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Lethal effect of sulfuryl fluoride on eggs of different age of the Indian meal moth *Plodia interpunctella* (Hübner) – demonstration of the no constancy of the ct product for control

Letaleffekte von Sulfurylfluorid auf Eier unterschiedlichen Alters der Dörrobstmotte *Plodia interpunctella* (Hübner) – Nachweis der Nichtkonstanz des ct-Produkts für die Bekämpfung

Abstract

One to four day old eggs of the Indian meal moth *Plodia interpunctella* served for an investigation of their tolerance towards sulfuryl fluoride (SF) fumigation at 27°C and 65% relative humidity and of the question of constancy of the lethal dosage of SF – the ct product (ctp) of the lethal concentration and the lethal exposure time – for different concentrations and exposure periods.

In many experimental series at fixed concentrations between 1 g/m^3 and 6.24 g/m^3 and exposure times between one and four days, egg age influenced the degree of mortality results. With few exceptions, one day old eggs tolerated the gas more than the other age groups of eggs at exposures of one to three days. 6.24 g/m^3 caused full mortality of all young and older eggs in the test after three days of fumigation [ctp_{100} = 450 gh/m^3] as well as 1.99 g/m^3 within four days [ctp_{100} = 191 gh/m^3].

Probit analysis and nonlinear regression of the mortality data resulted into an intensive discussion and into several tables and figures of lethal ct products as function of lethal exposure times and lethal concentrations. Ct products for 95% and 100% mortality, ctp_{95} and ctp_{100}, of all implemented eggs showed minima for four days exposure, strong dependency of the age of the treated eggs and strong variations depending on the lethal concentration of SF. Regression led to a final figure showing the lethal exposure time between one and four days of treatment as function of the corresponding necessary lethal concentration of SF.

Many accessible data from the literature and various tests confirmed the tendency of the presented results, showing the relative susceptibility of eggs of *Plodia interpunctella* in comparison with eggs of other stored product pest insects. For the first time, the lethal effect of SF was discussed in detail as a function of age of treated eggs of this moth for better understanding of the no constancy of the lethal ct product.

Key words: Sulfuryl fluoride, *Plodia interpunctella*, eggs, stored products, moths, ct product

Zusammenfassung

Ein bis vier Tage alte Eier der Dörrobstmotte *Plodia interpunctella* dienten zur Untersuchung ihrer Widerstandsfähigkeit gegen Begasung mit Sulfurylfluorid (SF) bei 27°C und 65% relativer Luftfeuchte sowie der Frage der Konstanz der letalen SF-Dosierung, dem ct-Produkt (ctp)

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aus letaler Einwirkzeit und letaler Konzentration für verschiedene Einwirkzeiten und Konzentrationen.

In vielen Experimentalreihen bei fester Konzentration zwischen 1 g/m^3 und 6,24 g/m^3 und Variation der Einwirkzeiten zwischen ein bis vier Tagen beeinflusste das Alter der eingesetzten Insekteneier deutlich den Grad der erzielten Mortalität. Mit wenigen Ausnahmen vertrugen einen Tag alte Eier bei Begasungen über ein bis drei Tage mehr Gas als Eier der anderen Altersgruppen. 6,24 g/m^3 führten im Dreitagetest zu vollständiger Mortalität aller 600 eingesetzten jüngeren und älteren Eier [ctp_{100} = 450 gh/m^3] wie auch 1,99 g/m^3 in vier Tagen mit insgesamt 450 eingesetzten Eiern [ctp_{100} = 191 gh/m^3].

Eine Transformation der Versuchsdaten mit Probit Analyse und nichtlinearer Regression mündete in eine ausführliche Diskussion und mehrere Tabellen und Grafiken mit den ct-Produkten als Funktion der letaler Einwirkzeiten und SF-Konzentrationen. Die ct-Produkte für 95% und 100% Mortalität, ctp_{95} und ctp_{100}, aller eingesetzten Eier nahmen für vier Tage Behandlung deutlich ein Minimum an, und zeigten darüber hinaus eine starke Abhängigkeit vom Alter der jeweils verwendeten Motteneier und von der jeweils eingesetzten konstanten SF-Konzentration. Die Daten dienten abschließend für eine Darstellung der Beziehung zwischen letaler Einwirkzeit von ein bis vier Tagen in Abhängigkeit von der jeweils erforderlichen letalen SF-Konzentration mit dem letalen ct-Produkt als Parameter.

Viele der zitierten Literaturdaten bestätigten die Tendenz der durchgeführten Studie, welche die relativ hohe Empfindlichkeit der Eier der Dörrobstmotte im Vergleich zu Eiern anderer vorratsschädlicher Insekten belegte. Erstmalig unterzog eine Studie die Abhängigkeit des letalen Effekts von SF auf Eier dieser Motte einer detaillierten Diskussion bezüglich des Eialters, auch im Hinblick auf ein besseres Verständnis der Nichtkonstanz des letalen ct-Produkts für verschiedene Einwirkzeiten oder Konzentrationen.

Stichwörter: Sulfurylfluorid, *Plodia interpunctella*, Ei, Vorrat, Falter, ct-Produkt

Introduction

Sulfuryl fluoride (SF) is one of the promising alternatives for fumigation with methyl bromide (MB) against many stored product pests (MBTOC, 2011). MB has been phased out in developed countries since 2004; in developing countries this phase out is envisaged to take place in 2015. Application of SF can be more convenient than that of methyl bromide, since SF is a gas at normal ambient temperatures.

One of the pest insects causing severe problems towards various stored products and massive economic losses world wide is the Indian meal moth *Plodia interpunctella* (Hübner). This moth is also a very common household pest, feeding on food and especially on bird feed. It infests apart from other products various raw and processed cereals, seeds, dried fruit, nuts, almonds, spices and a variety of processed food products like chocolate (Reichmuth et al., 2007).

Numerous insecticides (Arthur et al., 1990) and also cold treatment have been used to control Indian meal moth populations (Bell, 1975; Reichmuth, 1979; Stratil and Reichmuth, 1984). In a study evaluating the effectiveness of chlorpyrifos-methyl and chlorpyrifos-methyl together with the insect growth regulator methoprene (Arthur et al., 1990), it was found that even after treatment with these chemicals, living Indian meal moths were found in corn stores. Resistance of this moth towards insecticides was observed as for instance in studies performed with the microbial insecticide *Bacillus thuringiensis* (McGaughey, 1985).

The response of eggs of *P. interpunctella* towards treatment with sulfuryl fluoride has been studied in this research, because they are known to be by far the most tolerant developmental stage (Drinkall et al., 1996, 2003; Ducom et al., 2003; Reichmuth et al., 1997, 1999, 2003; Schneider and Hartsell, 1999; TEAP, 2011; Tsai et al., 2011). The picture from the published data on the response of eggs of this moth towards fumigation with SF is still contradicting.

The presented study tried to highlight in more detail the specific dependencies of concentration of SF and exposure period to obtain lethal effects on insect eggs. *P. interpunctella* at 27°C served as model system. The concept of using the ct product for judging the efficacy of SF for various exposure times and concentrations was investigated.

Materials and Methods

To further investigate systematically the dependency of the efficacy SF on egg age, exposure period and concentration, eggs of different age have been subjected to treatment with gas mixtures of SF and air with different concentrations for different exposure periods. The numbers of hatching larvae from the fumigated eggs served for the bioassay. The insects were obtained from the long running culture at the Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection of the Federal Research Centre for Cultivated Plants (Julius Kühn-Institut) in Berlin, Germany. For including eggs of different ages into the experiments, a separate culture of *P. interpunctella* was maintained on broken almonds, wheat bran, glucose, yeast, glycerin and water (5:20:2.4:4:1) at 25°C ± 3°C and 65% ± 5% relative humidity (r.h.). Each individual test was replicated three times. 50 eggs each, one, two, three and four day old, respectively, were treated according to the method of Baltaci et al. (2006) at six concentrations of sulfuryl fluoride (approximately 1, 1.48, 1.99, 4.19, 5.24 and 6.24 g/m^3, respectively), at 27°C. A Fourier Transform Infrared Spectrometer (FTIR) served to determine the SF concentrations. Mortality data were subjected to probit analysis (Ehabsoft, ldPline®-software, log-concentration probit model) to determine the values of lethal concentrations at the 95% mortality level (LC_{95}) (Finney, 1971). Since the probit analysis is not specifically suitable to process data exclusively on high degrees of mortality, nonlinear regressions with the TableCurve® program (2011) have alternatively been used to calculate lethal concentrations and exposure times at 95% and 100% mortality level (LC_{95}, Lt_{95}, LC_{100} and Lt_{100}), following an application of either a logistic dose response model [equation 8076 of the program: $y = a/(1 + (x/b)^c)$] or a Gauss cumulative model [equation 8075: $y = (a/2)\ (1 + \mathrm{erf}((x - b)/\mathrm{SQR}(2c))$] with average regression coefficients for the application of the two models for the mortality data of $r^2 = 0.8333 \pm 0.1211$ as standard deviation. Some regressions for the determination of ct products at 100% mortality level, ctp_{100}, were carried out by using the impulse transition equation [equation 8095: $y = a + b((x - c)/d)(|x - c|/d)^{-e}$]. The obtained data have also been used to calculate an estimate for the required ct products with multiplications of given concentrations and exposure periods leading to the indicated degree of mortality.

Results

The percent mortalities with their standard deviations of the three replicates resulting from the fumigation of eggs of different ages of *P. interpunctella* with SF are presented in Tab. 1. Data in this Tab. show the general tendency that the mortalities of the eggs of the same age increased with increasing concentration of SF at the same exposure time and for increasing exposure times. The influence of the egg age on the lethal effect varied for the various combinations of concentration and exposure period.

For **one day exposure** and the tested SF concentrations, the lethal effect increased with increasing concentration and age, with the exception of three day old eggs and concentrations above 4.19 g/m³, when they were more tolerant than younger and older eggs. After exposure to 1 g/m³ of SF, the one, two, and three day old eggs were with 2% mortality more tolerant than the four day old eggs with 18% mortality. On increasing the SF concentration towards 4.19 g/m³ and 5.24 g/m³, one day old eggs possessed the highest tolerance of all egg stages

Tab. 1. Percent mortalities as average of three replicates for 50 eggs each of *P. interpunctella* of different age after exposure to various concentrations of sulfuryl fluoride (SF) for different exposure periods at 27°C and 65% r.h.; 100%-values are indicated in grey; SD in the lane below the mortality data; below some 100% mortality values the according ct products for full control in [ ]

SF conc. in g/m³	Average mortality of eggs of different age SD in % or below some 100% figures ct product in gh/m³ in [ ]														
	1 day exposure, egg age in days				2 days exposure, egg age in days				3 days exposure, egg age in days				4 days exposure, egg age in days		
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3
1.00	2	2	2	18	12	54	68	73	78	88	92	89	98	88	100
	1.6	0	0	0	5.6	2.8	3.2	2.4	8.6	1.6	2.8	0.8	2.8	2.8	[96]
1.48	4	14	16	30	32	70	68	83	84	90	94	94	100	96	100
	2.8	3.2	1.6	1.6	2.8	5.8	1.6	2.5	4.3	1.6	4.3	3.3	[142.1]	5.6	[142.1]
1.99	12	22	28	43	60	72	90	88	94	96	96	96	100	100	100
	5.6	2.8	1.6	2.5	3.2	2.8	4.0	1.6	8.4	4.3	4.3	1.6	[191]	[191]	[191]
4.19	32	54	46	71	82	90	92	91	100	98	98	95	100	100	100
	5.8	0	2.8	2.5	7.5	7.4	2.8	0	[301.7]	2.8	1.6	4.1			
5.24	72	79	70	82	84	96	98	100	100	100	100	99	100	100	100
	5.9	1.4	1.6	2.8	8.4	3.2	1.6	[251.5]	[377.4]	[377.4]	[377.4]	1.9			
6.24	84	80	70	91	90	98	100	100	100	100	100	100	100	100	100
	8.4	1.6	3.2	1.4	0	1.6	[299.5]	[299.5]	[499.3]	[499.3]	[499.3]	[499.3]			
Untreated	0.3	1	0	8	0.3	3	1	3	0.3	0	1	2	0.3	0	0
	0.47	0.8		1.6	0.47	0.9	0.8	0.8	0.47		0.8	1.6	0.47		

at this exposure period. At 5.24 g/m^3 and 6.24 g/m^3, three day old eggs were more tolerant than one day old eggs. Meanwhile, four day old eggs showed the highest susceptibility at all applied concentrations. Only after exposures of one day, percent mortalities of two and three day old eggs ranged between those of one and four day old eggs.

After **two days exposure**, the percent mortalities increased clearly and reached even 100% at the two highest concentrations for all eggs of ages of three and four days. The corresponding ct products of 299.5 gh/m^3 and 251.5 gh/m^3, respectively, are given in [ ] in the line below (see numbers below the according 100% values in Tab. 1). One day old eggs were always least susceptible.

After increasing the exposure period to **three days**, even 1 g/m^3 was enough to kill 80 – 90% of all eggs of all four ages with one and two day old eggs being most tolerant. The highest tested concentration of 6.24 g/m^3 obtained full control of all egg stages tested within three days of exposure [ctp_{100}: 449.3 gh/m^3]. This data point with 100% mortality is based on investigations of four age groups with 50 eggs each and three replications amounting to 600 eggs in total. Four day old eggs required the highest tested dose.

The full lethal effect occurred already at 1.99 g/m^3 and **four days** of exposure with a ct product of 191 gh/m^3 for altogether 450 tested eggs. Four days of exposure were not carried out with four day old eggs, since hatch of larvae from the untreated eggs occurred already during this period. Therefore, these results are based on 450 eggs instead of 600 eggs. Two day old eggs were most tolerant at concentrations of 1 g/m^3 and 1.48 g/m^3, respectively.

Fig. 1 presents all mortality results together in one graph without indicating the standard deviations. Generally during the various experiments, the increase of mortality followed very clearly for all egg ages and tested exposure periods the increase in concentration of SF. Four days exposure resulted already at fairly low concentrations of 1.99 g/m^3 in 100% mortality of all eggs of all stages [ct = 191 gh/m^3], whereas 6.24 g/m^3 achieved the same degree of mortality of all tested eggs of all ages within three days of fumigation at 27°C [ct = 449.3 gh/m^3]. One and two day old eggs survived two days of exposure to 6.24 g/m^3, the highest concentration tested, whereas eggs of all ages survived this treatment when they were exposed for one day only.

As shown in Tab. 1, the standard deviations of the three replicates of the mortality results remained nearly always below 5%; only in very few series of experiments the SD increased up to 8.4%. SD of the mortalities in the untreated reference samples varied between 0% and 1.6%. This low mortality in untreated samples can be expected from reported studies with eggs of this moth (Reichmuth, 1979; Reichmuth et al., 1999, 2003).

As example, Fig. 2 demonstrates for three day old eggs the magnitude of the SD in relation of the various averages of the results. For one day exposure, the typical sigmoid function described the resulting mortality data for the increasing concentration. Four days of exposure at 1 g/m^3 [ct = 96 gh/m^3] or three days at 5.24 g/m^3 [ct = 377.3 gh/m^3] were sufficient for complete control of eggs of this age. This degree of control of these eggs was also achieved with 6.24 g/m^3 [ct = 299.5 gh/m^3] within two days of fumigation.

Fig. 3 reveals the increase of efficacy when the exposure period was expanded for up to four days. The increase of mortality from three days of treatment to four

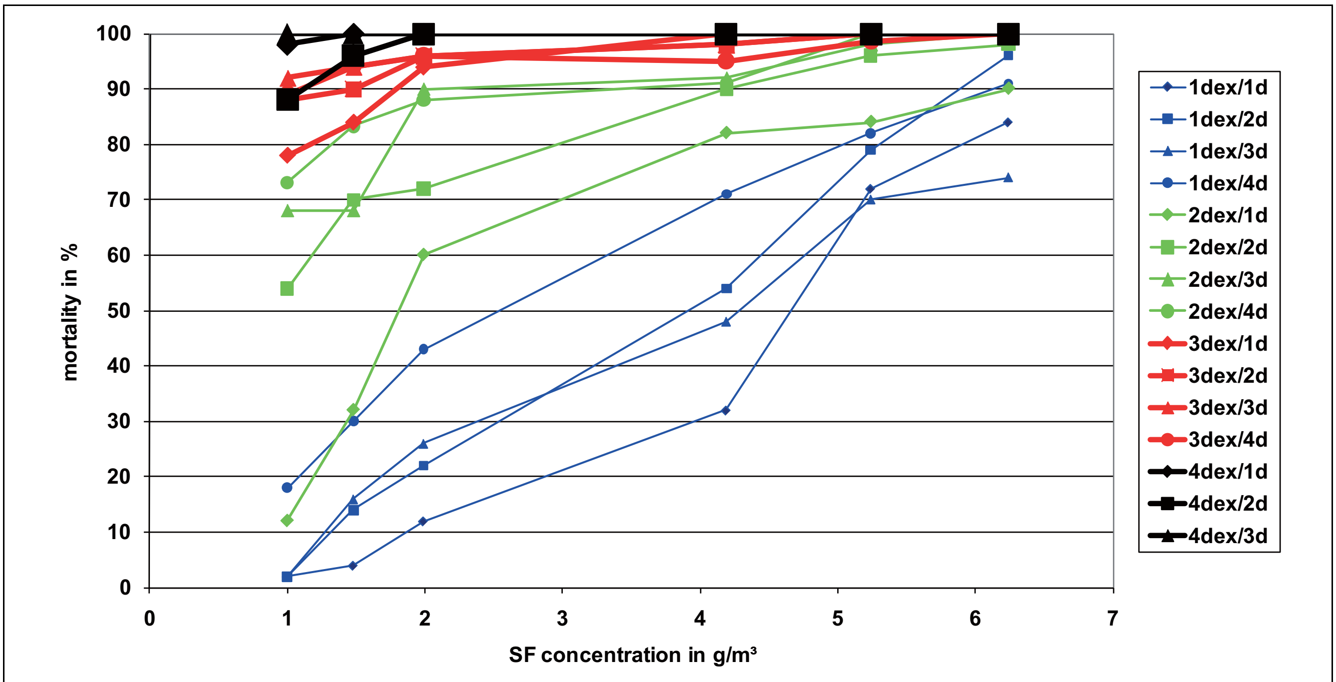

Fig. 1. Average mortality of eggs of *P. interpunctella* of different age (1d – 4d) after exposure to different concentrations of sulfuryl fluoride for different times of fumigation: 1 day exposure (1dex) to 4 days exposure (4dex); 27°C and 65% r.h., three replicates.

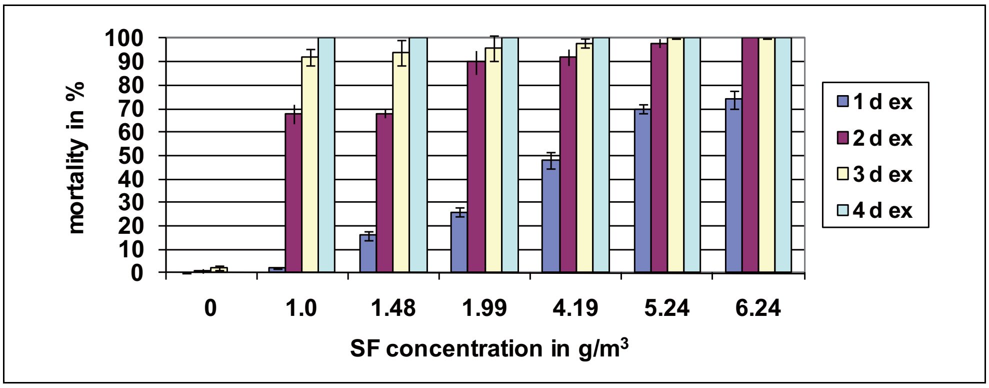

Fig. 2. Average mortality of three day old eggs of *P. interpunctella* after one to four days of exposure (1dex – 4dex) to different concentrations of sulfuryl fluoride; 27°C and 65% r.h., three replicates.

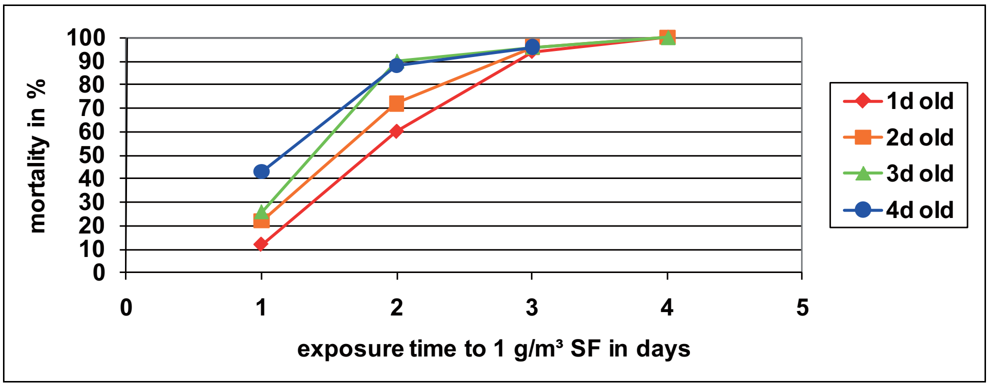

Fig. 3. Efficacy of 1 g/m³ of sulfuryl fluoride towards eggs of different age (one day to four days) of *P. interpunctella* at 27°C and 65% r.h., three replicates per average point with 50 eggs each.

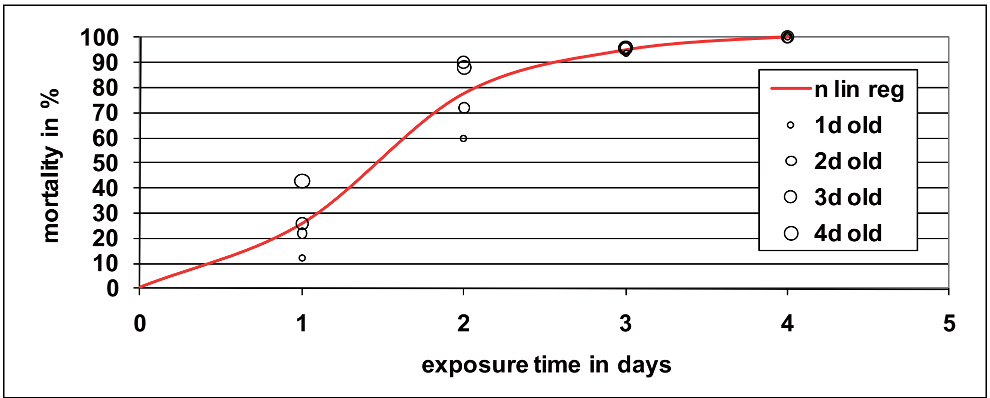

Fig. 4. Mortality of eggs of *P. interpunctella* of different age, 1 day (1d) to 4 days (4d) after exposure to 1.99 g/m³ of sulfuryl fluoride at 27°C.

days – following the typical sigmoid shape – was not tremendous. Two day old eggs could tolerate best the four day treatment. Duplication of the concentration from 1 g/m³ to 1.99 g/m³ [ct = 191 gh/m³] led to full control of eggs of all ages as shown in Fig. 4 (see also Tab. 1).

Discussion

The data in Tab. 1 offer two ways of discussing the mortality results and ct products of eggs of *P. interpunctella* after treatment with SF at 27°C: 1. increase of efficacy by extending the exposure period from one to four days at a fixed concentration between 1 g/m³ and 6.24 g/m³; 2. increase of efficacy due to increasing the concentration from 1 g/m³ to 6.24 g/m³ at a fixed period of exposure from one to four days. From methyl bromide it is known that the ct product for a given mortality is fairly constant, independent if the ct product contains high concentration and short exposure or low concentration and long exposure leading to the same figure and degree of control. This discussion should clarify for the selected insect stage and species and temperature, if the ct product of SF for complete kill is equally independent of the length of exposure and magnitude of concentration.

As can be expected from Fig. 3, the prolongation of the exposure to 1 g/m³ beyond four days may lead to full control within few more days than four. This new concept of SF fumigation would on one side save plenty of fumigant and help to reduce emission of the gas during and after the treatment and lead to less formation of residues in treated goods. On the other side, prolongation of the exposure time can be costly in many situations (e.g. mill fumigation, ship fumigation).

At the tested temperature of 27°C, effective fumigation against *P. interpunctella* can obviously be carried out at fairly low concentrations within fairly short treatment periods. One day exposure would theoretically require about 13 g/m³ for 95% control [ct_{p95} = 312 gh/m³] or about 16 g/m³ [ct_{p100} = 384 gh/m³] for full control, if the corresponding mortality data were extrapolated for instance with formula 8076 of the TableCurve program (r^2 = 9656; a = 110.9866, b = 4.4049, c = −1.7145). The fiducial limits of this regression were fairly high and indicated the necessity to determine these data more precisely by further fumigation experiments. Even if – for practical reasons – the concentration during a fumigation in the field would be increased by factor two to three, as proposed by Reichmuth and Klementz (2008), full control of eggs of this species could be obtained with ct products below 1,500 gh/m³, as registered in many countries.

The nonlinear regression of all corresponding average results for 1.99 g/m³ with TableCurve (Equation 8076, logistic dose response model, r^2 = 0.9418), shown as line in Fig. 4, evaluated 3.89 days as the necessary exposure time for full control [ct = 185.8 gh/m³], confirming the result in Tab. 1 with four days being sufficient for 100% mortality of all tested eggs [ct = 191 gh/m³].

Tab. 2 and Fig. 5 try to highlight the dependency of the ct product for control from the age of the treated eggs and the exposure time. An obvious shift in susceptibility of eggs of different age from one day to three days of exposure leads finally to nearly the same mortality of one, two and three day old eggs, respectively, after four days of exposure. The explanation could be the change in membrane permeability for SF of the egg shells with age and during the exposure (Outram, 1967a, b) and the development of hardly susceptible eggs into fairly susceptible young larvae within the eggs during the exposure period. During four days of exposure, also very young

Tab. 2. Concentration x time product and exposure time for control of eggs of different age of *P. interpunctella* with sulfuryl fluoride at 27°C; calculated lethal SF concentrations for each exposure period and egg age in brackets; Upper line: Data for 95% control calculated with probit analysis (Ehabsoft, ldPline®) where possible or nonlinear regression for four days of exposure (TableCurve; Equation 8076, $r^2_{(1d)}$ = 0.9999816, $r^2_{(2d)}$ = 0.99998667; *italic figures*); Lower line: Data for 100% mortality either directly from Tab. 1 (bold) or from nonlinear regression for those results without 100% mortality in Tab. 1 (TableCurve Equation 8095: y = a+ b (x-c/d) (x-c/d)^e for regression with r^2 between 0.9707 and 0.9992, *bold and italic*)

Exposure Time in days	ct_{p95}//ct_{p100} in gh/m³ (lethal concentration in brackets) egg age in days			
	1d	2d	3d	4d
1	258.24 (10.76) ***207.36 (8.64)***	250.56 (10.44) ***190.8 (7.95)***	374.88 (15.64) ***235.92 (9.83)***	*226.08 (9.42)* ***175.44 (7.31)***
2	374.88 (7.81) ***444 (9.25)***	356.16 (7.42) ***317.76 (6.62)***	251.52 (5.42) **299.5 (6.24)**	*307.68 (6.41)* **252 (5.24)**
3	176.4 (2.45) **301.7 (4.19)**	236.88 (3.29) **377.3 (5.24)**	198.72 (2.76) **377.3 (5.24)**	*241.92 (3.36)* **449.3 (6.24)**
4	*78.24 (0.815)* **142.1 (1.48)**	*66.91 (0.697)* **191 (1.99)**	–* **96 (1.00)**	–*

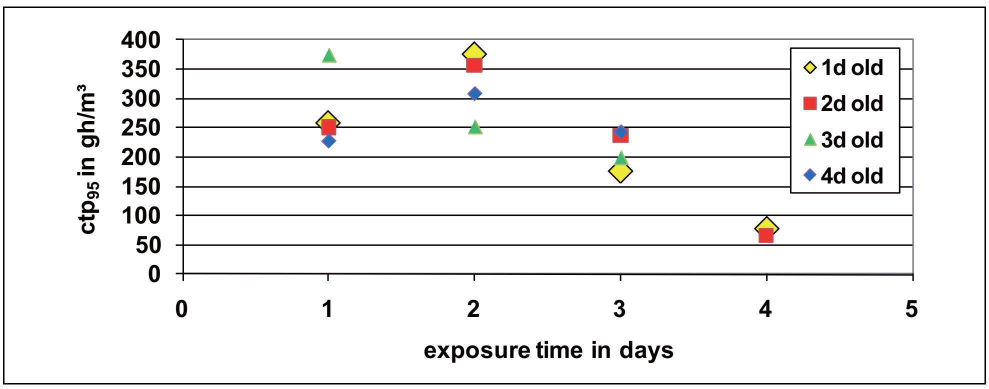

Fig. 5. ct product as function of the exposure period for control of 95% of the eggs of different age of *P. interpunctella* with sulfuryl fluoride at 27°C, data see Tab. 2.

eggs at the beginning of the treatment have presumably and obviously developed into more susceptible eggs.

Since the source of some data in Fig. 6 were those concentrations and exposure times in Tab. 1 where full control of all exposed 150 eggs was achieved, the theoretical lethal dosage may be a bit lower due to the step width of concentration and exposure time. On the other hand, 150 tested eggs as total cannot lead to mortality data with much better than 1% precision as pointed out by Reichmuth (2007). In this case of ctp_{100} – other than with ctp_{95} –, nonlinear regression for determination of missing data for 100% mortality for short exposure periods was not applied due to very large fiducial limits, indicating the low precision of the calculated data. Still it was obvious that expectedly like ctp_{95} also ctp_{100} dropped at longer exposures than three days. For one and two days of exposure, ctp_{100} data for were not produced and are missing in the Fig. 6. Tab. 2 contains these as extrapolated figures.

Fig. 6 combines the results of Tab. 1 and 2 in selecting only those ct product values that were required to control the most tolerant eggs of the tested ages at each exposure time. The formula $ctp_{95} = 967 - 197\ t - 395/t$ ($r^2 = 0.99998987$, for t = 1 to 4 days) describes the decline of the ct product with extended exposure beyond two days. In so far, it is demonstrated that the ct product for achieving 95% or 100% control is not constant for various exposure periods in the range of one to four days. Longer exposure required less SF for control than proportional would expected if the ct value would have been a constant.

As shown in Fig. 7, the ct product varied also as a function of the selected concentration and was not constant. Especially short exposure times required high concentrations for control.

The dependency of ctp_{100} on the concentration of SF contains Fig. 7 with those data directly taken from Tab. 2. Non bold symbols are based on data calculated by non linear regression.

Fig. 8 describes the exposure time as function of the concentration of SF during a fumigation of eggs of different age of *P. interpunctella* at 27°C leading to 100% mortality of 150 eggs each at all data points. Values of ctp_{100}

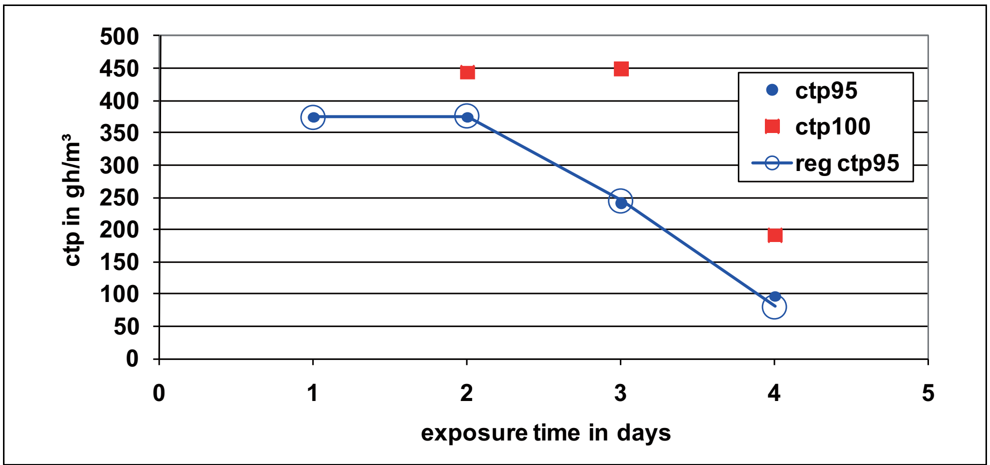

Fig. 6. ct products as function of the exposure period; data represent ctp values for control of the most tolerant eggs at each exposure time for control of *P. interpunctella* with sulfuryl fluoride at 27°C; data see Tab. 2; polynomial nonlinear regression of the dependency.

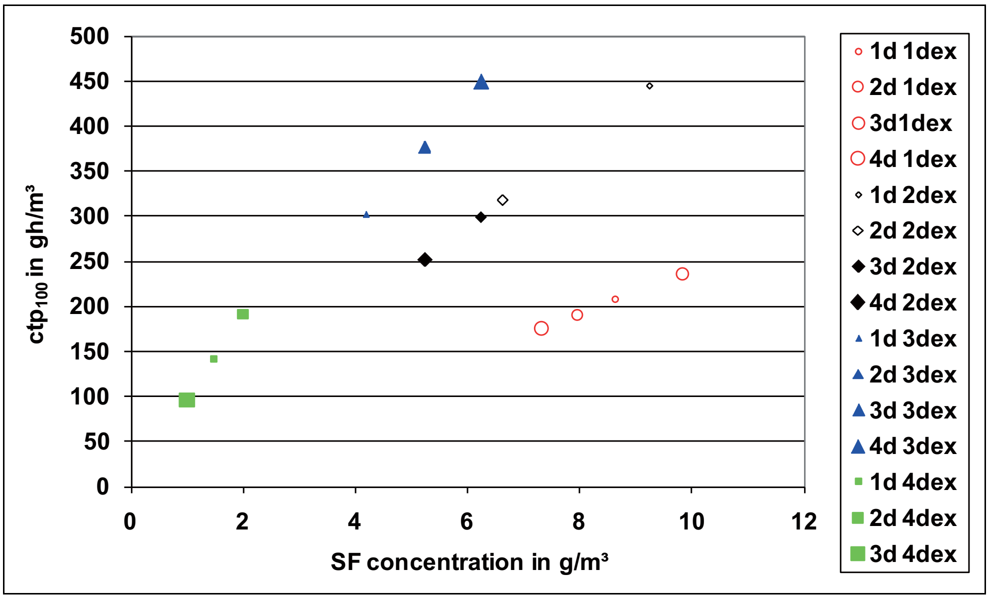

Fig. 7. ctp_{100} as function of the concentration of sulfuryl fluoride during one to four days of fumigation (1dex to 4dex) of eggs of different age (1d to 4d) of *P. interpunctella* at 27°C; at each exposure period, the larger symbols represent the older eggs; bold symbols derived directly from Tab. 1, some data (not bold symbols) determined with nonlinear regression with TableCurve (Equation. 8095).

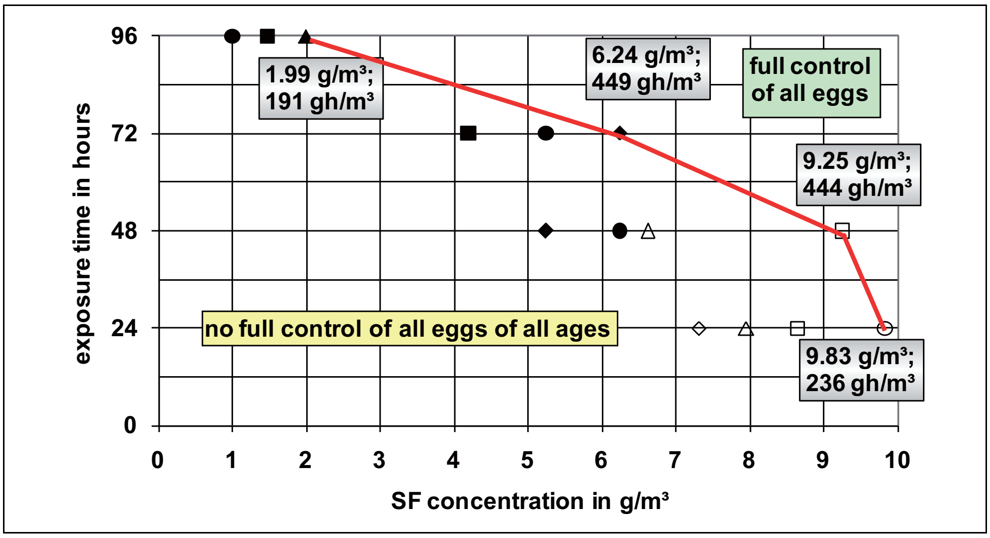

Fig. 8. Exposure time and concentration of sulfuryl fluoride to obtain full control of eggs of *P. interpunctella* at 27°C; bold symbols directly taken from Tab. 1; data for other symbols by non-linear regression; square: 1 day old eggs, triangle: 2 day old eggs, circle: 3 day old eggs; diamond: 4 day old eggs; lines indicate necessary conditions for full control of eggs of all ages: Multiplication of exposure time and the indicated figures results in the ctp_{100} for these conditions.

for eggs of different ages are indicated with different symbols. Data that were taken directly from Tab. 1 are presented in bold symbols; the other calculated data are indicated as hollow symbols. The lines connect those data at each exposure time that required the highest concentration to be fully effective against the respective most tolerant age group of eggs. These boundary concentrations and corresponding values of the ctp_{100} are presented as figures in the frames. Exposure periods and concentrations with coordinates under and left of the lines would not be sufficient for full control of all eggs of all age groups. Appropriate values with coordinates right of and beyond these lines would lead to full control. A fairly simple polynomial regression $y = a + bx + cx^2$ with the regression coefficient $r^2 = 0.9985$ describes this borderline: y = lethal exposure time in hours; x = lethal concentration of SF in g/m^3; a = 8.85, b = 0.07975 and c = −0.0015798611 are the constants of the equation.

According to the presented data, fumigation for one day or four days seems to require the smallest ct products with short exposure of one day needing the higher concentration (9.8 g/m^3) and longer exposure of four days only 1/5 (1.99 g/m^3) of this high concentration. According to the nonlinear regression for ctp_{100} at two days of exposure, this time or three days of exposure would require nearly the same ct product of about 450 gh/m^3. Of course, the concentration for only two days of effective treatment had to be 1.5 times higher than for three days exposure.

The general question was, as to whether the ct product of SF remains a constant number over a range of concentrations and exposure periods or not. As found in this study in the contrary (see Tab. 1 and Tab. 2), the ct product for control changed for eggs of different age at each exposure period. The rank of the magnitude of the mortality varied between exposure periods and concentrations (see Tab. 3; data for the ranking taken from Tab. 1). In the four blocks with six concentrations and four different exposure periods, it can be seen that the ranks varied clearly, with most often rank 1 for egg age one day and rank 4 for egg age four days. The ranking was slightly distorted by some occasions when the same mortality occurred at two different data points. In this case the same figure was given several times. The lower line in Tab. 3 presents the sum of ranks for all ages and exposure times. It indicates the stated trend for most tolerant eggs of one day and least tolerant eggs of four days with some exceptions. In so far, it can be expected that also the ct products are not constant.

The lethal ct product of the most tolerant egg age clearly increased from one day to two or three days of exposure and decreased again strongly from two and three days of exposure towards four days of treatment (Fig. 8). The mortality data for one day exposure and the concentrations tested remained far below 100%. So, only extrapolated data with very high fiducial limits could be used for this type of consideration. Over the whole investigated and extrapolated range of concentrations and exposure periods, the lethal ct product varied and fluctuated between 96 gh/m^3 and 450 gh/m^3, depending on exposure time, concentration and egg age (Tab. 2). It can be concluded that at 27°C a ct product of 450 gh/m^3 should be sufficient for control of all eggs of this moth in fumigations in the laboratory. All these data confirm the conclusion that the ct product to control eggs of *P. interpunctella* with SF at 27°C is not constant but strongly dependent on concentration, exposure period and age of the eggs.

Studies on various insect species showed that the egg is generally the most tolerant stage (TEAP report, 2011). Ciesla and Ducom (2009, 2010) in their studies confirmed the study of Reichmuth et al. (2003), that the dosage for an efficacious SF fumigation can be reduced "intelligently" for instance with increasing the temperature in the treated object or extending the treatment time. Even double treatment with low concentration may be an option to overcome the high tolerance of insect eggs towards treatment with SF. Karakoyun and Emekci (2010) showed in their results that the susceptibility of eggs of *Carpophilus hemipterus* to SF at 25°C varied according to age. One day old eggs of *C. hemipterus* seemed more tolerant than one to two day old eggs at all the tem-

Tab. 3. Rank of magnitude of the average mortality of eggs of different age in integer numbers from 1 (lowest number, highest tolerance) to 4 (highest number, lowest tolerance), original mortalities see Tab. 1

SF conc. in g/m³	Exposure time in days														
	1				2				3				4		
	rank of magnitude of average mortality of eggs of different age in integer numbers from 1 (lowest number) to 4 (highest number)														
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3
1.00	1	1	1	2	1	2	3	4	1	2	4	3	2	1	3
1.48	1	2	3	4	1	3	2	4	1	2	3	4	2	1	2
1.99	1	2	3	4	1	2	3	4	1	2	2	2	1	1	1
4.19	1	2	3	4	1	2	4	3	3	2	2	1	1	1	1
5.24	1	3	2	4	1	2	3	4	2	2	2	1	1	1	1
6.24	3	2	1	4	1	2	3	3	1	1	1	1	1	1	1
Σ	8	12	13	22	6	13	18	22	9	11	14	12	8	6	9

peratures tested. At 25 °C, BALTACI et al. (2009) found that a complete mortality of one to four day old eggs of *E. elutella* was obtained after one day exposure at 21.3 g/m³ [ct = 511.2 gh/m³]. Extrapolated from the results after one day exposure of this study at 27 °C, a ct product of only 235.92 gh/m³ (ctp_{100}) would have been sufficient for this degree of control for eggs of all age groups of *P. interpunctella*. In the presented study, a ct product of 449.3 gh/m³ achieved complete mortality of eggs of all ages of *P. interpunctella* at a concentration of SF of 6.24 g/m³ after three days of exposure at 27 °C.

BELL et al. (1999) investigated the response of eggs of the Mediterranean flour moth *Ephestia kuehniella* to fumigation with SF. They observed strong dependency on the temperature and also on the age of the treated eggs. The necessary ct products for control even at 25 °C were much higher than those determined for eggs of *E. elutella* (BALTACI et al., 2009) or *P. interpunctella* in this study. For effective treatment of eggs of a closely related pest moth, the rice moth *Corcyra cephalonica*, BARAKAT et al. (2009) needed 6.24 g/m³ (5.24 g/m³ and 4.19 g/m³, respectively) at 27 °C within three (four and five) days of exposure [ctp_{100}: 449.28 gh/m³ (3d), 503.04 gh/m³ (4d) and 502.8 gh/m³ (5d)]. Compared to this study with *P. interpunctella*, the ctp_{100} of 449.3 gh/m³ for three days of exposure was equal. Four days of exposure to only 1.48 g/m³ [ctp_{100} = 142.1 gh/m³] and 1.99 g/m³ [ctp_{100} = 191 gh/m³] were sufficient to control one and two day old eggs of *P. interpunctella*. Three day old eggs of *C. cephalonica* were not tested by Barakat et al. at this exposure period. In this study, *P. interpuctella* eggs of that age died already at ctp_{100} = 96 gh/m³.

WALSE (cited in TEAP, 2011) presented results for treatment over one day of eggs of *P. interpunctella* for control with SF at different temperatures. At 26.7 °C, 559 gh/m³ achieved 99% mortality of several thousands of eggs in the test.

Several field and laboratory studies with SF included all developing stages of *P. interpunctella* in their groups of tested insects (DRINKALL et al., 2003 [semolina mill, 22 h 15 min, 1101 gh/m³ – 1501 gh/m³, 28 °C – 31 °C], DUCOM et al., 2003 [three laboratory studies, 24 h, 1125 – 1877 gh/m³, 25 °C], REICHMUTH et al., 2003 [three mill fumigations, 36 h to 48 h, 1860 – 2055 gh/m³, 22 °C – 40 °C]) and observed 100% control in all samples with this species including eggs. Some overdosing for this species can be assumed, since also more tolerant species like the flour beetles *Tribolium* spec. had to be controlled in the tests.

In general, it can be concluded that the relationship between the ct product obtained with SF and the mortality of eggs of *P. interpunctella* is complex. The influencing factors include the levels of concentration, the period of exposure and the age of the eggs. Therefore the ct principle cannot be used as the only guide for practical fumigation work also due to very specific conditions of the fumigations. Always either the concentration or the exposure period must be mentioned together with the respective ct product to obtain a full picture.

Conclusion

One to four day old eggs of *P. interpunctella* could be controlled in the laboratory with sulfuryl fluoride at 27 °C with concentrations of 6.24 g/m³ within three days and 1.99 g/m³ within four days of treatment. Susceptibility of eggs of different age varied strongly.

The results of this study support the presumption that for fumigating eggs of pest insects with SF, short exposures at relatively high concentrations are not as effective as long exposures at relatively low concentrations. At a given temperature and exposure periods between one and four days, the lethal ct product is not constant but varies considerably with the tendency to decline for longer exposures than two days.

In so far, the concept of the constancy of the product for control of eggs of insects as valid for methyl bromide does not hold for SF. This gas is for many applications a promising alternative for methyl bromide but the determination of the appropriate dosage for control is more complex and needs more consideration.

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