The efficacy of two diatomaceous earths on the mortality of Rhyzopertha dominica and Sitophilus oryzae

Nadia Baldassari¹, Antonio Martini²

¹Dipartimento di Scienze e Tecnologie Agro-Alimentari - u.o.s. Cesena, Università di Bologna, Cesena, Italy ²Dipartimento di Scienze Agrarie - Entomologia, Università di Bologna, Italy

Abstract

The insecticidal activity of two commercial formulations of diatomaceous earths (DEs) was evaluated in the laboratory against two major pests of stored grain: *Rhyzopertha dominica* (F.) (Coleoptera Bostrichidae) and *Sitophilus oryzae* (L.) (Coleoptera Curculionidae). The formulations Protector® and SilicoSec® were tested at two different doses (500 and 1000 ppm) against adults of the two species. Bioassays were carried out using 2-liter-glass jars containing common wheat (with a moisture content ranging from 10.5 to 11.7%, for *R. dominica*, and 10.7 to 13.4% for *S. oryzae*) mixed with the DEs, and maintained at 26.7 °C and 60% RH. The efficacy of the treatment was evaluated by recording adult mortality after 2, 7 and 14 days. Both formulations have shown an adulticidal effect, although the mortality was influenced by species, dosages and exposure time. Protector® showed a significant insecticidal activity against the lesser grain borer and the rice weevil already after two days of treatment. After 14 days the mean mortality was greater than 95% even at a dosage of 500 ppm for *R. dominica*, and greater than 87 and 92% at a dosage of 500 ppm and 1000 ppm respectively, for *S. oryzae*. SilicoSec® caused less mortality than Protector® after 2 days of exposure, especially for the *S. oryzae*. However, there was no difference between formulations after 7 or 14 days.

Key words: grain protectans, diatomaceous earth, Protector[®], SilicoSec[®], Rhyzopertha dominica, Sitophilus oryzae.

Introduction

The use of inert dusts for the protection of stored grain has remote origins: the ancient Egyptians, Greeks, Romans (Grandori *et al.*, 1950; Quarles and Winn, 1996) and Aztecs mixed grain with these powders to protect them from the attack of insects (Golob, 1997), and this technique is still in use in small rural communities in developing countries (Stathers *et al.*, 2008). These powders of different origins (sand, lime, clay, wood ash, etc.) are mixed with stored grain, and they damage the insect's cuticle, resulting in dehydration and death.

In particular, the diatomaceous earths (DEs), made up of the frustula (siliceous skeletal remains) of unicellular algae from Eocene and Miocene periods (Athanassiou *et al.*, 2003), forming layers of sedimentary rock in sea water, fresh water and wetlands (Fields, 1999), have long been used in the protection of stored grains. These fossils, after mining, crushing and grinding, give rise to a fine powder predominantly consisting of amorphous silicon dioxide (SiO₂) with small amount of aluminum, iron oxide, magnesium, sodium and lime (Quarles and Winn, 1996; Korunic, 1997).

Insecticidal activity of DEs is principally due to dehydration of insects caused by the damage of the cuticular layers of the integument. In particular DEs particles are trapped on the body of insect and they absorb lipids in the waxy layer of the epicuticle and they abrade the exoskeleton (Ebeling, 1971; Korunic, 1998; Mewis and Ulrichs, 2001). However, their insecticidal efficacy differs significantly in relation to the physical, chemical and morphological characteristics of species of diatoms that compose the DE (Korunic, 1998; Rojht *et al.*, 2010) and to the particle size (Korunic, 1997; Subramanyam and Roesli, 2000). Other factors affecting the effectiveness of the treatment are the physical and chemical features of the

grain (Mvumi *et al.*, 2006); particularly the structure of the pericarp influences adherence of DE dust to kernels (Quarles, 1992; Subramanyam and Roesli, 2000; Kavallieratos *et al.*, 2005; Fields *et al.*, 2003), and a grain moisture content exceeding 14% allows the insect to recover the water lost through dehydration (Quarles, 1992; Quarles and Winn, 1996; Korunic, 1998). Insects' susceptibility to DEs is influenced by differences in their morphology, namely the body size and the presence of hairs (Quarles, 1992; Quarles and Winn, 1996; Korunic, 1998; Subramanyam and Roesli, 2000), and the chemical composition of the cuticle. Moreover the DE effectiveness is influenced by the developmental stage of insect, since adults are generally less sensitive than larval stages (Mewis and Ulrichs, 2001; Baldassari *et al.*, 2004).

The use of DEs to control grain stored pests give many advantages: they leave no chemical residues, they are not flammable, they have a very low mammal toxicity, they are inexpensive and can be easily removed with movements of grain (Korunic, 1998). Moreover due to their physical mode of action, the development of resistance is unlikely, and the reported cases of resistance are probably of behavioural rather than physiological origin (Vajas *et al.*, 2008). They have a rather long-term insecticidal efficacy, and their persistence is related to relative humidity, temperature and changes in internal characteristics or physical proprieties of treated grain, during the storage (Arthur, 2002; Stathers *et al.*, 2004; Athanassiou *et al.*, 2005a; Fields, 2006).

Because of the numerous factors influencing insecticidal activity of DE dusts, it appears necessary to test the different commercial products to analyze their efficacy against different insect species infesting stored-grain bulks. The purpose of this study is to investigate the insecticidal activity of two commercially available DEs, Protector® and SilicoSec®, against adult belonging

to two species of beetles infesting grains: *Rhyzopertha dominica* (F.) (Coleoptera Bostrychidae) and *Sitophilus oryzae* (L.) (Coleoptera Curculionidae). For each product the effectiveness of different concentrations and exposure intervals were evaluated on adult mortality.

Materials and methods

 $R.\ dominica$ and $S.\ oryzae$ adults used for the experiments were obtained from laboratory cultures, carried out inside climate chambers. The beetles were kept in the dark at a temperature of 25 ± 1 °C and a relative humidity of 60%. The tested commodity was the soft wheat cv. Bologna, for both species. The grains, before use, were kept for two days at -18 °C; then they were sieved, to remove dockages, and stored at room temperature in 50-litre PVC tanks, sealed with a hermetic screw caps, until use. The moisture content of wheat, as determined by a Aquasearch PM-600 moisture meter before and after the treatment, ranged from 10.5 to 11.8% for $R.\ dominica$ and 10.7 to 13.4% for $S.\ oryzae$.

The two commercial formulations of DE tested were: Protector® (Biogard, CBC, Italy) with a 50% in particle with size less than 9.46 μ M and SilicoSec® (Biofa, Germany), formulated from fresh water and with mean particle size between 8 and 12 microns (Athanassiou *et al.*, 2005a). The chemical composition of the two DEs are reported in table 1. The application rates tested for each product were 500 and 1000 ppm, and the exposure intervals of adults were two, seven and fourteen days.

The biological assays were performed within 2-literglass jars. The jar was closed with a screw cap, which had a 4.5 cm hole closed by a fine-mesh brass net to prevent insects from escaping and to allow air circulation.

Experimental unit consisted of glass jar containing 1 kg of soft wheat, to which DE was added, and then carefully mixed by hand. Thirty 1-3 weeks old unsexed adults of one of the two testing species were introduced to the jar. The same procedure was followed for each DE formulation, dose rate, exposure intervals and insect species. An additional jar containing untreated wheat were used as control for each trials set. The jars were placed in an incubator at a temperature of 26.7 ± 0.7 °C, and in the dark. For each trial (DE, dose and exposure interval) three treated samples, and three untreated controls were set up.

In correspondence of the end of each exposure interval considered, the contents of jars was sifted, live and dead insects were collected and counted and they were all discharged. Mortality was expressed as percentage of dead adults on the total number of beetles found in each jar. These data were classified according to the product tested, the applied dose and the exposure interval of adults to DE. Mortality percentages were transformed into arcsine $\sqrt{9}$ % to perform the statistical analysis.

For each species the mortality data were analyzed by using the one-way ANOVA, with insect mortality as the response variable, and DE formulation, dose rate and exposure interval as main effects. The means were compared by Tukey's test. The existence of an interaction between DE formulations and insect species was analyzed by using the two-ways ANOVA, with adult mortality, for each exposure interval, as the response variable.

Results

R. dominica

All main effects, DE formulation, dose rate and exposure interval, were statistically significant (table 2). For all treatments adult mortality was significantly higher than in untreated controls. After two days, 1000 ppm Protector[®] caused more mortality than other treatments. However, there were no differences at seven and fourteen days between treatments (table 3).

S. oryzae

All main effects, DE formulation, dose rate and exposure interval, were statistically significant (table 4). SilicoSec® at 500 or 1000 ppm after 2 and 7 days had between 3 and 43% mortality, however these values were not significantly different than the untreated controls. Protector® did significantly increase mortality under all treatments. After 14 days, there was over 88% mortality in all treatments, this was significantly different from the controls and there were no differences between treatments (table 5).

The interaction between species and treatments (formulates and application doses) was not significant (table 6). In fact mortality rate was significant higher for *R. dominica* in comparison to *S. oryzae* only after two and seven days of adults exposure to DEs treatments; but at the longest exposure interval, rate mortality was the same for both species. Moreover applied treatments showed a significant different adults mortality rate only after two-day exposure interval (tables 3 and 5).

Table 1. Contents (%) of the main oxides, biogenic amorphous silica (opal-A) and the silica gel for the tested DE formulations, and correlation between their insecticidal efficacy.

	DE composition												
	SiO_2	Al_2O_3	Fe_2O_3	MgO	CaO	Na_2O	K_2O	TiO_2	P_2O_5	MnO	Cr_2O_3	opal-A	silica gel
Correlation	++	_	_	_	+	n.s.	_	_	_	+	_	++	++
Protector [®]	69.7	5.89	1.05	n.d.	0.414	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	52.03	10
SilicoSec [®]	84.47	3.79	1.21	0.25	0.33	0.15	0.07	0.07	0.02	0.02	0.002	73.10	0

⁺⁺ highly significant correlation, + weak correlation, - negative correlation, n.s. no correlation, n.d. not detectable. Composition of SilicoSec[®] and correlation with the efficacy of each ingredient are taken from Rojht *et al.* (2010). Composition of Protector[®] was analyzed by Neotron Srl (Modena, Italy). The formula reported by Rojht *et al.*, 2010 (opal-A = $SiO_2 - 3 \times Al_2O_3$) was used to calculate the content of opal-A in Protector[®].

Table 2. Results of ANOVAs on adults mortality (performed on the square root of the arcsine of the percentage value) of *R. dominica* exposed to the two DE formulations at different doses and time intervals, and untreated control. Treatment: SilicoSec[®] at 500 and 1000 ppm, and Protector[®] at 500 and 1000 ppm.

Exposure interval	d.f.	s^2	d.f.	s^2	E	n
(days)	between treatments	between treatments	within treatments	within treatments	I.	Р
2	4	1731.29	10	57.92	29.89	< 0.0001
7	4	1533.60	10	54.63	28.07	< 0.0001
14	4	1788.59	10	75.67	23.64	< 0.0001

Table 3. Percent mortality of R. dominica adults (mean \pm SEM) treated with two DE formulations for different exposure intervals and at different doses. For each exposure interval means followed by the same letters do not differ significantly (Tukey's test).

	Dogo		Exposure interval							
Treatment	Dose		2 days	7 days n Mean mortality			14 days			
	ppm	n	Mean mortality			n	Mean mortality			
Control	0	3	2.22 ± 2.22 a	3	10.35 ± 5.24 a	3	20.24 ± 5.57 a			
SilicoSec®	500	3	$34.44 \pm 7.78 \text{ b}$	3	$79.16 \pm 5.12 \text{ b}$	3	$85.56 \pm 2.94 \text{ b}$			
Silicosec	1000	3	$58.47 \pm 4.25 \text{ b}$	3	$84.85 \pm 5.33 \text{ b}$	3	$97.70 \pm 2.30 \text{ b}$			
Protector®	500	3	54.60 ± 5.48 bc	3	$72.99 \pm 7.03 \text{ b}$	3	$95.48 \pm 2.26 \text{ b}$			
	1000	3	87.22 ± 6.41 c	3	$93.06 \pm 3.43 \text{ b}$	3	$95.40 \pm 4.60 \text{ b}$			

Table 4. Results of ANOVAs on adults mortality (performed on the square root of the arcsine of the percentage value) of *S. oryzae* exposed to the two DE formulations at different doses and time intervals, and untreated control. Treatment: SilicoSec® at 500 and 1000 ppm, and Protector® at 500 and 1000 ppm.

Exposure interval	d.f.	s^2	d.f.	s^2	E	n
(days)	between treatments	between treatments	within treatments	within treatments	1	Р
2	4	492.88	10	56.91	8.66	0.0028
7	4	622.67	10	139.35	4.47	0.0250
14	4	2609.09	10	87.57	29.79	< 0.0001

Table 5. Percent mortality of *S. oryzae* adults (mean \pm SEM) treated with two DE formulations for different exposure intervals and at different doses. For each exposure interval means followed by the same letters do not differ significantly (Tukey's test).

	Dose		Exposure interval						
Treatment			2 days	7 days			14 days		
	ppm	n	Mean mortality	n	Mean mortality	n	Mean mortality		
Control	0	3	0.00 ± 0.00 a	3	5.59 ± 3.99 a	3	4.52 ± 1.19 a		
SilicoSec®	500	3	3.33 ± 3.33 ab	3	24.67 ± 2.77 ab	3	$88.89 \pm 9.49 \text{ b}$		
	1000	3	13.33 ± 3.33 bc	3	42.80 ± 9.74 ab	3	$98.89 \pm 1.11 \text{ b}$		
Protector®	500	3	15.56 ± 5.88 bc	3	52.22 ± 16.81 b	3	$87.66 \pm 5.86 \text{ b}$		
	1000	3	$27.78 \pm 8.01 \text{ c}$	3	$46.67 \pm 12.02 \text{ b}$	3	92.22 ± 2.22 b		

Discussion

The experiments demonstrated that both DE formulates are effective in reducing adult populations of *R. dominica*, just after two days exposure interval. On the other hand, only Protector® at both experimental doses and SilicoSec® at the rate of 1000 ppm were effective after two days exposure interval. However adult mortality increased with the increase in exposure interval for the two species, and after 14 days exposure it was the same for both beetle species.

Therefore the insecticidal activity of DE formulations

is mainly influenced by exposure time, so that insects can pick up dust particles during their movement inside the bulk grain (Athanassiou *et al.*, 2005a), whilst the different formulations and doses of tested DEs affect adults mortality of both species when they are exposed to treated wheat for two days.

Apparently this difference could be due to the commercial DEs formulation: in fact Protector® contains 10% of silica gel which, as reported by Korunic and Fields (1995), would increase the efficacy compared to formulations containing only diatomaceous earth, such as SilicoSec®. On the other hand Rojht *et al.* (2010)

Table 6. Two-way ANOVA parameters for main effects and associated interactions for adults mortality (performed on the square root of the arcsine of the percentage value) at each exposure intervals. Species: *R. dominica*, *S. oryzae*. Treatment: SilicoSec[®] at 500 and 1000 ppm, and Protector[®] at 500 and 1000 ppm.

Adults mortality after 2-days exposure interval							
Effects	d.f.	Mean square	F	P			
Species	1	5628.17	90.14	0.0001			
Treatment	3	899.25	14.40	0.0001			
Species × treatment	3	51.74	0.83	n.s.			
Residuals	16	62.44					
	Adults mor	tality after 7-days exposu	re interval				
Effects	d.f.	Mean square	F	P			
Species	1	4234.73	43.11	0.0001			
Treatment	3	165.89	1.69	n.s.			
Species × treatment	3	134.38	1.37	n.s.			
Residuals	16	98.24					
Adults mortality after 14-days exposure interval							
Effects	d.f.	Mean square	F	P			
Species	1	28.55	0.30	n.s.			
Treatment	3	214.60	2.26	n.s.			
Species × treatment	3	100.17	1.06	n.s.			
Residuals	16	94.88					
	·		·	·			

reported that, of the chemical components of DEs, mainly the SiO₂ content and biogenic amorphous silica (opal-A) are positively related with adults mortality of *S. oryzae*. The content of these components is higher in SilicoSec[®] compared to Protector[®]; so that they could compensate for the absence of silica gel in the first commercial compound.

The research showed that the rice weevil *S. oryzae* proved less susceptible to tested DEs than the lesser grain borer *R. dominica*, as already pointed out by Baldassari *et al.*, 2002; in fact adults mortality for *S. oryzae* is lower than for *R. dominica* after 2 and 7-days exposure interval. This result is opposite to what generally observed by other authors for the same two species (Fields and Muir, 1996; Korunic, 1998; Fields and Korunic, 2000).

Acknowledgements

We would like to thank Dr. Massimo Benuzzi (Biogard, CBC Europe, Italy) for providing the SilicoSec® and the Protector®.

This paper reports the results of research only. Mention of a proprietary chemical or a trade name does not constitute a recommendation or endorsement by *Alma Mater Studiorum* - Università di Bologna.

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Authors' addresses: Nadia BALDASSARI, Dipartimento di Scienze e Tecnologie Agro-Alimentari - u.o.s. Cesena, *Alma Mater Studiorum* - Università di Bologna, p.zza G. Goidanich 60, 47521 Cesena (FC), Italy; Antonio MARTINI (corresponding author: antonio.martini@unibo.it), Dipartimento di Scienze Agrarie - Entomologia, *Alma Mater Studiorum* - Università di Bologna, viale G. Fanin 42, 40127 Bologna, Italy.

Received July 24, 2013. Accepted January 14, 2014.