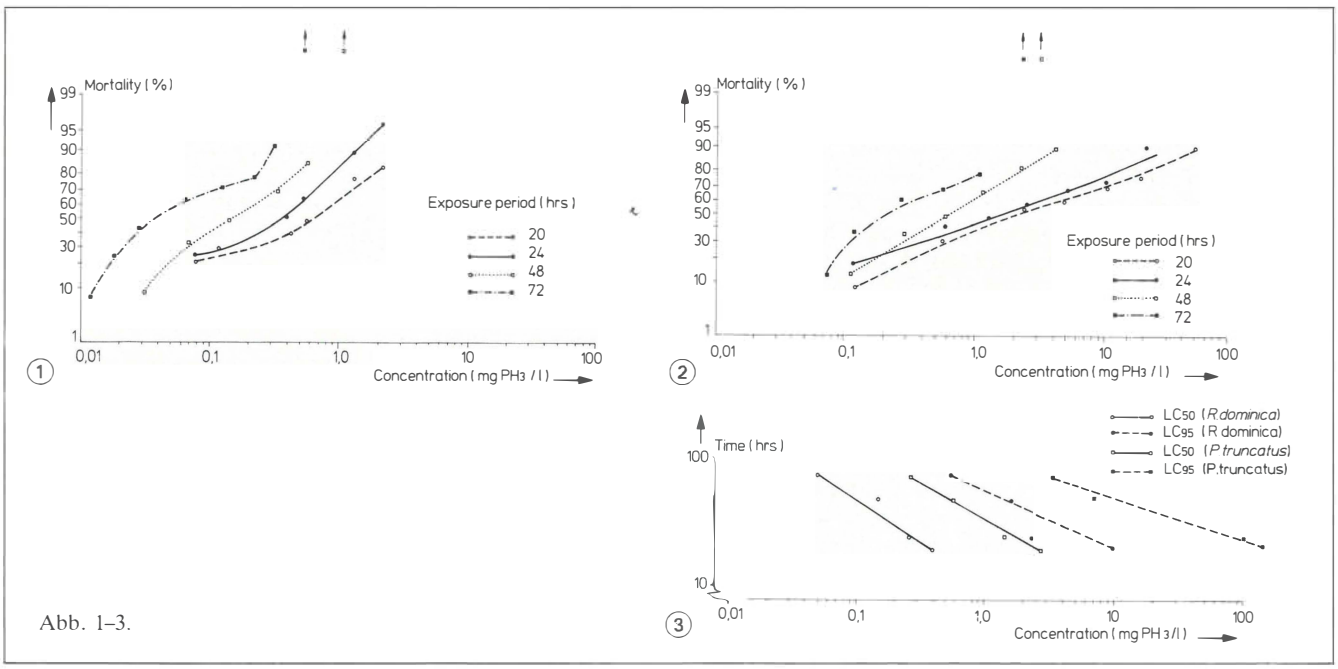

Abb. 1–3.

eggs of *P. truncatus*. They required for complete mortality 120, 96 and 72 hours at 0.13, 0.32 and 0.54 mg/l, respectively, compared with 168, 144 and 120 hours at 0.12, 0.29 and 0.60 mg/l, respectively, for eggs of *P. truncatus* (Table 3). It is clear throughout the present tests, that longer exposures were much more effective in achieving control than shorter ones as has already been widely reported (BARKER, 1969; BELL and GLANVILLE, 1973; and HOLE et al., 1976).

4. Discussion

The results presented here are further evidence of the high tolerance towards phosphine of 0–48 hr old eggs of stored product pest insects. For the first time this has been shown for *P. truncatus* in comparison to *R. dominica* with eggs of the same age. HOLE et al. (1976) have demonstrated that there is a tolerant period during the first two days at 25 °C in the eggs of the lesser grain borer *R. dominica* eggs. QURESHI et al. (1965) found with Cadelle, *Tenebroides mauritanicus* (L), that eggs were tolerant to phosphine at 25 °C and 70 % r.h. during the first 4 days of development. BARKER (1969) found 0–24 hr old eggs of *Cryptolestes ferrugineus* (Stephans) highly tolerant to exposure to phosphine to 24 hr at 24 °C. VINCENT and LINDGREN (1972) obtained some survival of 0–1 day old eggs of 3 Dermestidae, *Trogoderma glabrum* (Herbst), *T. sternale* Jayne and *T. variabile* Ballion, exposed to 6 mg phosphine/l for 24 hr exposures at 21 ± 1 °C.

High tolerance to phosphine in the egg stage can be regarded as a common phenomenon, and is undoubtedly linked with metabolism during embryological development (BELL, 1976). BOND et al. (1967 and 1968) showed that oxygen was essential for phosphine to exert a toxic effect and stages which can survive without oxygen for a period of time are likely to be tolerant.

This probable ability of stages to disregard the first part of an exposure period is another factor contributing to the highly individual ct-relationship which operates for phosphine. Oxygen consumption during the egg stage varies with the species under consideration (WIGGLESWORTH, 1972), and at the present time too few data exist to correlate low oxygen consumption and high tolerance to phosphine during the egg stage (BELL, 1976).

As can be seen from the results reported here, phosphine is markedly less effective to control eggs of *P. truncatus* than those of *R. dominica* at 20 °C. The findings of the present work confirm that with phosphine, the duration of exposure is more critical than gas concentration for ensuring insect control (WINKS, 1982). BELL et al. (1984) found with *Trogoderma granarium* eggs that the exposure times required at 20 °C or below are likely to be too long to be recommended. Suggested exposure times are at least a week at 15 °C, and at least 3–6 days at temperature over 20 °C (PREVETT and BLATCHFORD, 1972). BELL and GLANVILLE (1970) established that eggs of *Ephestia elutella* (Hübner), *E. kuehniella* (Zeller), *E. Cautella* (Walker) and *Plodia interpunctella* (Hübner) were tolerant to phosphine at 25 °C, if the exposure time did not exceed 2 days.

Similar tolerance to 24-hr fumigations has been reported by other workers for *E. cautella* (BASKARAN and MOOKHERJEE, 1971, and MUTHU, 1973). BELL (1976) indicated the position and length of the period during which eggs of *E. cautella, E. kuehniella, E. elutella* and *P. interpunctella* are tolerant to phosphine at a range of temperature. However, the kill obtained after short exposures was not always proportional to concentration, possibly different levels of susceptibility occur during the tolerant phase and variation in the exact age of eggs in different age groups of 0–24 hr may be critical. One reason as to why phosphine is more efficient after long than after short exposures is that insects continue to develop while being fumigated and that extra time increases the chance of reaching a susceptible stage (BELL, 1979). For phosphine, differences between the tolerance of stages are extremely great (HOWE, 1973, and BELL, 1976). Tolerant stages continue development and do not succumb until a susceptible stage is reached (REYNOLDS et al., 1976).

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Zum Vorkommen von Kartoffelvirus V in Kartoffeln

Incidence of potato virus V in potatoes

Von Hans-Ludwig Weidemann

Zusammenfassung

Stichproben aus 139 Kartoffelsorten und aus sekundär infizierten Kartoffelpflanzen aus langjährigen Nachbauten wurden mit ELISA auf Gehalt mit Kartoffelvirus V (PVV) untersucht. Dabei wurde das Virus in der Kartoffelsorte „Hela" nachgewiesen. Die infizierte Pflanze zeigte keine Symptome. In Übertragungsversuchen mit *Myzus persicae* wurden das Isolat „Hela" und ein irisches PVV-Isolat miteinander verglichen. Beide Virusisolate wurden mit ähnlichem Erfolg auf 52 % bzw. 46 % der Testpflanzen übertragen.

Summary

Random samples of 139 potato cultivars and secondary infected potato plants were investigated for potato virus V (PVV) by ELISA. The virus was detected in a plant of the potato cultivar "Hela" which remained symptomless. Transmission tests with *Myzus persicae* were carried out in comparison with an Irish isolate of PVV. Both, the isolate "Hela" and the Irish isolate were transmitted with a similar efficiency of 52 % and 46 % respectively of infected test plants.

Einleitung

Das Kartoffelvirus V (potato virus V, PVV) ist ein Potyvirus und somit verwandt mit den Kartoffelviren A und Y (PVA, PVY). Das Virus wurde erstmals in den Niederlanden gefunden (ROZENDAAL et al., 1971), später auch in Nordirland (CALVERT et al., 1980) und Frankreich (BOUDAZIN et al., 1984). Zunächst wurde dieses Virus irrtümlich der Stammgruppe C des PVY^C) zugeordnet, weil es ebenso wie PVY^C auf Kartoffelsorten, die das Gen Nc tragen, Überempfindlichkeitsreaktionen verursachte. Anhand eines Isolates aus peruanischen Kartoffeln wurde es jedoch als ein eigenständiges Virus erkannt und erhielt die Bezeichnung potato virus V (FRIBOURG et al., 1984). PVV wurde auch in verschiedenen Kartoffelsorten, die in Großbritannien angebaut werden, nachgewiesen (JONES und FULLER, 1984; JONES, 1987), was darauf hinweist, daß es in Europa offenbar weiter verbreitet ist.

Im Gegensatz zu PVA und PVY verursacht PVV im A_6-Test keine Lokalläsionen. In den siebziger Jahren, als der A_6-Test noch als Nachweismethode für diese Viren in der Kartoffeltestung verbreitet war, versagte dieser Test verschiedentlich bei Pflanzen, deren Symptome auf PVY-Infektionen hinwiesen (WEIDEMANN und KOENIG, 1979). Es liegt jetzt der Verdacht nahe, daß es sich dabei um PVV-infizierte Pflanzen gehandelt hat. Um zu überprüfen, ob dieses Virus auch in deutschen Kartoffeln vorkommt, wurden Stichproben aus dem Kartoffelsortiment auf Befall mit PVV untersucht.

Material und Methoden

Stichproben von jeweils 5 Knollen aus 139 Kartoffelsorten, die uns freundlicherweise von Herrn Dipl. agr. PETER KRÄTZIG, Saatbauinspektion Soltau, zur Verfügung gestellt wurden, sowie von etwa 150 Proben aus langjährigen Nachbauten, die als Virusquellen bei Resistenzprüfungen dienten, wurden vom Augensteckling mit ELISA auf PVY untersucht. Zur Herstellung des Antiserums wurde ein irisches PVV-Isolat aus der Kartoffelsorte „Arran Banner" (PVV:AB) verwendet. Außerdem wurden als Antikörper ein polyklonales Immunglobulin gegen PVV:AB eingesetzt, das zusammen mit dem Virusisolat freundlicherweise von Dr. R. COPELAND, Belfast, zur Verfügung gestellt wurde, sowie ein monoklonales Antikörperkonjugat von Bioreba, Basel. Zur Viruspräparation wurde PVV in