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Initial and delayed mortality of *Rhyzopertha dominica* F. (Col: Bostrychidae) and *Tribolium castaneum* Herbst (Col: Tenebrionidae) exposed to Silico-Sec[®] formulation of diatomaceous earth

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Abstract

One of the most promising alternatives to control stored product insects is diatomaceous earth which is obtained from deposits of diatomite. Diatomaceous earth particles absorb wax from insect's cuticle resulting in water loss and death through desiccation. Effects of temperature and exposure interval on the toxicity of Silico-Sec[®], a commercial DE product produced in Germany, were determined against 7-14 day old adults of *Rhyzopertha dominica* (F.), lesser grain borer, and *Tribolium castaneum* (Herbst), red flour beetle. Experiments were carried out at 22, 27, 32 °C and 65 ± 5 % r.h. in the dark. Adults were exposed for 8, 16, 24, 48 and 72 hours to Silico-Sec[®] at the rate of 1 mg/cm² (10 g/m²) on filter paper inside plastic Petri dishes separately and clean Petri dishes served as a control with four replicates. After exposure intervals, initial mortality was counted and live individuals were held for a week in clean Petri dishes under the same conditions to determine delayed mortality. The initial mortality results of both species were generally lower at 22 °C but significantly increased at higher temperature of 27, 32 °C and at longer exposure intervals. Mortality of both storage beetles was greater after they were held for 1 week in clean Petri dishes than the initial mortality. The LT₉₉ values of initial mortality for *R. dominica*

were 147, 63 and 33 hours at 22, 27, 32 °C, respectively and in the case of *T. castaneum* LT₉₉ values were 249, 75 and 38 hours at 22, 27 and 32 °C, respectively. The LT₉₉ values indicated *R. dominica* was more sensitive to Silico-Sec[®] than *T. castaneum* exposed to the same conditions. A field test using a similar design is essential to confirm the laboratory findings.

Key words: Initial mortality, Delayed mortality, Diatomaceous earth, *Rhyzopertha dominica*, *Tribolium castaneum*

Introduction

One of the most promising alternatives to chemical insecticides and fumigants is the use of diatomaceous earths, which are made up of fossilized diatoms (Korunic, 1998). The main advantages of DE are its low toxicity to mammals and its stability; DE is extremely stable and doesn't produce toxic chemical residues or reacts with other substances in the environment (Fields et al., 2002). Diatomaceous earth and synthetic silica dusts have been reported to be effective alternatives to chemical pesticides for control of insect pests in stored products, e.g. when applied to grain or to the walls of storage facilities (McLaughlin, 1994; Golob, 1997). Dust

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applications are confined to grain-handling machinery, ducts and vertical silos, and slurries are applied to horizontal grain stores to avoid exposure to very dusty atmospheres which would be created if the dry dust was applied (Golob., 1997). In addition, slurry applications of desiccant dusts reflect the preference for clean application methods with minimal operator exposure to dusts (McLaughlin, 1994). It also allows a much closer control of application rate to treated surfaces, as the deposit rate is easily identifiable. Exceptionally, only one or two specific DE products are effective when applied in a slurry form however most DE products can be applied as a dust for surface treatment on walls and floors. Few studies have investigated the effectiveness of diatomaceous earth formulations applied as a surface treatment to control stored product insects. White and Loschiavo (1989) conducted studies in which the effects of exposure intervals, type and quantity of food after exposure to Silica aerogel was determined on the survival of the merchant grain beetle and the confused flour beetle, the mortality of beetles increased with exposure interval but the efficacy of Silica aerogel was reduced when beetles were provided with food after they were exposed. McLaughlin (1994) exposed *Sitophilus granaries* (L.) and *Sitophilus oryzae* (L.) to several DE samples at the rate of 2 g/m² in aluminum pans for 30 and 26 h, respectively at 25 °C, 56 % r.h. and recorded the initial mortality and mortality after 1 and 6 d after the beetles were removed and allowed to recover on clean wheat. Mortality was dependent upon the specific product and exposure periods. Arthur (2000a) carried out a similar experiment to determine the effects of temperature and relative humidity on the toxicity of Protect-It placed on filter paper inside plastic Petri dishes to red flour beetles and confused flour beetles, he has shown that mortality of both species increased as temperature and exposure interval increased, and within each temperature decreased as humidity increased. Arthur (2000b) in the other research investigated the impact of food source on survival of red flour beetles and confused flour beetles. He reported accumulated food material reduce residual efficacy of the diatomaceous earth treatment.

The aim of current study was to determine (1) the effect of length of exposure of *Rhyzopertha dominica* F. and *Tribolium castaneum* Herbst to Silico-Sec[®], followed by a period without food ; (2) to determine if beetles would subsequently die even if they were still alive after they were exposed (3) in addition, influence of temperature on beetles mortality.

Materials and methods

Insects

Adults of *R. dominica* were reared on whole wheat, while *T. castaneum* adults were reared on wheat flour plus 5 % brewers yeast (by weight) at 28 °C and 65 ± 5 % r.h. in the dark. All adults were used in experiments were 7-14 day old, of mixed sex.

DE formulation

Silico-Sec[®] is a freshwater formulation of diatomaceous earth obtained from Biofa GmbH and is composed of 92% SiO₂, 3 % Al₂O₃, 1 % Fe₂O₃ and 1 % Na₂O. The median particle size is between 8-12 µm. DE was stored in the laboratory at ambient conditions, until the beginning of the experiment (approximately one month).

Bioassay

Laboratory experiments were conducted to assess the effects of temperature and exposure time on the toxicity of Silico-Sec[®] against *R. dominica* and *T. castaneum*. The method of our experiment was similar to that of Arthur (2000a). Plastic Petri dishes with an internal diameter of 8.8 cm and area of 60.7 cm² were used in the test. Adults were exposed for 8, 16, 24, 48 and 72 h to Silico-Sec[®] at the rate of 1 mg/cm², therefore 60.7 mg of DE was placed on filter paper inside plastic Petri dishes. The dishes were shaken to distribute the DE to filter paper, static electricity causes the DE to stick to the filter paper, and then Petri dishes were left undisturbed after shaking for at least 1 min.

Subsequently, fifteen adults were placed in each dish and the dishes were covered with lids. Petri dishes were placed in incubators set at 22, 27 and 32 °C and 65 ± 5 % r.h. There were four replicates plus an untreated dish that served as a control for each exposure interval (Twenty four Petri dish for each temperature and insect). After exposure, initial mortality was counted and live individuals were held for a week in clean Petri dishes at the same conditions to determine delayed mortality.

Data analysis

Control mortality was rare for both insects and no corrections were necessary. The data were analyzed using Analysis of Variance and the T_ test was used to determine significant differences between mortalities of *R. dominica* versus *T. castaneum* (SAS, 2000). To equalize variances, mortality percentage was transformed using the square root

of the arcsin (Snegecor and Cochran, 1989). The time required to kill 99 % of the exposed insects (LT_{99}) was estimated using probit analysis (SPSS, 1999). Means were separated by using the Duncan's multiple range test, at $P=0.05$.

Results

The main effects of initial and delayed, recorded after 1 week, mortality were all significant. In addition, all associated interactions, were also significant (Table 1, 2). Mortality of both species after initial exposure increased as temperature and exposure interval increased and was highest for 24, 48 and 72 h at 32 °C (Figure 1). All beetles were dead 1 week after being exposed to DE at 32 °C and after 24 h of exposure at any temperature (Figure 2).

Table 1. ANOVA parameters for main effects and interactions for initial mortality of *R. dominica* and *T. castaneum* exposed to Silico-Sec® at the rate of 1 mg/cm².

Source	df	F	P
Insect	1	231.15	< 0.001
Temperature	2	148.70	< 0.001
Exposure interval	4	333.04	< 0.001
Temperature × Exposure interval	8	18.04	< 0.001
Insect × Exposure interval	4	14.58	< 0.001
Temperature × Insect	2	5.08	< 0.01
Temperature×Exposure interval×Insect	8	11.99	< 0.001

Table 2. ANOVA parameters for main effects and interactions for delayed mortality of *R. dominica* and *T. castaneum* exposed to Silico-Sec® at the rate of 1 mg/cm².

Source	df	F	P
Insect	1	58.47	< 0.001
Temperature	2	75.00	< 0.001
Exposure interval	4	116.14	< 0.001
Temperature × Exposure interval	8	32.72	< 0.001
Insect × Exposure interval	4	28.49	< 0.001
Temperature × Insect	2	16.29	< 0.001
Temperature×Exposure interval×Insect	8	8.32	< 0.001

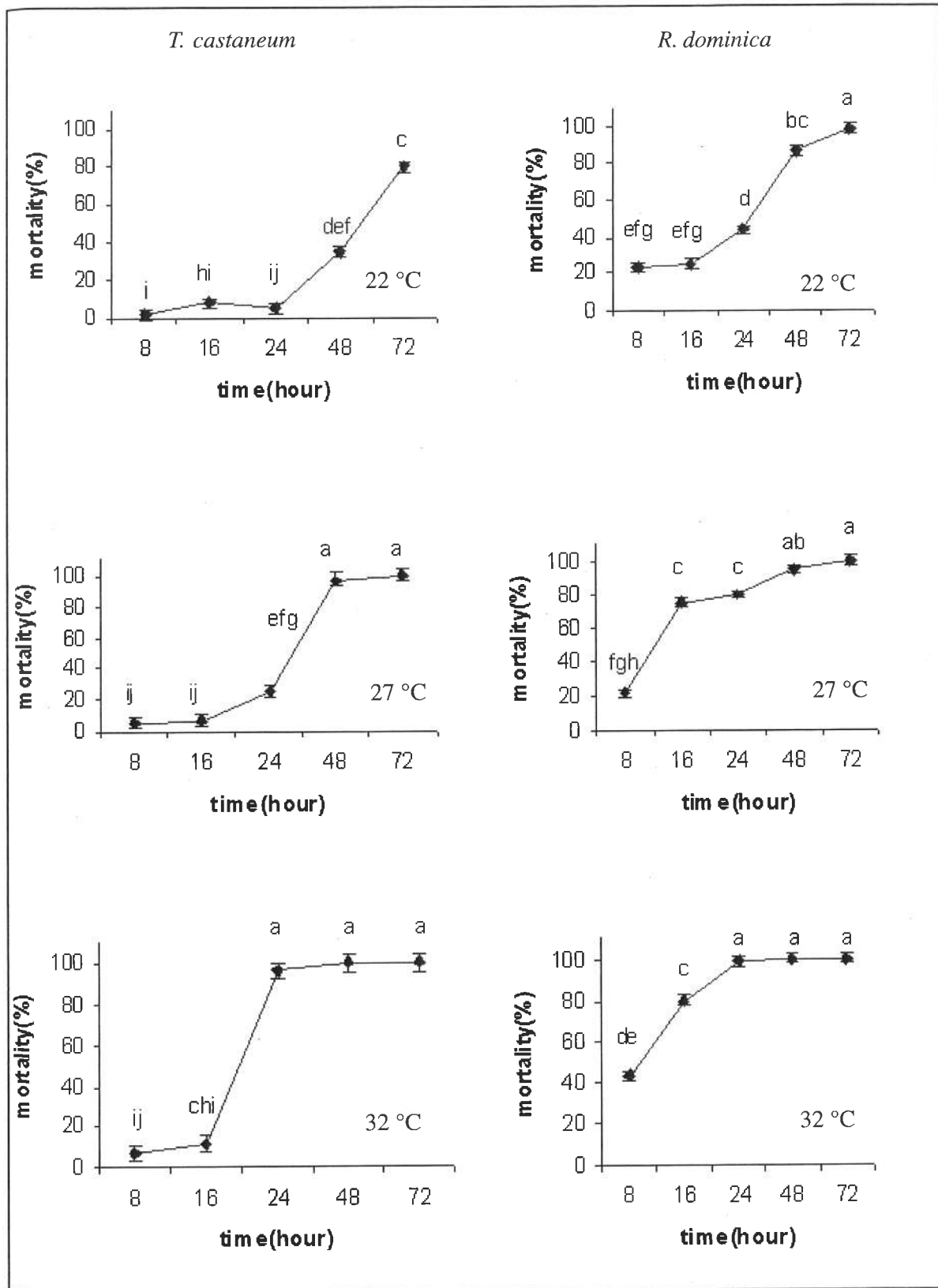


Figure 1. Initial mortality (%) \pm SEM of *R. dominica* at the right and *T. castaneum* on the left exposed to 1 mg/cm² Silico-Sec[®] at 22, 27 and 32 °C. Means followed by the same letter are not significantly different; Duncan's multiple range test at $P = 0.05$.

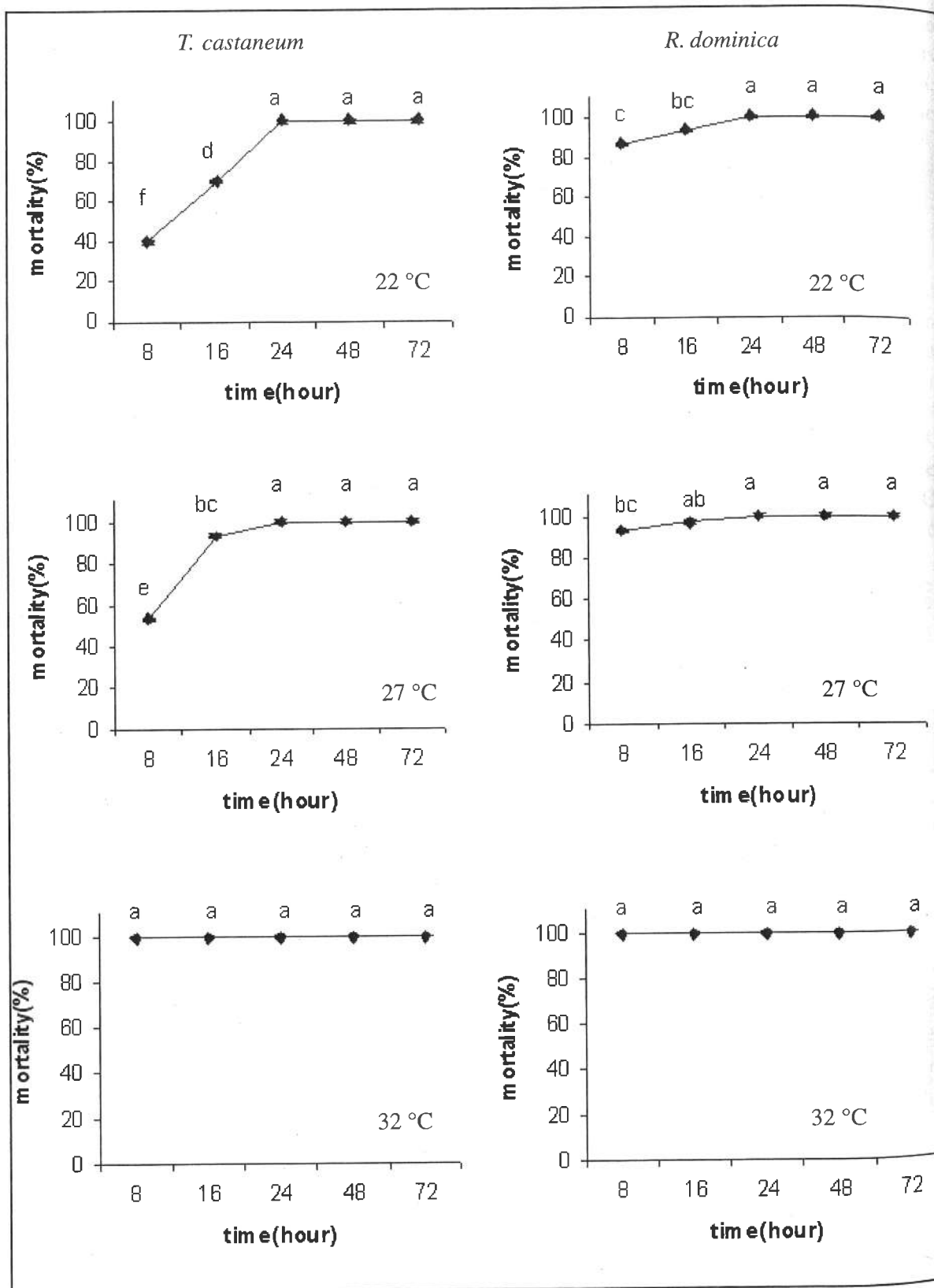


Figure 2. Delayed mortality (%) \pm SEM of *R. dominica* at the right and *T. castaneum* on the left exposed to 1 mg/cm² Silico-Sec® at 22, 27 and 32 °C. Means followed by the same letter are not significantly different; Duncan's multiple range test at $P = 0.05$.

The LT_{99} values for initial mortality of *R. dominica* and *T. castaneum* exposed to 1 mg/cm² Silico-Sec® were recorded in Table 3.

The initial mortality of the lesser grain borer was more than mortality of red flour beetle; but after the 1 week holding period, there was no significant difference between mortality of both species for 24, 48 and 72 h of exposure at any temperature (Table 4).

Discussion

Our results suggest that, regardless of the species, and the temperature, long exposure to DE particles is required to obtain high mortality levels of the exposed beetles; this stands in accordance with previous reports by other researchers, (White and

Loschiavo, 1989; Arthur 2000a, b). McLaughlin reported that the two aerogels and Dryacide® UF gave virtually 100 % kill by the 6 d (end-point) for both *Sitophilus* species. Arthur (2000a) stated that mortality increased as the exposure interval increases; furthermore, beetles exposed to diatomaceous earth subsequently died after they were removed from the treated environment.

Our study indicates mortality of both species was positively related to temperature. Fields and Korunic (2002) reported that increased temperature would increase insect movement, causing increased contact with DE and greater cuticular damage; this is in the agreement with results obtained later (Aldryhim, 1990, 1993; Arthur, 2000a). However results of our test in which both species were exposed directly to Silico-Sec® showed that mortality was generally lower at 22 °C but significantly increased at higher

Table 3. The LT_{99} (hour) values for initial mortality of *R. dominica* and *T. castaneum* exposed to Silico-Sec® at the rate of 1 mg/cm² at 22, 27, 32 °C.

Insect	Temperature		
	22 °C	27 °C	32 °C
<i>R. dominica</i>	147.1 (58.4 - 148069.3)	62.9 (47.9 - 94.98)	32.6 (25.6 - 49.0)
<i>T. castaneum</i>	249.5(100.7-1985942.0)	75.52(*)	38.37(*)

* g < 0.90, confidence limits could not be calculated.

Table 4. Mortality of *R. dominica* versus mortality of *T. castaneum* after the initial and delayed exposure.

Temperature	Exposure	Exposure interval (h)				
		8	16	24	48	72
22 °C	Initial	**	*	**	*	*
	Delayed	*	*	Ns	Ns	Ns
27 °C	Initial	*	**	*	Ns	Ns
	Delayed	**	Ns	Ns	Ns	Ns
32 °C	Initial	*	*	Ns	Ns	Ns
	Delayed	Ns	Ns	Ns	Ns	Ns

Differences between beetles at each temperature and exposure interval determined by the T_{-} test (SAS, 2000). Ns no significant difference; * and ** indicate that mortality of *R. dominica* < mortality of *T. castaneum* at $P < 0.05$ and $P < 0.01$, respectively.

Mortality percentage was transformed using the square root of the arcsin to equalize variances.

temperature of 27, 32 °C. Aldryhim (1990, 1993) found that *S. granarius* and *R. dominica* are more susceptible to Dryacide® at 30 °C than at 20 °C but *T. confusum* is less sensitive at the higher temperatures. Arthur (2000a) stated that both *Tribolium* species were exposed to Protect at controlled temperatures showed a progressive increase in mortality as temperature increased from 22 to 27 and 32 °C.

Stored-product insects show a wide range of susceptibility to DE (White and Loschiavo, 1989; Aldryhim, 1990, 1993). Korunic (1998) indicated *Tribolium* spp. appeared more resistant to DE than other storage pests and this is in agreement with our results. In our study comparing the LT₉₉ values after the initial exposure of *R. dominica* and *T. castaneum* indicated that length of exposure to Silico-Sec® to achieve 99 % mortality of *R. dominica* was less than the LT₉₉ values of *T. castaneum* and the times required to kill both species decreased as temperature increased.

The results confirm that the mortality of both storage beetles was greater after they were left starved for 1 week in clean Petri dishes than the initial mortality. White and Loschiavo (1989) exposed merchant grain beetles and the confused flour beetles to silica aerogel at the rate of 0.72 mg/cm². The beetles were either starved after exposure or given 125 mg to 1 g of whole rolled oats, ground rolled oats, or ground bread crumbs. All starved beetles were dead within 3 d, whereas mortality of both species in the food treatments was greatly reduced. Therefore, the presence of the food material may have reduced desiccation and provided a physical means for the exposed beetles to remove the DE particles from the cuticular surface (Arthur, 2000b). Le Patourel (1986) suggests that, whilst feeding, the individual may be able to compensate for the water loss by the production of metabolic water, which would not be produced whilst the insect is deprived of food or simply unable to feed. Arthur (2000b) emphasizes the importance of sanitation to reduce the occurrence of food material within the storage environment and to eliminate harborage sites and refuge when possible.

The main conclusion of our work is: 1) longer exposure times increase mortality of *R. dominica*

and *T. castaneum* 2) higher temperatures increases mortality 3) insects left to starve after exposure increases mortality and 4) *T. castaneum* is less sensitive to desiccation by DE dusts than *R. dominica*.

Future works should be done to investigate the effect of food on survival of *R. dominica* and *T. castaneum*. In addition, a field test using a similar design is essential to confirm the laboratory findings.

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