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The effect of chitin metabolic effectors on the population increase of stored product mites

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Abstract The study tested the effect of the chitin metabolic effectors, teflubenzuron, diflubenzuron, and calcofluor, and a combination of a chitinase and soybean trypsin inhibitor (STI) on the population growth of eight species of stored product mites under laboratory conditions. The compounds were incorporated into the diets of the mites in concentrations ranging from 0.01 to 50 mg g^{-1}. The final populations of mites were observed after 21 days of growth in controlled conditions. Diflubenzuron and calcofluor suppressed the growth of all the tested species more effectively than the other compounds. The doses required to suppress the mite populations to 50% (rc_{50}) in comparison to the control ranged from 0.29 to 12.68 mg g^{-1} for diflubenzuron and from 1.75 to 37.7 mg g^{-1} for calcofluor, depending on the mite species. When tested at the highest concentration (10 mg g^{-1}), teflubenzuron also suppressed all of the tested mite species in comparison to the control. The addition of chitinase/STI into the diet influenced population growth in several ways. When the highest concentration of chitinase in a cocktail of chitinase and STI (12.5 mg g^{-1} of diet) was compared to the control, populations of *Acarus siro*, *Aleuroglyphus ovatus* and *Aëroglyphus robustus* decreased significantly, whereas populations of *Tyroborus lini* and *Sancassania rodionovi* increased significantly. The sensitivity of species to the tested compounds differed among species. The most tolerant species was *S. rodionovi*, the most sensitive was *A. ovatus*. The results confirmed that calcofluor and diflubenzuron have a toxic effect on stored product mites.

Keywords Chitinase · Teflubenzuron · Diflubenzuron · Calcofluor · Mite · Storage

Introduction

Recent research on the control of stored product mites has focused on searching for an alternative to organophosphorous pesticides (Collins 2006). This trend includes innovative

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applications of less toxic and more selective alternative pesticides (Downing et al. 1993; Palyvos et al. 2006). Chitin metabolic effectors are one type of alternative and are targeted mainly against insects (Ishaaya et al. 2007) but are also effective against mites (Collins 2006). Chitin is a compound found in mite cuticles, and inhibition of chitin synthesis affects molting in mites (Alberti and Coons 1999). Chitin is found in the peritrophic membrane (PM), as demonstrated by the labeling of wheat germ agglutinin with colloidal gold (Sobotnik et al. 2008a) and chitin effectors can act to destroy the PM structure in the mite midgut (Peters 1992). The PM is a permeable barrier between the food and the midgut epithelium that enhances the digestive processes, compartmentalizes the digestive tract and protects the midgut cells from abrasion and pathogen infection (Peters 1992; Terra 2001; Bolognesi et al. 2001, 2008; Hegedus et al. 2009). In *Acarus siro* (L.), the PM is formed in the ventriculus, and the packed food bolus passes through the rest of the gut (intercolon, colon and postcolon; Sobotnik et al. 2008b).

Diflubenzuron suppressed the larval development of *Tyrophagus putrescentiae* (Schrank) (Lipa and Chmielewski 1976) and inhibited the population growth of *T. putrescentiae*, *Lepidoglyphus destructor* (Schrank) and *A. siro* (Collins et al. 2001). A similar suppression was reported for flufenoxuron (Collins 2003a, b). Species differ in their sensitivity to both of these compounds (as reviewed in Collins 2006). The tested species represent the most frequent and abundant mites found in grain stores (Athanassiou et al. 2005, Palyvos et al. 2008), but the species spectra in stored commodities is wider (Hughes 1976) and their sensitivity to diflubenzuron could be variable.

In a previous study, it was found that the chitin metabolic effectors, calcofluor, diflubenzuron, and chitinase, and a combination of chitinase with soybean trypsin inhibitor suppresses the population growth of *A. siro* (Sobotnik et al. 2008a). These results indicated that there are more acaricidal compounds that need to be tested before application.

The aim of this study was to compare the effect of the chitin metabolic effectors (diflubenzuron, calcofluor, teflubenzuron and the cocktail of chitinase/STI) on eight species of stored product mites. To obtain comparable results, we have chosen a routinely used laboratory assay (Hubert et al. 2007, Erban et al. 2009).

Materials and methods

Experimental mites

Eight species of stored product mites of pest importance were chosen for the study: Acaridae: *A. siro*, *Aleuroglyphus ovatus* (Troupeau), *Sancassania rodionovi* (Zachvatkin), *T. putrescentiae*, *Tyroborus lini* (Oudemans); Carpoglyphidae: *Carpoglyphus lactis* (L.); Glycyphagidae: *L. destructor, Aëroglyphus robustus* (Banks). The mites were mass reared on the wheat and fish food-derived diet in controlled conditions (Erban and Hubert 2008).

Chitin metabolic effectors

The inhibitors of chitin synthesis included diflubenzuron, which is an active ingredient of the commercial insecticide Dimilin 25 W (Crompt Crop Protection, Middlebury, USA), and teflubenzuron (Sigma–Aldrich, cat. no. 45756). Calcofluor (Fluorescent Brightener 28, Sigma–Aldrich, cat no. F3543,) is a chitin-binding compound. The chitinase used in this study originated from *Streptomyces griseus* (Sigma–Aldrich, cat no. C6137), it is the most active at pH 6.0. In experimental diet, the chitinase was combined with soybean trypsin

inhibitor (STI; Sigma–Aldrich, cat no. T9128) to protect the chitinase against degradation by endogenous mite proteases in mite gut (Sobotnik et al. 2008a).

The experimental diets were derived from rearing diets with addition of tested compounds. The compounds were diluted in distilled water (chitinase, STI, calcofluor) or ethanol (teflubenzuron and diflubenzuron) to obtain the appropriate concentrations of active ingredients (a.i.). The concentrations of diflubenzuron and teflubenzuron used in the diets were: 10, 5, 1, 0.5, 0.1 and 0.01 mg g^{-1} of diet. For calcofluor, the concentrations were 50, 20, 10, 5, 1 and 0.1 mg g^{-1} of diet. The cocktail of chitinase/STI was composed of 5 mg of STI per g diet and of the following concentrations of chitinase: 12.5, 2.5 and 0.25 mg g^{-1} of the diet. The controls were diets with the addition of solvents that did not contain these compounds. The solutions were properly mixed using a MS1 Minishaker (IKA®, Staufen, Germany) and were then lyophilized in a PowerDry LL3000 (Thermo, Shanghai, China). The lyophilized material was ground into a powder in a pottery grinding mortar, and before the experiment the material was rehydrated for 24 h in a desiccator containing distilled water (Erban et al. 2009).

The 50 mg of each diet was placed in the rearing chambers along with 50 adult mites (unsexed), and they were incubated in the dark in desiccators at 85% r.h. and 25 ± 0.5°C for 21 days. In general, 10 replicates were used for each concentration and species, and the experiment was terminated by the addition of 10 ml of 80% ethanol to the chambers. The mites were directly counted under a STEMI 2000 C dissection microscope (Zeiss, Jena, Germany).

Data analysis

Mite population growth was different among tested species on control diets. To eliminate the effect of the different population growth, the final population numbers were transformed into relative values, by considering the highest obtained density of particular species as 100%. We applied the angular transformation to obtain a normal distribution in data. The data were analyzed by redundancy analysis (RDA) (Jongman et al. 1987), with 'transformed density' as dependent variable and 'compound' and 'transformed concentrations' as independent variables. In addition, regression models were applied for each compound and species separately, in which 'transformed density' was used as a dependent variable and 'concentration of the tested compound' as the independent variable. In preliminary analyses, the highest R^2 was observed when the concentration was transformed as ln(concentration + $1*10^{-7}$). From the regression models, the rc_{50} doses (concentration for 50% suppression of the final mite population in comparison to the control) were estimated along with 95% confidence intervals for the fit. In some cases, the highest concentration of the tested compound was compared to the control using a *t*-test. The analyses were done in XLStat2007 (Addinsoft, New York, NY, USA) and QC-Expert (TriloByte Statistical Software, Pardubice, Czech Republic).

Results

In general, the addition of the chitin metabolic effectors to the diet decreased the population growth of mites in comparison to the control in all species, and the population growth decreased with increasing concentrations of the compounds. As clearly illustrated by redundancy analysis (Fig. 1), the concentration of the compounds (on the first axis) explains 80% of the variability in the data sets. The second axis explained 13% of

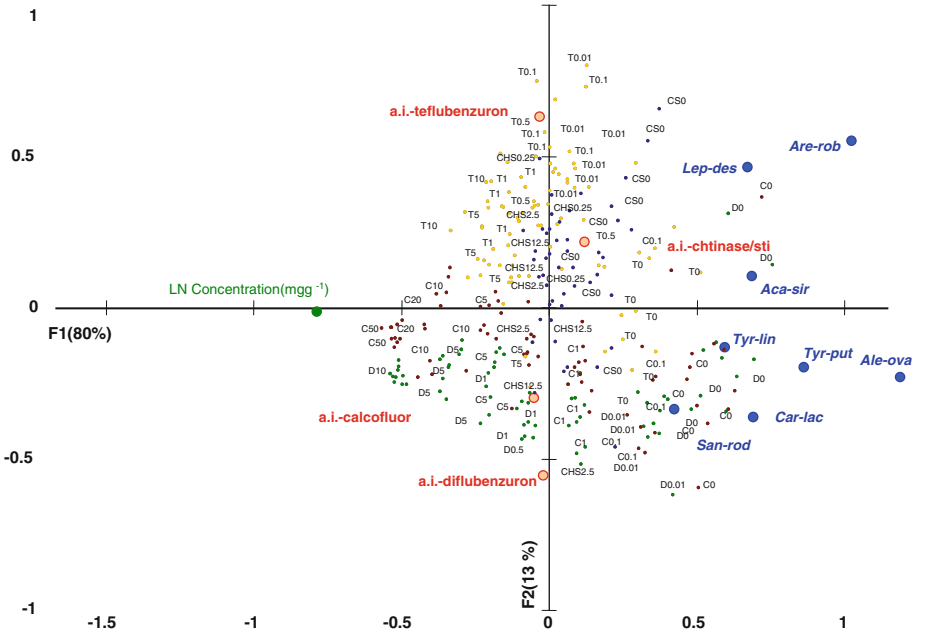

Fig. 1 Comparison of the a.i. and the transformed concentration (ln[concentration + $1*10^{-7}$] (mg g^{-1})) to the final mite density (tN), expressed as relative numbers of mites after angular transformation provided by RDA. The first two axes explain 93% of the variability in the data set, the scores are shown. *Legend*: Aca-sir: *Acarus siro*; Ale-ova: *Aleuroglyphus ovatus*; Aer-rob: *Aëroglyphus robustus*; San-rod: *Sancassania rodionovi*; Car-lac: *Carpoglyphus lactis*; Lep-des: *Lepidoglyphus destructor*; Tyr-lin: *Tyroborus lini*; and Tyr-put: *Tyrophagus putrescentiae*

variability and differentiates between the compounds. Chitinase/STI is located near the zero for both of the axes due to the low effect of the chitinase concentration on the growth of mites. The second axis also separates the transformed density of mites influenced by teflubenzuron from calcofluor- and diflubenzuron-treated diets. The position of the various species on the first axis illustrates differences in their tolerance to the chitin metabolic effectors, which had the most effect on *A. ovatus* and the least on *S. rodionovi*.

In the next step, the effects of the concentrations of the chitin metabolic effectors were compared separately for each compound and species. Diflubenzuron suppressed the population growth of all of the tested species (Fig. 2). The sensitivity to diflubenzuron decreased in the following order: *A. robustus*, *A. ovatus*, *L. destructor*, *A. siro*, *T. putrescentiae*, *T. lini*, *C. lactis* and *S. rodionovi*. The rc_{50} doses ranged from 0.29 to 12.68 mg g^{-1} (Table 1). The effects of calcofluor on the population growth of all mite species were similar to those of diflubenzuron (Fig. 3). The sensitivity to calcofluor decreased in the following order: *A. robustus*, *A. ovatus*, *C. lactis*, *T. lini*, *A. siro*, *L. destructor*, *T. putrescentiae* and *S. rodionovi*. The rc_{50} doses ranged widely from 1.75 to 37.7 mg g^{-1} (Table 2).

Teflubenzuron suppressed the population growth of *A. ovatus, A. robustus* and *T. putrescentiae* (Fig. 4) as indicated by the rc_{50} doses (Table 3). The rest of the species showed no realistic fits of the rc_{50} values. However, when the population growth of mites at the highest concentration (i.e., 10 mg g^{-1}) of teflubenzuron and control was compared using a *t*-test, the suppressive effect of teflubenzuron was significant, i.e., *A. siro*:

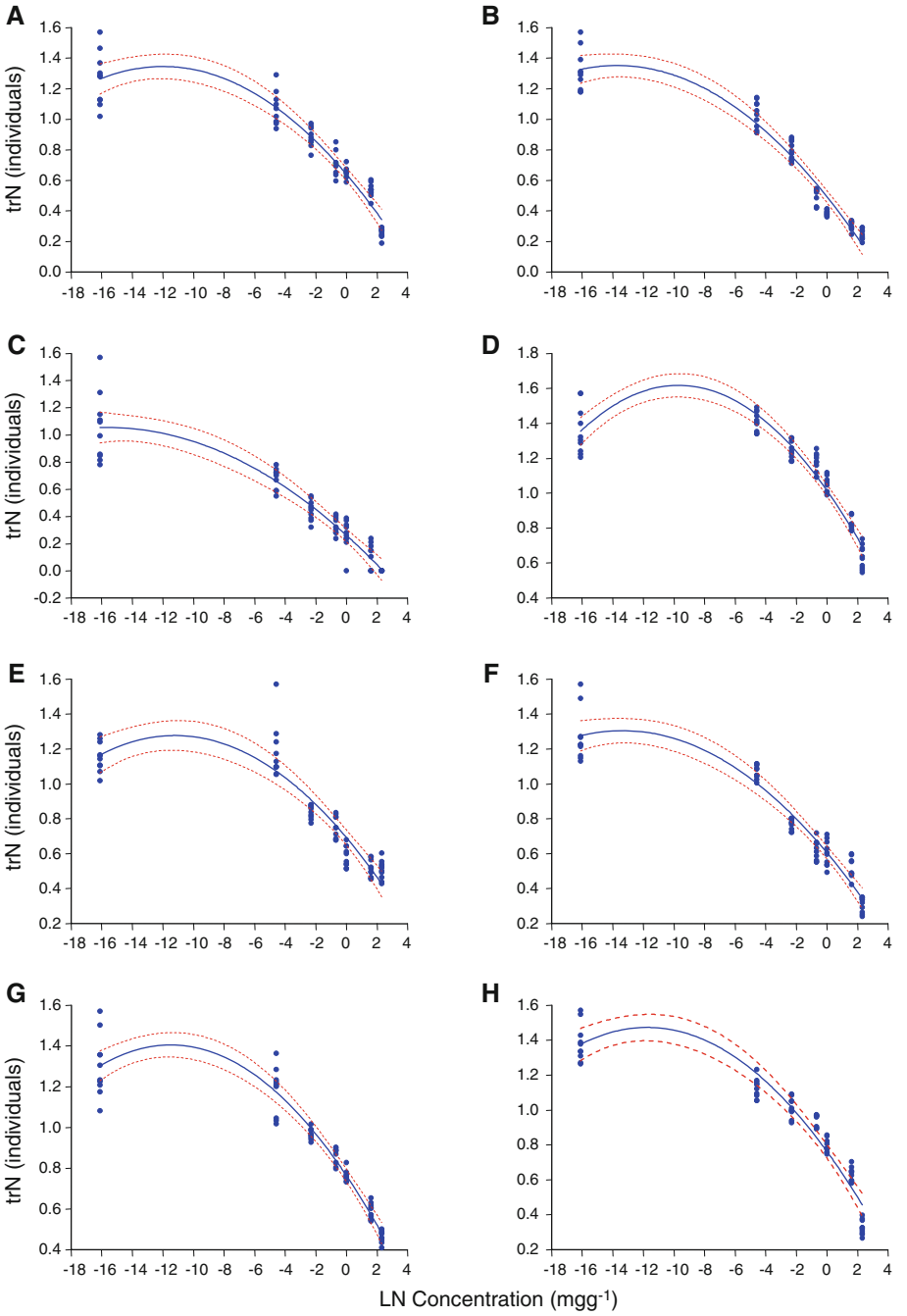

Fig. 2 Regression model describing the effect of diflubenzuron concentration on final mite density; **a** *Acarus siro*; **b** *Aleuroglyphus ovatus*; **c** *Aëroglyphus robustus*; **d** *Sancassania rodionovi*; **e** *Carpoglyphus lactis*; **f** *Lepidoglyphus destructor*; **g** *Tyroborus lini*; and **h** *Tyrophagus putrescentiae*. The points represent actual data, *middle line* is fitted, and *dashed lines* are 95% confidence intervals

Table 1 Parameters of the regression model describing the effect of diflubenzuron concentration on final mite density (trN = a*ln[conc]^2 + b*ln[conc] + c)

Species	Regression model				
	R^2	a	b	c	Fit rc_{50}
Acarus siro	0.91	−0.0049	−0.12	0.64	1.21
		(−0.0057/−0.0041)	(−0.13/−0.10)	(0.61/0.67)	(0.82/1.67)
Aleuroglyphus ovatus	0.94	−0.0046	−0.13	0.49	0.29
		(−0.0057/−0.0041)	(−0.14/−0.11)	(0.47/0.52)	(0.18/0.43)
Aëroglyphus robustus	0.89	−0.0033	−0.10	0.26	0.12
		(−0.0053/−0.0038)	(−0.12/−0.09)	(0.23/0.29)	(0.05/0.22)
Sancassania rodionovi	0.91	−0.0064	−0.12	1.01	12.68
		(−0.0071/−0.0057)	(−0.13/−0.11)	(0.99/1.04)	(8.94/19.11)
Carpoglyphus lactis	0.86	−0.0047	−0.10	0.69	4.06
		(−0.0055/−0.0038)	(−0.12/−0.09)	(0.66/0.72)	(2.53/6.69)
Lepidoglyphus destructor	0.92	−0.0039	−0.10	0.61	0.96
		(−0.0047/−0.0032)	(−0.12/−0.09)	(0.58/0.63)	(0.66/1.43)
Tyroborus lini	0.93	−0.0049	−0.11	0.76	3.06
		(−0.0055/−0.0042)	(−0.12/−0.10)	(0.74/0.78)	(2.14/4.35)
Tyrophagus putrescentiae	0.92	−0.0051	−0.12	0.76	1.95
		(−0.0059/−0.0044)	(−0.13/−0.11)	(0.74/0.79)	(1.43/2.80)

Final mite density (trN) is expressed in relative numbers of mites after angular transformation and after the a.i. concentration (ln[conc]) was transformed by ln[concentration + $1*10^{-7}$] (mg g^{-1}). Parameters are expressed with 95% confidence intervals in parentheses. The rc_{50} value shown is the concentration required for 50% suppression of the population relative to the control, and the 95% confidence intervals are given in parentheses

$t_{1,22} = 10.6$, $P < 0.0001$; *S. rodionovi*: $t_{1,22} = 3.6$, $P = 0.001$; *C. lactis:* $t_{1,22} = 9.8$, $P < 0.0001$; *L. destructor*: $t_{1,22} = 6.7$, $P < 0.0001$; and *T. lini*: $t_{1,22} = 7.4$, $P < 0.0001$.

The effect of the chitinase provided in cocktails of chitinase/STI on the population growth of mites was variable and depended on the chitinase concentrations and species tested. The mite density at the highest concentration (i.e., 12.5 mg g^{-1} of chitinase and 5 mg g^{-1} of STI) was compared to the mite density of the control using a *t*-test (Table 4). The chitinase/STI inhibited *A. siro*, *A. ovatus* and *A. robustus*, and had no effect on *C. lactis*, *L. destructor* and *T. putrescentiae*. The final population densities of *S. rodionovi* and *T. lini* were higher for the mites given the chitinase/STI cocktail than for the control (Table 4).

Discussion

The inhibitors of chitin synthesis, including teflubenzuron and diflubenzuron, showed a toxic effect and are therefore considered to be suitable for the control of stored product beetles (Elek and Longstaff 1990; Clarke and Jewess 1990; Mondal and Parween 2000). The results confirmed the previously published finding that some chitin metabolic effectors have a suppressive effect on stored product mites (Lipa and Chmielewski 1976; Collins et al. 2001, 2003a, b; Sobotnik et al. 2008a). In spite of that, there are studies reporting failures of control of astigmatic mites by chitin metabolic effectors. Downing et al. (1990)

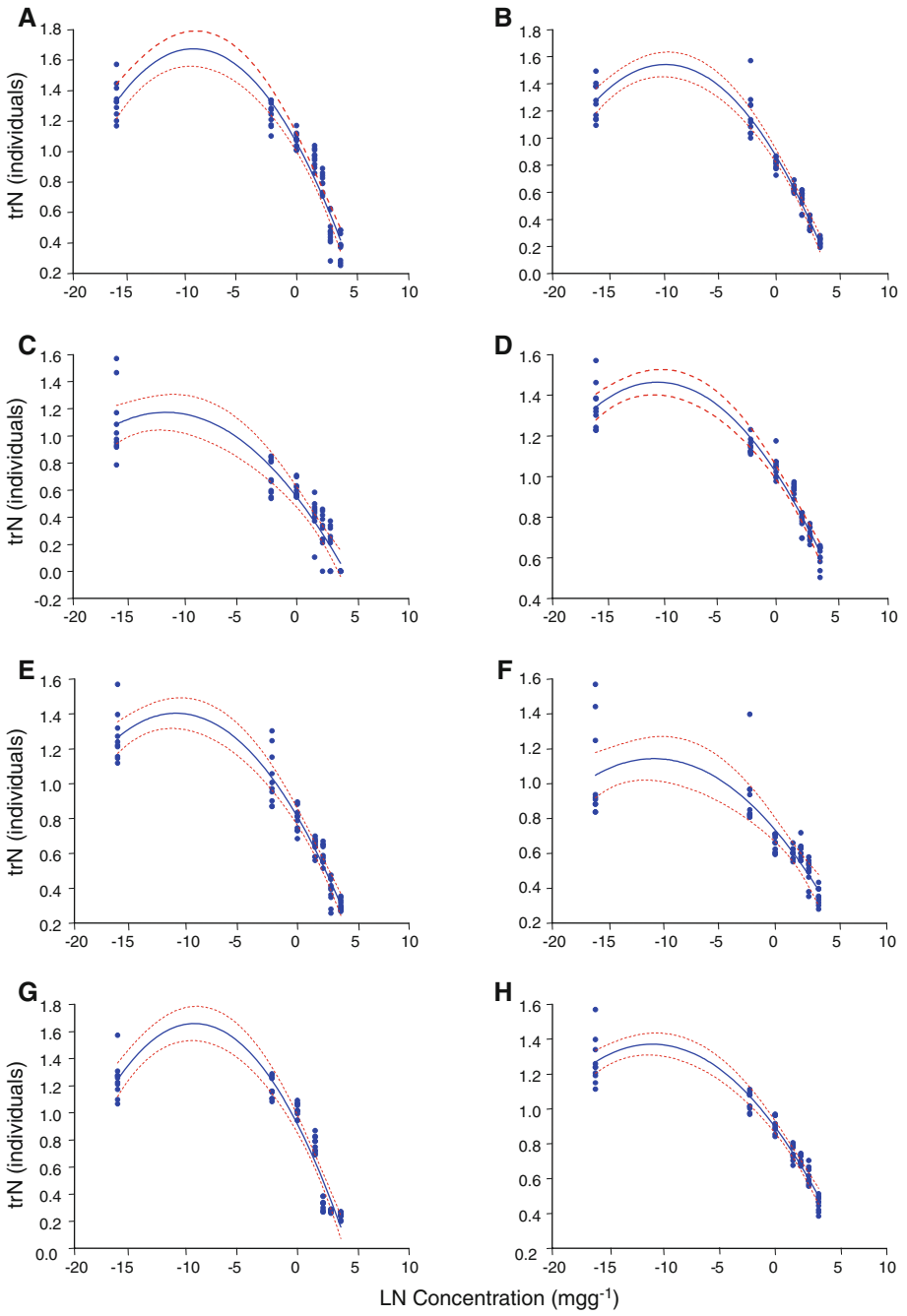

Fig. 3 Regression model describing the effect of calcofluor concentration on final mite density; **a** *Acarus siro*; **b** *Aleuroglyphus ovatus*; **c** *Aëroglyphus robustus*; **d** *Sancassania rodionovi*; **e** *Carpoglyphus lactis*; **f** *Lepidoglyphus destructor*; **g** *Tyroborus lini*; and **h** *Tyrophagus putrescentiae*. The points represent actual data, *middle line* is fitted, and *dashed lines* are 95% confidence intervals

Table 2 Parameters of the regression model describing the effect of calcofluor concentration on final mite density (trN = a*ln[conc]^2 + b* ln[conc] + c)

Species	Regression model				
	R^2	a	b	c	Fit rc_{50}
Acarus siro	0.89	−0.0073	−0.134	1.06	15.03
		(−0.0082/−0.0063)	(−0.147/−0.121)	(1.02/1.09)	(11.13/21.12)
Aleuroglyphus ovatus	0.94	−0.0069	−0.137	0.86	5.81
		(−0.0077/−0.0062)	(−0.147/−0.127)	(0.83/0.89)	(4.62/7.39)
Aëroglyphus robustus	0.84	−0.0046	−0.107	0.55	1.75
		(−0.0057/−0.0034)	(−0.124/0.091)	(0.49/0.60)	(0.09/2.89)
Sancassania rodionovi	0.93	−0.0040	−0.084	1.02	37.71
		(−0.0045/−0.0034)	(−0.124/0.091)	(1.00/1.04)	(27.11/56.26)
Carpoglyphus lactis	0.92	−0.0050	−0.109	0.81	6.05
		(−0.0058/−0.0043)	(−0.120/−0.099)	(0.78/0.84)	(4.48/8.33)
Lepidoglyphus destructor	0.73	−0.0034	−0.075	0.73	16.12
		(−0.0046/−0.0024)	(−0.092/−0.077)	(0.69/0.78)	(8.85/38.09)
Tyroborus lini	0.91	−0.0087	−0.161	0.92	6.36
		(−0.0098/−0.0077)	(−0.175/−0.146)	(0.88/0.96)	(4.95/8.25)
Tyrophagus putrescentiae	0.94	−0.0039	−0.086	0.90	20.70
		(−0.0045/−0.0034)	(−0.094/0.078)	(0.87/0.92)	(15.64/29.67)

The final mite density (trN) is expressed as a relative number of mites after angular transformation and after the a.i. concentration (ln[conc]) was transformed by ln[concentration + 1*10^{-7}] (mg g^{-1}). Parameters are expressed with 95% confidence intervals in parentheses. The rc_{50} value shown is the concentration required for 50% suppression relative to the control, and the 95% confidence intervals are given in parentheses

did not find any reduction in the number of mites in populations of *Dermatophagoides farinae* Hughes treated with diflubenzuron and triflumuron at a dose of 50,000 ppm. Sánchez-Ramos and Castañera (2003) recorded a low mortality (16%) of the immature stages of *T. putrescentiae* treated with hexaflumuron at a dose of 1,000 ppm. Both doses are in the range of those used in the present study.

Although it is well established that these compounds affect chitin synthesis, their mode of action is still unclear (Merzendorfer and Zimoch 2003). Diflubenzuron has been reported to suppress the population growth of stored product mites (as reviewed in Collins 2006). This result was also observed in our study; diflubenzuron suppressed the population growth of all tested species, and the rc_{50} values ranged from 0.12 to 3.06 mg g^{-1} of diet. For *A. siro* we found similar rc_{50} doses as in the previous study (Sobotnik et al. 2008a), i.e., the means and 95% confidence limits were 1.21 (0.82–1.67) and 0.71 (0.64–0.83) mg g^{-1} of diet.

A previous study showed differences in the responses of species: *A. siro* was sensitive to diflubenzuron, and *T. putrescentiae* and *L. destructor* were more tolerant species (Collins 2006). However, we found no differences in sensitivity of *A. siro*, *L. destructor* and *T. putrescentiae* as indicated by the overlapping 95% confidence intervals in Table 1. These results were obtained based on different methods, i.e., the timing of compound addition to the grain was different, and whether or not it was homogenously mixed into the rearing diet.

Teflubenzuron is used to control spider mites, even when resistance has been developed (Gorman et al. 2002). However, the toxicity of this compound on stored product mites and

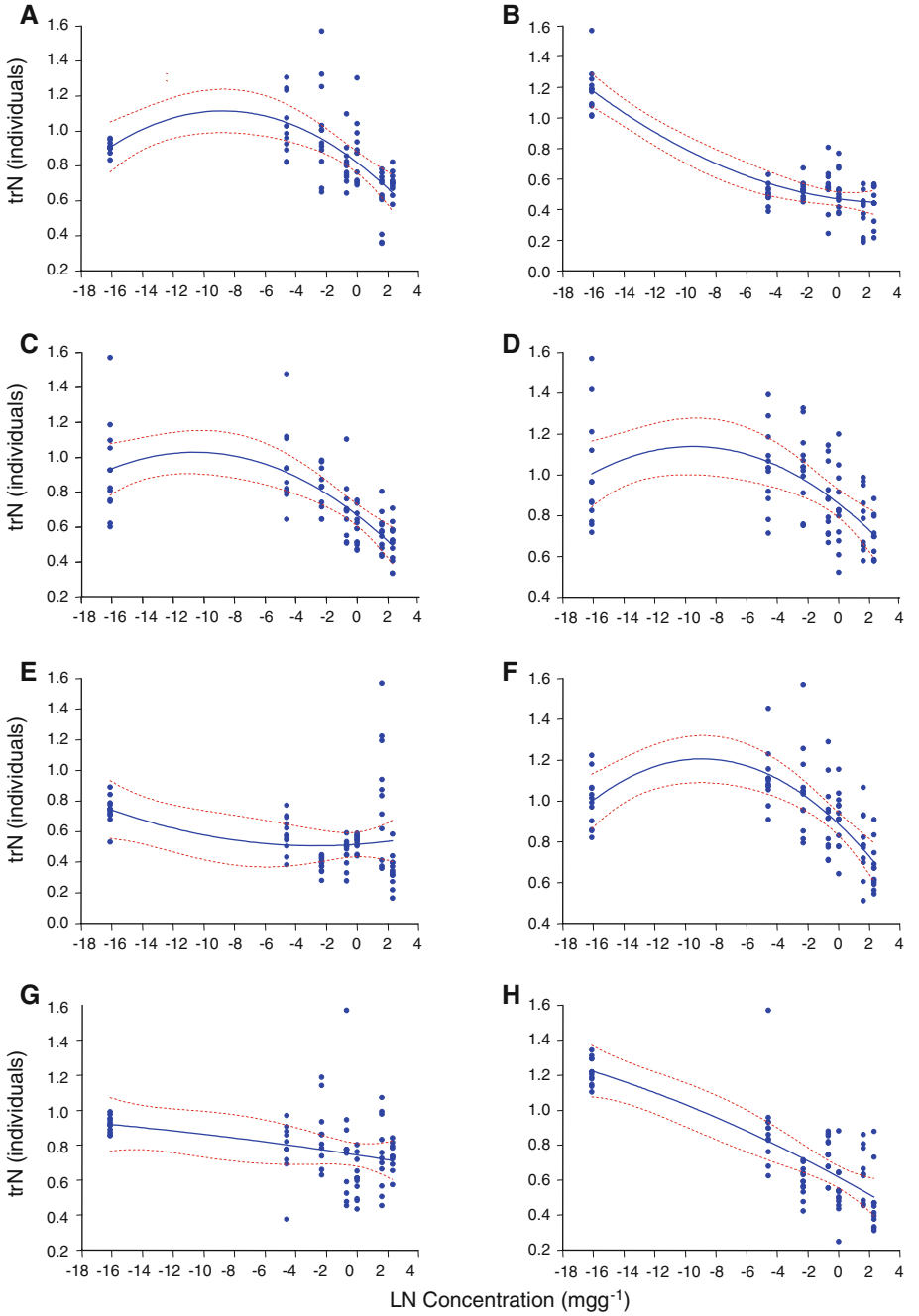

Fig. 4 Regression model describing the effect of teflubenzuron concentration on final mite density; **a** *Acarus siro*; **b** *Aleuroglyphus ovatus*; **c** *Aëroglyphus robustus*; **d** *Sancassania rodionovi*; **e** *Carpoglyphus lactis*; **f** *Lepidoglyphus destructor*; **g** *Tyroborus lini*; and **h** *Tyrophagus putrescentiae*. The points represent actual data, *middle line* is fitted, and *dashed lines* are 95% confidence intervals

Table 3 Parameters of the regression model describing the effect of teflubenzuron concentration on final mite density (trN = a*ln[conc]^2 + b* ln[conc] + c)

Species	Regression model				
	R^2	a	b	c	Fit rc_{50}
Acarus siro	0.40	−0.0038	−0.067	0.82	78.26
		(−0.0051/−0.0026)	(−0.086/−0.048)	(0.78/0.86)	(20.91/NR)
Aleuroglyphus ovatus	0.80	0.0019	−0.014	0.47	0.0046
		(0.0009/0.0028)	(−0.028/0.0006)	(0.44/0.50)	(0.0004/0.04)
Aëroglyphus robustus	0.48	−0.0032	−0.068	0.67	16.12
		(−0.0045/−0.0020)	(−0.087/−0.049)	(0.62/0.71)	(5.75/149.9)
Sancassania rodionovi	0.31	−0.0031	−0.059	0.86	149.90
		(−0.0045/−0.0017)	(−0.08/−0.038)	(0.81/0.90)	(24.53/NR)
Carpoglyphus lactis	0.13	0.0013	0.007	0.52	NR
		(−0.0003/0.0029)	(−0.018/0.032)	(0.46/0.57)	
Lepidoglyphus destructor	0.47	−0.0040	−0.072	0.89	NR
		(−0.0052/−0.0029)	(−0.089/−0.054)	(0.84/0.92)	
Tyroborus lini	0.12	−0.0002	−0.013	0.74	NR
		(−0.0015/0.0012)	(−0.034/0.007)	(0.70/0.79)	
Tyrophagus putrescentiae	0.66	−0.0006	−0.048	0.62	1.62
		(−0.0019/0.00064)	(−0.067/−0.028)	(0.58/0.66)	(0.47/16.95)

The final mite density (trN) is expressed as a relative number of mites after angular transformation and after the a.i. concentration (ln[conc]) was transformed by ln[concentration + 1*10^{-7}] (mg g^{-1}). Parameters are expressed in 95% confidence intervals in parentheses. The rc_{50} value is the concentration required for 50% suppression relative to the control, and the 95% confidence intervals are given in parentheses. NR indicates unrealistic fitted concentrations of a.i.

Table 4 Comparison of final mite densities with the control and chitinase/STI diet

Species	Concetration of chitinase mg g^{-1}				*T*-test (0 and 12.5)	
	0 (control)	0.25	2.5	12.5	*t* (observ.)	*P*
Acarus siro	0.91 ± 0.10	1.11 ± 0.20	0.99 ± 0.10	0.79 ± 0.15	2.205	0.038
Aleuroglyphus ovatus	1.18 ± 0.15	0.37 ± 0.03	0.38 ± 0.03	0.46 ± 0.09	5.907	<0.001
Aëroglyphus robustus	0.95 ± 0.27	0.66 ± 0.16	0.59 ± 0.08	0.44 ± 0.08	5.907	<0.001
Sancassania rodionovi	0.70 ± 0.16	0.83 ± 0.09	1.12 ± 0.18	0.87 ± 0.15	−2.610	0.016
Carpoglyphus lactis	0.80 ± 0.15	0.85 ± 0.26	0.83 ± 0.26	0.71 ± 0.21	2.074	0.148
Lepidoglyphus destructor	1.01 ± 0.13	1.05 ± 0.19	1.02 ± 0.20	1.02 ± 0.13	2.074	0.765
Tyroborus lini	0.64 ± 0.09	0.68 ± 0.10	0.73 ± 0.06	0.99 ± 0.23	−5.089	<0.001
Tyrophagus putrescentiae	0.98 ± 0.12	1.00 ± 0.14	0.98 ± 0.12	1.05 ± 0.22	−1.056	0.302

Final mite density was expressed as a relative number of mites after angular transformation. Means ± standard deviations are shown. *T*-test compared the control (chitinase/STI at 0 mg g^{-1}) and chitinase/STI at 12.5/5 mg g^{-1}. DF error = 22 for all species

related species has rarely been evaluated, with the exception of *Tyrophagus similis* Volgin (Kasuga and Amano 2003). In this study, we found a suppressive effect of teflubenzuron at a concentration of 10 mg g^{-1}. Non-realistic fitted rc_{50} values were observed and indicated the low suppressive effect of teflubenzuron on the tested mites.

Wang and Granados (2000) experimentally demonstrated that calcofluor, a chitin binding agent, completely inhibits PM formation in *Trichoplusia ni* larva. The lack of PM inhibits growth due to increases in metabolite costs associated with the conversion of food into body mass (Bolognesi et al. 2008). Supporting previous results from studies on *A. siro* (Sobotnik et al. 2008a), we confirmed the suppressive effect of calcofluor on seven other species of stored product mites. The rc_{50} ranged from 1.75 to 37.7 mg g^{-1}. For *A. siro* we found similar rc_{50} doses as in previous study (Sobotnik et al. 2008a), i.e., the means and 95% confidence limits were 15.03 (11.13–21.12) and 8.85 (4.34–20.71) mg g^{-1} of diet. The results showed that although calcofluor is less effective than diflubenzuron, it is still effective against stored product mites.

Chitinase hydrolyzes chitin fibrils in the PM. The application of exogenous chitinase completely blocked the formation of PM in the study species (Wiwat et al. 2000; Filho et al. 2002). The combination of chitinase and soybean trypsin inhibitor (STI) is suggested for the protection of chitinase by the inhibition of some endogenous mite proteases in the ventriculus. In this study we found a small effect of chitinase concentration on the density of mites and mite species had different sensitivities to cocktails of chitinase/STI. In some species, the high concentration of both proteins stimulated the growth of mites (Table 4). The decrease in the suppressive effect that was observed as the chitinase concentration in the cocktail increased could be due to the limitation of available chitin in the PM. In addition, STI is not able to inhibit all of the digestive proteases of mites (Ortego et al. 2000; Sánchez-Ramos et al. 2004). When the inhibition effect of STI is overcome by the mite proteases, both proteins (chitinase and STI) could be used as a source of nutrients and could accelerate the population growth of mites. Similar growth accelerations are observed when the diet is enriched by the protein additives albumin or casein (unpublished observation).

The main finding in this study was that the tested compounds suppressed eight stored product mite species that are important pests infesting stored grain, oil seeds, dried milk and dried fruits, cheese, ham, flour, herring meal, etc. (Hughes 1976). For practical applications, diflubenzuron and calcofluor showed the most potential for controlling stored product mites because they are toxic for all tested species and because the rc_{50} doses had realistic values. However, neither of these compounds eliminated all individuals in a short time period. The combination of the diflubenzuron and calcofluor with other compounds with acaricidal activity or biological means of control could increase the suppressive effect. For example, the additive effect was observed in the laboratory, when bean flour added into grain and the predator *Cheyletus malaccensis* Oudemans suppressed *T. putrescentiae* (Hubert and Pekar 2009). However, no additive effect in suppression of *A. siro* was found, when a cocktail of calcofluor and diflubenzuron was tested (Sobotnik et al. 2008a).

The laboratory assay used in this study was carried out under optimal conditions for the growth of mites, including optimal values for humidity, temperature and food availability. The real situation could be different. For example, during the ripening of some types of stored ham, temperature and relative humidity may occasionally exceed 27°C and 90%, respectively (García 2004), which is optimal for the population increase of mites like *T. putrescentiae* (Sánchez-Ramos and Castañera 2001, 2005). In stored grain, temperature and humidity are usually suboptimal for mite development (Athanassiou et al. 2005). Models describing the effect of temperature on mite growth rearing diets and grain show lower increase of mites in grain (Pekar and Hubert 2008). It is expected that under the conditions of stored grain, the toxicity of calcofluor and diflubenzuron would be greater. On the other hand, the compounds and rearing diets in this study were properly mixed before the experiments. This homogeneous distribution of the chitin effectors within the

experimental diet may be hard to achieve when applied in the grain. An additional study of application of the compounds into grain is therefore recommended.

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