Bulletin of Insectology ***57*** *(2): 95-102, 2004*
ISSN 1721-8861

Analysis of the sensitivity of different stages of *Rhyzopertha dominica* and *Tribolium castaneum* to diatomaceous earth

Nadia BALDASSARI[1], Alessandro BERLUTI[1], Antonio MARTINI[2], Piero BARONIO[2]
[1]*Campus Universitario Scienze degli Alimenti, Università di Bologna, Cesena, Italy*
[2]*Dipartimento di Scienze e Tecnologie Agroambientali - Entomologia, Università di Bologna, Italy*

Abstract

Diatomaceous earth (DE) products, composed of microscopic fossils of diatoms, can be an alternative to chemical methods in controlling stored products infestations. Insecticidal activity depends on the DE capacity to damage insects' cuticle and cause water loss from their bodies, so that they die of desiccation. The efficacy of DE products depends on several parameters, such as insect morphology, type of grains, DE physical parameters, temperature and relative humidity. The insecticidal activity of Protector® was tested against the eggs and the larval instars of *Rhyzopertha dominica* (L.) (Coleoptera Bostrychidae) (the lesser grain borer) and *Tribolium castaneum* (Hebst) (Coleoptera Tenebrionidae) (the red flour beetle). Preliminary tests were carried out to assess the development time from egg to adult for both insects at 23 °C and 34 °C. The experiments with DE were performed at 23 ± 0.2 °C and 34 ± 0.2 °C within one-liter glass jars using, as feeding substrate, soft wheat grains for *R. dominica* and maize (corn grains) for *T. castaneum* where they had previously laid their eggs. Samples were treated with a Protector® dosage of 0.5 g/Kg. The most sensitive larval instars of *T. castaneum* were identified at both temperatures. The results showed that Protector® can prevent infestations of both insects, especially *T. castaneum*. The efficacy of the product depended on the biological and morphological characteristics of the insects and on the temperature. The temperature hid the efficacy of the treatment at 23 °C against *R. dominica* because dead insects were collected in controls, but were not found in untreated samples at 34 °C; the mortality rate in treated jars at 23 °C was 74% and in untreated jars 8%. At 23 °C for *T. castaneum* no adults emerged, while at 34 °C we counted some adults. However, the number of the latter was considerably lower than that obtained in untreated samples. The sensitivity of the larval instars to Protector® showed that it was efficacious against *T. castaneum* larvae at both temperatures. We also noticed that the product was lethal for first instar larvae but not for older larval stages. The persistence of product efficacy was observed against *R. dominica* but not for *T. castaneum*.

Key words: Inert dust, diatomaceous earth, stored product protection, *Rhyzopertha dominica*, *Tribolium castaneum*, lesser grain borer, red flour beetle.

Introduction

Parasitic damage can occur in storage structures because as a result of attacks by animals and other organisms which produce a reduction in grain substances, and an almost always irreparable change in its properties (Gelosi and Süss, 1991; Süss and Locatelli, 2001). Insects are among the most responsible organisms for these phenomena, as they not only eat the grain but also filth it with faeces, larvae cuticles and silk thread made by moth larvae.

A valid alternative to residual insecticides in stored-grain protection is the use of inert dusts, which are mixed into the grain as previously employed by the Egyptians, the Chinese and the Romans (Quarles and Winn, 1996; Grandori *et al*., 1950).

There are five groups of inert dusts that are different in chemical composition and efficacy:

- *Non-silica dusts* such as katelsous, lime, sodium chloride, magnesium and zinc oxide are used to protect grain in various regions of the world;
- *Sand, kaolin, paddy husk ash, wood ash and clay* are used for small-quantities of foodstuffs, but have to be employed on a large scale so as to have a significant effect;
- *Diatomaceous earths* (DE) are the fossilized remains of diatoms composed mainly of amorphous hydrated silica, which usually contain about 90% of SiO_2 and also aluminium, iron oxide, magnesium, sodium and lime;
- *Silica aerogels* are light, hydrophobic powders that are obtained by drying aqueous solutions of sodium silicate (Golob, 1997);
- *Zeolites* are highly absorbent minerals, which have an open three-dimensional tetrahedron structure, inside of which there are some hollows containing cations and water (Contessi and Mucciolini, 1998).

The mode of action of these substances has not been completely explained. It is known shells damage the cuticular layer of the arthropod integument, as a result of physical properties and of their peculiar pointed, geometric shapes. This occurs because the shells absorb the lipids which make up the epicuticular waxy layer and abrade the exoskeleton, especially in its thinnest zones (flexible membranes and joints) (Alexander *et al.*, 1944; Quarles and Winn, 1996; Golob, 1997; Korunic, 1998). It has not yet been proved whether DE affects the respiratory system and whether it has a damaging action on the digestive system when ingested with food (Alexander *et al*., 1944).

Insect morphology, especially the exoskeleton, has an important function in the efficacy of the treatment. Hairy and rough insects are more susceptible to DE than those with smooth cuticles. The thickness of the waxy

layer is also important, thus, the thicker the waxy layer the more susceptible the insect is to DE (Subramanyam and Roesli, 2000).

The efficacy of DE is connected to several factors like: type of grain and its moisture, temperature and relative humidity, application rates, distribution of the product, particle size and shell shape, oil absorption capacity, active surface area and chemical composition of the DE (Quarles and Winn, 1996).

It is also worth noting that the species and type of grain play an important role in the application dosage. The efficacy of the DE is connected to the degree of adhesion of silica particles to the grain surface and to the quantity of product picked up by insects (Aldryhim, 1993). Thus, a higher concentration of product is necessary to protect corn than for wheat, for example (Quarles and Winn, 1996).

The insecticidal activity of these products is also affected by relative humidity, temperature and the combination of both, as has been found for the adults of *Sitophilus oryzae* (L.) (Coleoptera Curculionidae), *Tribolium castaneum* (Herbst) (Coleoptera Tenebrionidae) (Arthur, 2000) and *Rhyzopertha dominica* (F.) (Coleoptera Bostrychidae) (Aldryhim, 1993).

The application rate of the various kinds of DE also has an important function. In fact, the adults of *S. oryzae* and *Sitophilus granarius* (L.) (Coleoptera Curculionidae) treated with Perma Guard have shown a mortality rate of 95%-99% with doses of 2.268 kg and 2.721 kg per ton of wheat respectively. On the contrary, the adults of *T. castaneum* and *Tribolium confusum* Jacquelin du Val (Coleoptera Tenebrionidae) have shown a mortality rate of 9% and 4.6% with a dose of 3.175 kg per ton of wheat (Carlson and Ball, 1962). To reach a 100% mortality rate of *S. granarius* adults, 1.5-2 g of Dryacide (a mixture of DE and silica aerogels) per kilo of wheat is necessary. The same result for *S. oryzae*, *R. dominica* and *T. castaneum* can be obtained with a dose of 1g/kg of product; this is the same quantity necessary to prevent *S. oryzae* and *R. dominica* from having progeny, while *T. castaneum* and *S. granarius* require 0.5 g/kg and 3 g/kg respectively (Desmarchelier and Dines, 1987).

Another important element for a successful treatment of DE is a uniform distribution of the product (Arthur, 2000). The mortality of the fed and unfed adults of *T. castaneum* treated with DE was about 21% and 89% respectively after a week (Dowdy, 1999).

The insecticidal activity of DE is usually considerably affected by particle size and absorption capacity of lipids. Generally, smaller particles, for example those of 1-2 μm, are more efficacious because they adhere more to the cuticle, although similar results have been obtained with particles of nearly 200 μm. This is to prove that the assessment of the insecticidal efficacy of DE products is influenced by all the parameters affecting activity. Nevertheless, it has already been noticed that the most efficacious types of earth are 10-12 μm, flat shaped, with a high wax absorption capacity, a high active surface area and a high percentage of amorphous silica (Quarles and Winn, 1996; Korunic, 1997).

The use of DE to control stored product pests gives several advantages: it leaves no chemical residues; it is not inflammable; it is safe for mammals (these products are already used in cleaning processes, as a food additive up to 2% and as an anticaking agent); it does not affect food characteristics; it is unlikely the development of insect resistance; it is inexpensive. Its effectiveness does not diminish over time and the removal of DE is extremely easy. In fact the movement of grains reduces the quantity of the added DE (Korunic, 1998; Quarles and Winn, 1996). The use of DE also has some disadvantages: it reduces bulk density and grain flowability that causes machinery strain; it produces dusts and causes complications in people suffering from allergies. These disadvantages are slight compared with the above-mentioned advantages (Quarles and Winn, 1996).

The aim of this study is to analyse the insecticidal activity of Protector® against the eggs and the larval instars of *T. castaneum* (the red flour beetle) and *R. dominica* (the lesser grain borer), which are two of the most harmful pests of stored products. The former eats grain fragments and completes its metamorphosis outside the grains (Good, 1933; 1936); the latter eats grain content and its biological cycle is completed when the first instar larva gets into the grain and remains there until the adult emerges (Potter, 1935).

Materials and methods

The adults of *T. castaneum* and *R. dominica* with which were obtained the eggs used for these experiments came from a laboratory culture carried out within two-liter glass jars. The jars were closed with a metal cap which had a 4.5-cm diameter hole and a fine-mesh brass net to prevent insects from escaping and to allow air circulation. The jars were kept in a climate chamber at 27 ± 1 °C.

The DE formulation used in this study is the commercial product Protector® that was supplied by Intrachem Italia S.r.l. and has the following technical characteristics: 50% of particles smaller than 9.46 μm, 69.7% of SiO_2, 5.89% of Al_2O_3, 0.414% of CaO and 1.05% of Fe_2O_3 (these characteristics were analysed by Neotron S.r.l. of Modena).

The trials were carried out employing climate chambers, one-liter glass jars prepared as described above, and 11-cm glass Petri dishes.

The adults obtained from the samples in the jars were examined under a JEOL 5200 scanning electron microscope, in order to observe any possible DE particles used in the experiment.

Before performing DE experiments, preliminary tests were made to assess the development time from egg to adult for both insects at 23 °C and 34 °C. These trials were carried out by putting in an 11-cm Petri dish and 40 g of feeding substrate, as well as 10 unsexed adults of mixed ages. Three replications were conducted for each species and temperature. The dishes were checked daily. Once the eggs had been laid, the adults were removed and the dishes with the eggs were kept in the climate chamber until the new adults emerged. The ob-

served mean length of the cycle (from egg to adult) was about 60 days at 23 °C and 25 days at 34 °C for *R. dominica* and 77 days at 23 °C and 28 days at 34 °C for *T. castaneum*.

In order to identify by head measurements the larval stage for *T. castaneum*, that generally live outside the grain, we reared the insect within 15 ml glass test tubes closed with fine-mesh tulle and fixed with a strip of Parafilm®. Three grams of wheat flour and one adult of each sex (recognized at pupa stage) were placed inside each test-tube. The samples prepared as indicated were kept in a climate chamber at 27 ± 0.2 °C and 60 ± 2 relative humidity. The test-tubes were checked daily and the first instar larvae were isolated in another test-tube with 0.10 g of wheat flour. This sample was checked daily to verify its moult and to remove the old cuticle. This method was employed for each instar until the adult emerged. Ten specimens of each larval instar were killed with ethyl ether and the head measurements were performed using a micrometric ocular. The collected data were analyzed using the significance double-tailed curve test for a normal distribution, with a significance level of 95% (z = 1.96) (table 1).

The complete development cycle of *T. castaneum*, under the parameters described above, lasted 29.7 days on average. The average incubation period of an egg was 4.75 days and a larval development cycle of 22.1 days, with a different duration for each instar. It was also established that the pupal stage required an average of 7.6 days (table 2).

The experiments with DE were carried out at 23 ± 0.2 °C and 34 ± 0.2 °C; these temperatures were chosen as they are approximately the minimum and the maximum development temperature of the two insects (Birch, 1945; Howe, 1956). Inside the climate chambers, the relative humidity was 60 ± 2 at the lower temperature and 69 ± 2 at the higher temperature. These values were kept constant by putting a tub with a saturated solution of potassium nitrate in the climate chambers. To record the environmental parameters within the climate chambers a μMetos® electronic thermohygrometer was used.

The eggs employed in the experiments were obtained by putting 10 unsexed adults of mixed ages in a 11-cm Petri dish with 40 g of feeding substrate. These samples were kept in climate chambers at both experimental temperatures. The samples were checked daily for eggs.

Table 1. Head mean diameter (mm) of larval instars of *Tribolium castaneum*, reared in wheat flour, at 27 ± 0.2 °C and 60 ± 2 RH.

Larval stage	Mean ± zσ
First	0.15 ± 0.047
Second	0.18 ± 0.027
Third	0.26 ± 0.031
Fourth	0.35 ± 0.099
Fifth	0.46 ± 0.078

The chosen experimental feeding substrate consisted of soft wheat grains for *R. dominica* and national corn grains for *T. castaneum*. The grains, before use, were heated for two hours at 80 °C for disinfestation and kept in 50-litre PVC tanks with a hermetic seal screw cap. After the disinfestation the moisture content of the grain, recorded by an Aquasearch PM-600 moisture analyser, was of about 11.9 ± 1% for soft wheat and about 12.7 ± 1% for national corn.

The experiments, at both temperatures, were performed in jars as already explained. The following method was used: a preparation of a grains sample where the adults had laid the eggs, a pouring of the grains into a jar which was then topped up to 500 g and an addition of 0.25 g of Protector® uniformly mixed with grains in the treated jars. These jars were kept in two climate chambers at two temperatures (23 °C and 34 °C). This method was performed for both tested species.

The effect of the DE on the larval instars was assessed for *T. castaneum,* as it is an insect which lives and completes its cycle outside the grains. The trials were carried out by mixing 0.02 g of Protector® with 40 g of corn inside the Petri dishes where the insects had laid eggs, only after the removal of the adults. The samples were kept in two separate climate chambers at each temperature.

The insecticidal efficacy of Protector® against both species was examined after 90 days for samples at 23 °C and after 30 days for those at 34 °C. The adults were collected after sifting the jar contents. The adults were collected by using two sieves with a 1.7 mm and 0.4 mm mesh respectively, stacked on a purpose-made container. The collected adults were counted and separated into living and dead. The above-described opera-

Table 2. Biological cycle (days) of *Tribolium castaneum* reared in wheat flour at 27 ± 0.2 °C and 60 ± 2 RH.

Stage	Development cycle in days		
	Minimum	Maximum	Mean
Egg	4	5	4.75
1st instar	1	1	1
2nd instar	2	10	5
3rd instar	2	8	4.6
4th instar	1	7	4
5th instar	1	21	7.5
Complete larval development	7	47	22.1
Pupa	7	8	7.6
Complete development	14	55	29.7

tion was carried out under a laboratory aspirator to prevent allergic substances from being inhaled. For each species and for both temperatures the number of adults (living and dead) obtained from the treated jars were compared with those (living and dead) obtained from the untreated jars; these values were analysed with Yates's corrected χ^2 test.

The determination of the mortality of the different larval instars of *T. castaneum* was conducted by checking the samples weekly, while the collection of the adults was made after 90 days for trials at 23 °C and after 30 days for those at 34 °C. Periodically, the content of the Petri dishes was examined under a light microscope to collect the dead larvae that were kept in vials until head measurements were taken. These values, compared with those obtained from the study of *T. castaneum* biology, allowed for the exact larval instar killed by Protector® to be identified. Moreover, the number of dead larvae plus leaving larvae (i.e. number of emerged adults) counted in treated samples was compared with the corresponding total values obtained from controls and analysed with Yates's corrected χ^2 test.

The procedure to examine the adults under a scanning electron microscope, in order to check for any possible particles of Protector® on their bodies, was performed as follows. The insects collected in the samples were subjected to three 15-minute wash cycles with three alcohol-solutions, of 70, 80 and 90% respectively, each of them 15 minutes lasting. Then, two further 15-minute wash cycles were effected with a 100% ethanol-solution. The treated adults were kept in pure acetone previous to specific treatment for S.E.M. observation. This treatment consisted of removing the acetone from the insects' bodies by evaporation. They were subsequently glued to S.E.M. stubs and coated with gold.

Results

The mortality rate of *R. dominica* treated with Protector® (i.e. number of dead adults within treated grains, where the product was applied after the eggs had been laid) was significantly higher than in the control. This effect was more notable in the samples kept at 34 °C than in those at 23 °C. As seen in the control, the temperature of 23 °C was cause of mortality and hid the efficacy of the tested product. The insecticidal effect of the DE tested against larval instars of *R. dominica* is shown in table 3 for samples at 23 °C and in table 4 for those at 34 °C.

The efficacy of DE against *T. castaneum* was evaluated in the same way used for *R. dominica*, in addition to determining at what stage the larval instar died. As already stressed, tenebrionidae are different from bostrichidae in that they complete their metamorphosis outside the grains. To evaluate the efficacy of the product, this study took into consideration the numbers of living and dead adults obtained from treated grains after egg-laying. The results showed that progeny had all been killed at 23 °C. Thus, it was not possible to compare the numbers obtained from treated samples with those obtained from the controls using the χ^2 test. This comparison was possible for samples at 34 °C, where controls and treated trials had the same low mortality rate. However, the total number of living and dead adults collected in treated jars was notably lower than in the control. Table 5 shows the results obtained at 23 °C and table 6 those at 34 °C.

Table 3. Number of *Rhyzopertha dominica* living and dead adults collected in the jars kept at 23 °C and χ^2 test to compare the number of adults obtained from treated and untreated jars.

	Dead adults		Living adults		d.f.	χ^2	p
	No.	%	No.	%			
Treated	134	74.03	47	25.97	1	278.89	< 0.0001
Untreated	36	8.11	408	91.89			

Table 4. Number of *Rhyzopertha dominica* living and dead adults collected in jars kept at 34 °C and χ^2 test to compare the number of adults obtained from treated and untreated jars.

	Dead adults		Living adults		d.f.	χ^2	p
	No.	%	No.	%			
Treated	26	53.32	21	44.68	1	80.48	< 0.0001
Untreated	0	0	131	100			

Table 5. Number of *Tribolium castaneum* living and dead adults collected in jars kept at 23 °C.

	Dead adults		Living adults	
	n	%	n	%
Treated	0	-	0	-
Untreated	0	0	74	100

Table 6. Number of *Tribolium castaneum* living and dead adults collected in the jars kept at 34 °C and χ^2 test to compare the number of adults obtained from treated and untreated jars.

	Dead adults		Living adults		d.f.	χ^2	p
	No.	%	No.	%			
Treated	0	0	16	100	1	0.56	0.4532 (n.s.)
Untreated	2	1.32	150	98.68			

The results for both trial temperatures relative to the effect of the DE product on larval instars of *T. castaneum* are shown in tables 7 and 8.

The total number of dead larvae collected in treated samples was significantly higher than that found in the controls. Furthermore, the number of adults was significantly higher in untreated than in treated samples.

At 23 °C all the dead larvae collected were of the first instar, while at 34 °C the 7.14% of larvae founded dead were of the third instar.

Discussion

The most important result to emerge from this study was the efficacy of the DE product against the immature stages of *R. dominica* and especially against those of *T. castaneum*.

The thorough analysis of the obtained results for both species showed some of the ways in which the product worked in relation to the biological and morphological characteristics of the coleoptera and to the temperature. This parameter hid the efficacy of the product against *R. dominica*, so that at 23 °C we had a mortality of the adults in untreated samples. However, in treated samples there were 74% dead adults compared with 8% in the controls (calculated out of the total number of emerged adults). The above-mentioned explanation was confirmed by the mortality observed only in treated jars kept at 34 °C. The effect of temperature on adult vitality had already been explained by a study that evaluated the insecticidal efficacy of this physical parameter on the adults' longevity (Visini, 2000); Birch (1953), Vardell and Tilton (1980) had also explained the effect of temperature on the survival of *R. dominica* adults.

Another result observed was the persistence of the product, whose insecticidal activity was constant during the 90-day of the trials at 23 °C. This proved that Protector®, as a wheat protectant, was effective especially in controlling the infestation by *R. dominica* adults. The persistence of Protector® efficacy was not observed in other DE products, as they were no longer effective four weeks after being applied to wheat grains, with yeast and a relative humidity of 58% (Nielsen, 1998). Anyhow, the obtained duration of Protector® insecticidal activity was not definitive, as its efficacy might be longer than the 90 days of the experiment. In fact, a DE product applied on wheat grains, with a relative humidity of 60%, had been active for six months (Aldryhm, 1990).

The observed Protector® efficacy against *T. castaneum* was different from that against *R. dominica*, as the temperature had a synergic effect. In fact, the temperatures of 23 °C and 34 °C caused almost no mortality in controls but caused a different percentage of mortality in treated samples. At 23 °C no adult emerged and all larvae died at first instar, while at 34 °C only a few adults emerged from the larvae that survived the most susceptible instars, that is from the first instar to the third. In fact the highest larval mortality was at the first instar and the lesser at the third instar. The same result was observed for other pests like *Plodia interpunctella* (Hübner) (Lepidoptera Phycitidae), *Oryzaephilus mercator* Fauvel, *O. surinamensis* (L.) (Coleoptera Silvanidae),

Table 7. Number of *Tribolium castaneum* living and dead larvae kept at 23 °C and χ^2 test to compare the larval mortality in treated and untreated Petri dishes.

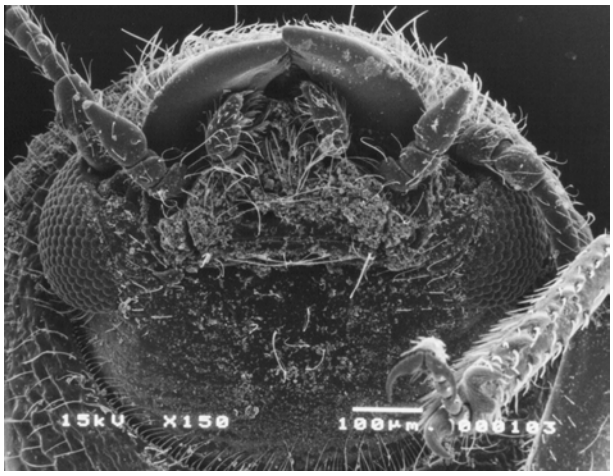
	Dead larvae		Living larvae [(a)]		d.f.	χ^2	p
	No.	%	No.	%			
Treated	225	100	0	0	1	109.22	< 0.0001
Untreated	67	57.26	50	42.74			

[(a)] Number of living adults counted after 90 days.

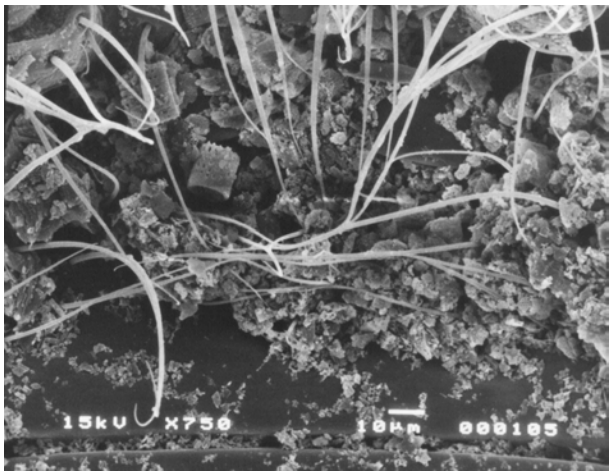
Table 8. Number of *Tribolium castaneum* living and dead larvae kept at 34 °C and χ^2 test to compare the larval mortality in treated and untreated Petri dishes.

	Dead larvae		Living larvae [(a)]		d.f.	χ^2	p
	No.	%	No.	%			
Treated	149	99.33	1	0.67	1	173.07	< 0.0001
Untreated	23	20.54	89	79.46			

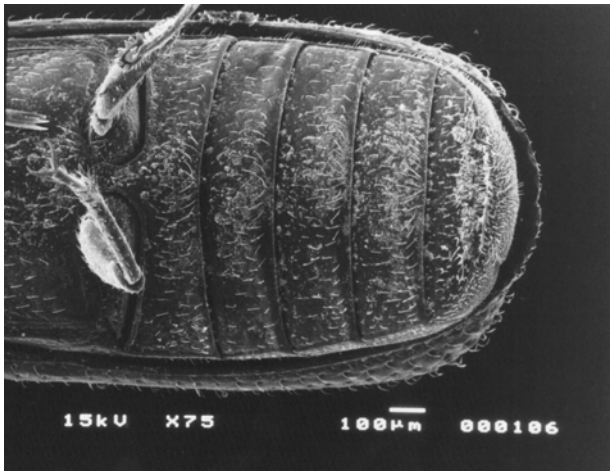
[(a)] Number of living adults counted after 30 days.



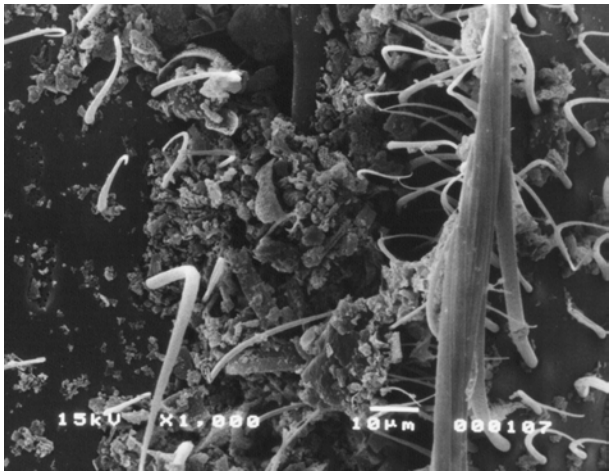
a



b



c



d

Figure 1. *Rhyzopertha dominica.* Scanning electron micrographs showing the adhesion of silica particles on insect's body parts: **a.** Ventral view of mouthparts (150x); **b.** Detail of the ventral side of labium (750x); **c.** Ventral side of metathorax and abdomen (75x); **d.** Detail of the ventral side of abdomen (1000x).

T. castaneum, *T. confusum*, *Tenebrio molitor* L. (Coleoptera Tenebrionidae), *S. granarius* and *Epilacna varivestis* Mulsant (Coleoptera Coccinellidae), (Hunt, 1947; White and Loschiavo, 1989; Subramanyam *et al.*, 1998; Mewis and Ulrichs, 2001a, 2001b).

The temperature and the DE products did not have the same effect on the various species of insects. Thus, for instance, the higher the temperature, the lower the quantity of progeny for *S. oryzae* (Arthur, 2002), while the opposite result occurred for *S. granarius* (Aldryhim, 1990).

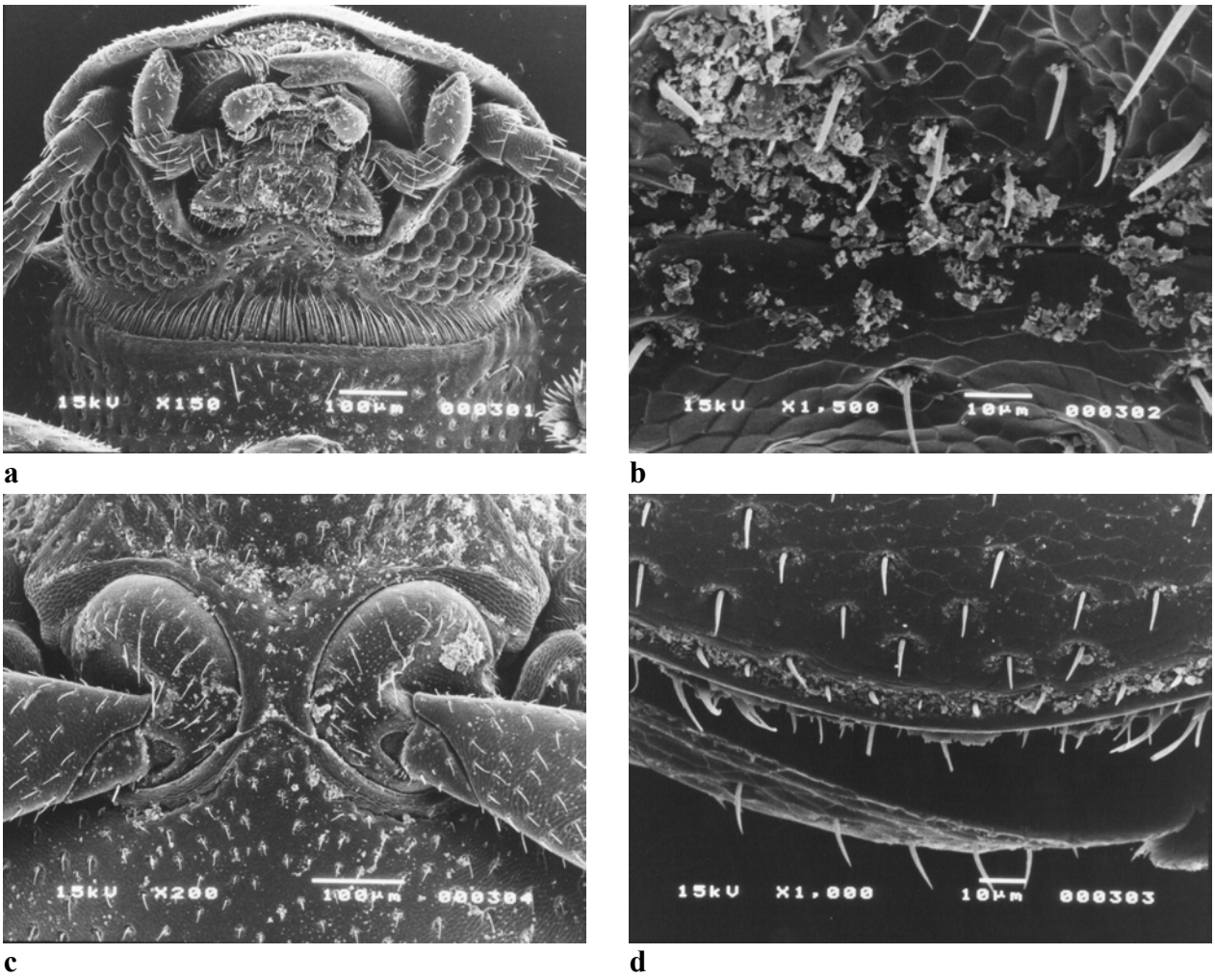
Finally, it was also noted that there was a partial mortality in the *R. dominica* adults emerging from the larvae which had completed their metamorphosis in the treated wheat, while all *T. castaneum* adults could survive. This result highlighted that the persistence of Protector® activity on both species was related to the insecticidal activity of silica-based products. The efficacy of Protector® was due to its capacity to remain on insects' bodies and cause water loss leading to death. *R. dominica* adults, which proved to be more sensitive to the DE product (Baldassari *et al.*, 2002), were effectively more covered with DE particles (figure 1) than *T. castaneum* adults (figure 2).

All in all, DE products like Protector®, as proved by the results of this study, could prevent insect infestation as they were lethal for the early larval instars of insects living outside the grains. This result proved to be impossible for species living inside the grains, which were highly unlikely to be affected by the product.

It was also proved that the product had a different efficacy against the larval instars compared with adults, as observed for *T. castaneum.* Generally, this is due not only to the capacity of the exoskeleton structure to retain the product on its surface, but also to the chemical composition of the cuticular layers and the different physiology of each development stage (Mewis and Ulrichs, 2001b).

Acknowledgements

The authors are grateful to Profs. Mario Marini and Gabriella Rocchetta of the Department of Experimental Evolutionary Biology (*Alma Mater Studiorum*, University of Bologna), for taking S.E.M. photographs and statistic advising respectively. We also thank Giovanni Baldoni from Intrachem Italia S.r.l. for supplying the DE product used in this study.



a **b**

c **d**

Figure 2. *Tribolium castaneum.* Scanning electron micrographs showing the adhesion of silica particles on insect's body parts: **a.** Ventral view of mouthparts (150x); **b.** Detail of the ventral side of labium; **c.** Detail of metasternum (200x); **d.** Ventral view of the last segment of abdomen (1000x).

References

ALDRYHIM Y. N., 1990.- Efficacy of the amorphous silica dust, Dryacide, against *Tribolium confusum* Duv. and *Sitophilus granarius* (L.) (Coleoptera: Tenebrionidae and Curculionidae).- *Journal of Stored Products Research,* 26 (4): 207-210.

ALDRYHIM Y. N, 1993.- Combination of classes of wheat and environmental factors affecting the efficacy of amorphous silica dust, Dryacide, against *Rhizopertha dominica* (F.).- *Journal of Stored Products Research*, 29 (3): 271-275.

ALEXANDER P., KITCHENER J. A., BRISCOE H. V. A., 1944.- Inert dust insecticides. Part I. Mechanism of action.- *Annals of Applied Biology*, 31: 143-149.

ARTHUR F. H., 2000.- Impact of food source on survival of red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae) exposed to diatomaceous earth.- *Journal of Economic Entomology*, 93 (4): 1347-1356.

ARTHUR F. H., 2002.- Survival of *Sitophilus oryzae* (L.) on wheat treated with diatomaceous earth: impact of biological and environmental parameters on product efficacy.- *Journal of Stored Products Research,* 38: 305-313.

BALDASSARI N., BALDONI G., BARONIO P., 2002.- Efficacia di diverse terre diatomacee nel controllo di insetti adulti.- *Tecnica Molitoria,* 53 (12): 1201-1207.

BIRCH L. C., 1945.- The influence of temperature on the development of the different stages of *Calandra oryzae* L. and *Rhizopertha dominica* Fab. (Coleoptera).- *Australian Journal of experimental Biology and Medical Science,* 23 (2): 29-35.

BIRCH L. C., 1953.- Experimental background to the study of the distribution and abundance of insects. I. The influence of temperature, moisture and food on the innate capacity for increase of three grain beetles.- *Ecology*, 34 (4): 698-711.

CARLSON S. D., BALL H. J., 1962.- Mode of action and insecticidal value of a diatomaceous earth as a grain protectant.- *Journal of Economic Entomology*, 55 (6): 964-970.

CONTESSI A., MUCCIOLINI G., 1998.- Prove comparative di efficacia insetticida di polveri silicee a base di zeoliti e di farina fossile di diatomee, pp. 425-433. In: *Atti 6° Simposio Difesa Antiparassitaria nelle Industrie Alimentari.* Piacenza, 24-26 settembre 1997, Chiriotti Editori, Pinerolo.

DESMARCHELIER J. M., DINES J. C., 1987.- Dryacide treatment of stored wheat: its efficacy against insects, and after processing.- *Australia Journal of Experimental Agriculture*, 27: 309-312.

DOWDY A. K., 1999.- Mortality of red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae) exposed to high temperature and diatomaceous earth combinations.- *Journal of Stored Products Research,* 35: 175-182.

GELOSI A., SÜSS L., 1991.- *Insetti e acari dei cereali in magazzino*. Edagricole, Bologna.

GOLOB P., 1997.- Current status and future perspectives for inert dusts for control of stored product insects.- *Journal of Stored Products Research*, 33 (1): 69-79.

GOOD N. E., 1933.- Biology of the flour beetles, *Tribolium confusum* Duv. and *T. ferrugineum* Fab.- *Journal of Agricultural Research*, 46 (4): 327-334.

GOOD N. E., 1936.- The flour beetles of the genus *Tribolium*.- *USDA Technical Bulletin*, 498: 1-57.

GRANDORI R., GRANDORI L., DOMENICHINI G., CARÈ E., 1950.- La bentonite come disinfestante del frumento immagazzinato.- *Bollettino di Zoologia Agraria e di Bachicoltura*, 6 (11): 51-84.

HOWE R. W., 1956.- The effect of temperature and humidity on the rate of development and mortality of *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae).- *Annals of Applied Biology*, 44 (2): 356-368.

HUNT C. R., 1947.- Toxicity of insecticide dust diluents and carriers to larvae of the mexican bean beetle.- *Journal of Economic Entomology*, 40 (2): 215-219.

KORUNIC Z., 1997.- Rapid assessment of the insecticidal value of diatomaceous earths without conducting bioassays.- *Journal of Stored Products Research*, 33 (3): 219-229.

KORUNIC Z., 1998.- Review diatomaceous earths, a group of natural insecticides.- *Journal of Stored Products Research*, 34 (2/3): 87-97.

MEWIS I., ULRICHS CH., 2001a.- Treatment of rice with diatomaceus earth and effects on the mortality of the Red flour beetle *Tribolium castaneum* (Herbst).- *Anzeiger für Schädlingskunde*, 74: 13-16.

MEWIS I., ULRICHS CH., 2001b.- Action of amorphous diatomaceus earth against different stages of the stored product pests *Tribolium confusum*, *Tenebrio molitor*, *Sitophilus granarius* and *Plodia interpunctella*.- *Journal of Stored Products Research,* 37: 153-164.

NIELSEN P. S., 1998.- The effect of diatomaceus earth formulation on the larvae of *Ephestia kuehniella* Zeller.- *Journal of Stored Products Research,* 34 (2/3): 113-121.

POTTER C., 1935.- The biology and distribution of *Rhizopertha dominica* (Fab.).- *Transactions of the Royal Entomological of London*, 83 (4): 449-482.

QUARLES W., WINN P. S., 1996.- Diatomaceous earth and stored product pests.- *The IPM Practioner*, 18 (5/6): 1-10.

SUBRAMANYAM B., ROESLI R., 2000.- Inert dusts, pp. 321-380. In: *Alternatives to pesticides in stored product IPM* (SUBRAMANYAM B., HAGSTRUM D. W., Eds).- Kluwer Academic Publishers, Boston.

SUBRAMANYAM B. H., MADAMANCHI N., NORWOOD S., 1998.- Effectiveness of Insecto applied to shelled maize against stored-product insect larvae.- *Journal of Economic Entomology*, 91 (1): 280-286.

SÜSS L., LOCATELLI D. P., 2001.- *I parassiti delle derrate: riconoscimento e gestione delle infestazioni nelle industrie alimentari*. Calderini Edagricole, Bologna.

VARDELL H. H., TILTON E. W., 1980.- Heat sensitivity of the lesser grain borer, *Rhizopertha dominica* (F.).- *Journal of the Georgia Entomological Society,* 16 (1): 116-121.

VISINI P., 2000.- *Effetti delle basse temperature sulla sopravvivenza e attività riproduttiva degli adulti di* Rhyzopertha dominica *F. (Coleoptera Bostrychidae) e sviluppo delle uova*.- Tesi, a.a. 1999-2000, Facoltà di Agraria, C.d.L. Scienze e Tecnologie Alimentari, Università di Bologna.

WHITE N. D. G., LOSCHIAVO S. R., 1989.- Factors affecting survival of the merchant grain beetle (Coleoptera: Cucujidae) and the confused flour beetle (Coleoptera: Tenebrionidae) exposed to silica aerogel.- *Journal of Economic Entomology*, 82 (3): 960-969.

Authors' addresses: Nadia BALDASSARI (corresponding author, e-mail: nbaldas@foodsci.unibo.it), Alessandro BERLUTI, Campus Universitario di Scienze degli Alimenti, *Alma Mater Studiorum* Università di Bologna, p.zza Goidanich 60, 47023 Cesena, Italy; Antonio MARTINI, Piero BARONIO, DiSTA - Entomologia, *Alma Mater Studiorum* Università di Bologna, viale G. Fanin, 42, 40127 Bologna, Italy.

Received June 24, 2004. Accepted September 8, 2004.