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Insecticidal efficacy of a diatomaceous earth formulation against a mixed age population of adults of *Rhyzopertha dominica* and *Tribolium castaneum* as function of different temperature and exposure time

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Abstract

The insecticidal efficacy of the inert dust Protector® was evaluated against specimen of mixed ages of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera Bostrichidae) and the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera Tenebrionidae). The experiment were performed exposing adults of both species to cereals treated with Protector®, at the dose of 0.5 g/kg, for three exposure intervals (2, 7 and 14 days) and at four temperatures (24, 27, 30 and 33 °C). The adult mortality was measured just after the exposure to the diatomaceous earth (DE) (initial mortality); the adults that survived were then held on untreated grains for a week and the mortality was measured again (1-week mortality). The adult mortality did not increase for all temperatures and exposure intervals; at the temperature of 33 °C for *R. dominica* and at 30 °C for *T. castaneum* the initial mortality was greater after 7 days in comparison with 14 days of exposure to DE. However, *R. dominica* adults were the most sensitive to Protector®. Moreover, the 1-week mortality for *R. dominica* adults at 24 and 27 °C decreased with the increase of the exposure intervals. These trials showed that the use of Protector®, against the two coleopteran species, did not allow to obtain the 100% mortality in grouping of mixed age adults.

Key words: Diatomaceous earth, Protector®, stored product protection, *Rhyzopertha dominica*, *Tribolium castaneum*.

Introduction

The insecticidal activity of the diatomaceous earth (DE) formulations is largely a consequence of insect desiccation, which derives from wax absorption in epicuticle layer of the integument and cuticle abrasions to which the powder adheres (Alexander *et al*., 1944; Quarles and Winn, 1996; Golob, 1997; Korunic, 1998). The efficacy of a treatment with DE depends on a variety of factors: the morphological and physical properties of diatomaceous skeletons (Korunic, 1998), the dimension of the particles (Korunic, 1997; Subramanyam and Roseli, 2000), grain proprieties, like the structure of kernel's integument which influence the adhesion of DE (Quarles, 1992; Subramanyam and Roseli, 2000) and grain moisture content (m.c.): grains with an m.c. above 14% allow the insect to replenish water loss caused by the treatment (Quarles, 1992; Quarles and Winn, 1996; Korunic, 1998). Other factors influencing the efficacy are obviously the moisture and temperature of storage environment. Moreover, insect characters, like body size (Korunic, 1998; Subramanyam and Roseli, 2000), morphology (Quarles, 1992; Quarles and Winn, 1996; Korunic, 1998) physiology and the chemical composition of cuticle, can influence the sensitivity to DE. Finally, the efficacy of a treatment is influenced by the stage of insect, as the adults are usually less sensitive than larval stages (Mewis and Ulrichs, 2001; Baldassari *et al*., 2004).

The aim of this study was to evaluate a specific formulation of DE using mixed age adults of two serious cereal pests, the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera Bostrichidae) and the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera Tenebrionidae), and examining the effect of exposure interval and temperature on insect mortality. In order to test the delayed effects of DE on survived insects, mortality was also examined after one week.

Materials and methods

Adults of *T. castaneum* and *R. dominica* employed in the present work were taken from cultures kept in incubators at the constant temperature of 25 ± 1 °C. The beetles were reared inside 2-liter glass jars closed with a metal cap which has a 4.5-cm diameter hole closed with a fine-mesh brass net to prevent insects from escaping and to allow air circulation. The feeding substrates were corn for *T. castaneum* and soft wheat for *R. dominica*. The DE formulation used in this study is the commercial product Protector® supplied by Intrachem Italia S.r.l. with 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 (all the characteristics were analysed by Neotron S.r.l. of Modena).

Trials were performed using incubators and glass jars alike those above described. The adhesion of DE's particles on the insect's bodies was checked by a scanning electron microscope (Philips SEM 505). Grain moisture content was checked by the Aquasearch PM-600 moisture analyzer and the experimental temperatures, inside the bulk, were monitored using the thermometric probe

μMetos®. Inside the incubators, relative humidity was maintained by $NaNO_3$ and KNO_3 saturate solutions. In order to collect dead insects during the trials, two sieves of 10 and 40 mesh (ASTM, USA) put in series were used.

Cereals used for experiments were soft wheat of cvs. Genio and Serio mixed together (10.8-10.9% m.c.) for *R. dominica*, and national corn (10.3-10.7% m.c.) for *T. castaneum*. Grains were heated for 2 hours at 80 °C into a disinfestation stove and then kept in 50-litre PVC tanks with a hermetic seal screw cap until use. Experiments were carried out with temperatures of the grain bulks of 24, 27, 30 and 33 °C. In order to get these values, temperatures were checked daily inside jars, containing 1 kg of grains, and incubator; so that temperature of climatic chambers could be adjusted in connection to that taken at the centre and at 10 cm of deep into grain mass. Steadiness of temperatures was monitored for a week before starting trials. Owing to these regulation, the temperatures inside the incubator, corresponding to those chosen for mass grains, were: for wheat, 24.3 ± 0.1 °C and 72 ± 3% RH; 27 ± 0.2 °C and 69 ± 4% RH; 29.8 ± 0.1 °C and 63 ± 1% RH; 36.2 ± 0.3 °C and 66 ± 2% RH. For corn: 24.6 ± 0.2 °C and 67 ± 1% RH; 27.9 ± 0.2 °C and 75 ± 1% RH; 30.1 ± 0.3 °C and 67 ± 3% RH; 32.5 ± 0.9 °C and 59 ± 7% RH.

Adults of each insect species were maintained in contact with treated kernels for 2, 7 and 14 days respectively. Three treated and three untreated replicates for each combination of temperature and exposure interval were set for the two coleopteran species. Each treated replicate consisted of 1 kg of the feeding cereals put inside jars together with 0.5 g of Protector® (500 ppm); in order to distribute as uniformly as possible the DE, the jars were then rolled by hand. Thirty adults of unknown age and sex were put inside each treated and untreated jars. This procedure was performed for each species.

In order to obtain the initial mortality, at the end of each exposure interval the jars content was sieved with the above mentioned sieves and dead insects were removed and counted.

The delayed mortality, i.e. a mortality eventually caused by delayed effects of DE on survived insects was checked by putting the live insects, into new jars containing 1 kg of untreated kernels. The new jars were then maintained for another week at the previous experimental temperatures and moistures. Afterwards the jars content was sieved and dead adults counted (1-week mortality). Data for dead adults were expressed as percentage on the total number of specimen inside each jar.

Values from initial mortality were transformed by taking the square root of the arcsin of the percentage values and then analyzed using a factorial linear model ANOVA with DE treatment, exposure intervals and temperatures as fixed effects.

The 1-week mortality was analyzed using a generalized linear model ANOVA with binomial distribution of data and errors. This analysis considers the different number of survived adults in each jar at the end of the treatment. The dependent variable is the number of dead and alive adults after they were reared for one week on untreated kernels; the main effects are treatment, temperature and exposure time to the DE. As data and residuals had not a normal distribution the "F" tests could not be applied, but the significance of each effect and of their interactions were compared by using the χ^2 tests. All the analyses in this work were performed with R software, version 2.4.1 (R Development Core Team, 2006).

In order to check for particles of Protector® covering their bodies, some specimen collected in the treated samples were examined under the scanning electron microscope. For this purpose, the collected adults were subjected to three wash cycles with three alcohol-solutions, of 70, 80 and 90% respectively, each of them 15 minutes lasting. Two further 15-minute wash cycles were then effected with a 100% ethanol-solution. The treated adults were kept in pure acetone previous to specific treatment for SEM observation. This treatment consisted of removing the acetone from the insect's bodies by evaporation. They were subsequently glued to SEM stubs and coated with gold.

Results

The ANOVA on the initial mortality has shown that the main effects (treatment, temperature and exposure interval) were all significant for both the considered coleopteran species; the same results were obtained for the interactions "treatment by exposure interval" and "temperature by exposure interval" (table 1). In both insect species and for all temperatures and exposure intervals, the percentages of dead adults were significantly higher in treated samples than in control ones. At all temperatures and for all exposure intervals tested, the mortality in untreated controls did not exceed 3.41 ± 1.99% (mean ± SE) for *R. dominica*, and 5.52 ± 1.15% for *T. castaneum* (tables 2 and 3).

For *R. dominica* adults, the significantly lowest mortality (13.5 ± 2.09%) was recorded after 2 days of exposure to DE at 27 °C, whereas the highest mortality (93.33 ± 3.33%) was recorded after 7 days of exposure to DE at 33 °C (table 2). Moreover, temperature influenced the efficacy of treatment with Protector® only at 33 °C for 7 days of exposure, whereas for the other three temperatures the adult mortality increased with the increasing of exposure interval to DE (interaction "treatment by temperature" not significant, table 1).

For *T. castaneum* adults the initial minimum mortality was 3.33 ± 1.92% and the maximum was 57.28 ± 4.92% after 2 and 14 days respectively of exposure to DE at 33 °C (table 3). The adult mortality at 24 and 33 °C after 14 days of exposure to Protector® was significantly higher in comparison to that obtained at all the four temperatures after 2 days of treatment; moreover the adults exposed to Protector® at the temperatures of 24 and 33 °C for 14 days showed the same percent of mortality. The mortality of treated adults of *T. castaneum*, like for *R. dominica*, increased as exposure interval increased at the temperatures of 24, 27 and 33 °C.

The comparison of the initial mortality of the two different species, analysed adding the factor "species" in the same factorial ANOVA already used, was statistically significant ($F_{(1,96)} = 52.17$; $p < 0.001$). In particular, the

Table 1. Results of the factorial model ANOVAs‡ on the initial mortality (performed on the square root of the arcsine of the percentage value) of adults of *R. dominica* and *T. castaneum*. Treatment: control and treated with Protector®. Temperature: 24, 27, 30 and 33 °C. Exposure interval: 2; 7 and 14 days.

Effects	DF	*R. dominica*		*T. castaneum*	
		Mean Sq	F	Mean Sq	F
Treatment	1	35895.80	631.53***	10845.10	160.52***
Temperature	3	306.62	5.39**	230.60	3.41*
Exposure interval	2	1698.92	29.89***	1744.00	25.81***
Treatment x Temperature	3	109.26	1.92	90.87	1.34
Treatment x Exposure interval	2	1166.15	20.52***	1104.92	16.35***
Temperature x Exposure interval	6	162.98	2.87*	201.77	2.99*
Treatment x Temperature x Exposure interval	6	91.23	1.61	49.86	0.74
Residuals	48	56.84		67.56	

DF, degree of freedom; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

‡Model: $X_{ijkr} = \mu + C_i + T_j + D_k + CT_{ij} + CD_{ik} + TD_{jk} + CTD_{ijk} + E_{r(ijk)}$.

The measure of a generic data X, belonging to the treatment C_i, to the temperature T_j, to the exposure interval D_k and to the replicate E_r, is equal to the sum of the general mean μ, the effects of the treatment i, the temperature j, the exposure time k, all the interactions and the residuals r.

Table 2. Initial percent mortality of *R. dominica* adults (mean ± SE) treated with Protector® for different exposure intervals and at different temperatures.

Exposure interval (days)		Temperature			
		24 °C	27 °C	30 °C	33 °C
2	control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.11 ± 1.11
	treated	25.38 ± 5.15	13.52 ± 2.09	30.31 ± 6.87	34.44 ± 11.11
7	control	0.00 ± 0.00	2.38 ± 2.38	1.11 ± 1.11	3.41 ± 1,99
	treated	43.68 ± 21.47	58.74 ± 3.54	48.89 ± 5.56	93.33 ± 3.33
14	control	2.30 ± 2.30	0.00 ± 0.00	1.11 ± 1.11	1.11 ± 1.11
	treated	70.46 ± 1.02	72.41 ± 3.45	76.67 ± 6.94	73.98 ± 5.69

Table 3. Initial percent mortality of *T. castaneum* adults (mean ± SE) treated with Protector® for different exposure intervals and at different temperatures.

Exposure interval (days)		Temperature			
		24 °C	27 °C	30 °C	33 °C
2	control	0.00 ± 0.00	0.00 ± 0.00	1.11 ± 1.11	0.00 ± 0.00
	treated	5.67 ± 3.03	3.57 ± 3.57	13.49 ± 7.70	3.33 ± 1.92
7	control	3.33 ± 1.92	0.00 ± 0.00	5.52 ± 1.15	0.00 ± 0.00
	treated	37.78 ± 4.84	14.67 ± 5.67	47.78 ± 20.21	19.37 ± 4.92
14	control	2.30 ± 1.15	2.22 ± 1.11	0.00 ± 0.00	0.00 ± 0.00
	treated	53.56 ± 11.24	30.00 ± 3.33	29.43 ± 7.98	57.28 ± 4.93

interaction "species by treatment" was significant ($F_{(1,96)} = 58.5$; $p < 0.001$), with the higher percentage of mortality between the *R. dominica* adults compared to *T. castaneum*. This result was obtained at all temperatures, but only for the 2 and 14 days of exposition to DE (tables 2 and 3).

The analysis of the number of death and alive adults of *R. dominica* reared for one week on untreated grains (1-week mortality) showed significant effects for treatment (comparison between the untreated controls and treated specimen); the mean percent mortality for the untreated specimen, 1.06 ± 0.41%, was significantly lower than for the treated ones, 6.73 ± 2.89%. The interactions "treatment by temperature", "treatment by exposure interval" and "temperature by exposure interval" were also statistically significant (table 4). The 1-week mortality of the adults exposed to Protector® for 2 days, at 24 and 27 °C, and for 7 days, at 27 °C, was significantly greater than the 1-week mortality of the untreated ones (table 5). The number of death adults after one week of rearing on untreated wheat at 24 °C decreased as the original exposure interval increased. A similar result, but no so manifest, was obtained for treated adults at 27 °C.

Mortality of the originally treated adults of *T. castaneum* was greater than for the untreated specimen also after one week on untreated corn (table 6). In particular, the mean percent 1-week mortality of adults survived to the exposition to Protector® (5.83 ± 1.04%) was significantly greater than the untreated ones (2.21 ± 0.48%).

Table 4. Results of the generalized linear model ANOVAs‡ with binomial distribution of errors on the one week mortality (performed on the number of dead and alive adults) of *R. dominica* and *T. castaneum*; the means were separated using χ^2. Treatment: control and treated with Protector®. Temperature: 24, 27, 30 and 33 °C. Exposure interval: 2; 7 and 14 days.

Effects	DF	*R. dominica*		*T. castaneum*	
		Mean Sq	χ^2	Mean Sq	χ^2
Treatment	1	28.84	7.87e-08***	11.51	6.93e-04***
Temperature	3	2.31	0.07	1.33	0.26
Exposure interval	2	1.52	0.22	2.10	0.12
Treatment x Temperature	3	3.76	0.01*	0.14	0.94
Treatment x Exposure interval	2	4.11	0.02*	0.54	0.58
Temperature x Exposure interval	6	2.21	0.04*	1.22	0.29
Treatment x Temperature x Exposure interval	6	1.44	0.20	1.00	0.43
Residuals	48	0.90		1.61	

DF, degree of freedom; * P < 0.05; ** P < 0.01; *** P < 0.001.
‡Model: $X_{ijkr} = \mu + C_i + T_j + D_k + CT_{ij} + CD_{ik} + TD_{jk} + CTD_{ijk} + E_{r(ijk)}$.

Table 5. 1-week percent mortality of *R. dominica* adults (mean ± SE) survived to treatment and holding on untreated wheat for one week.

Exposure interval (days)		Temperature			
		24 °C	27 °C	30 °C	33 °C
2	control	0.00 ± 0.00	0.00 ± 0.00	1.11 ± 1.11	0.00 ± 0,00
	treated	16.78 ± 3.85	11.60 ± 7.66	1.39 ± 1.39	0.00 ± 0.00
7	control	2.22 ± 2.22	0.00 ± 0.00	1.15 ± 1.15	4.60 ± 3.04
	treated	3.06 ± 1.55	6.27 ± 3.29	1.96 ± 1.96	33.33 ± 33.33
14	control	2.47 ± 2.47	0.00 ± 0.00	0.00 ± 0.00	1.15 ± 1.15
	treated	0.00 ± 0.00	0.00 ± 0.00	3.33 ± 3.33	3.03 ± 3.03

Table 6. 1-week percent mortality of *T. castaneum* adults (mean ± SE) survived to treatment and holding on untreated wheat for one week.

Exposure interval (days)		Temperature			
		24 °C	27 °C	30 °C	33 °C
2	control	1.11 ± 1.11	0.00 ± 0.00	2.22 ± 2.22	1.11 ± 1.11
	treated	1.19 ± 1.19	3.82 ± 2.32	7.47 ± 3.93	3.41 ± 1.93
7	control	1.19 ± 1.19	2.22 ± 1.11	5.87 ± 2.42	3.33± 0.00
	treated	1.75 ± 1.75	7.80 ± 3.98	8.75 ± 5.91	7.58 ± 5.46
14	control	1.23 ± 1.23	3.45 ± 3.45	1.23 ± 1.23	3.53 ± 2.06
	treated	10.77 ± 2.37	4.35 ± 4.35	5.26 ± 5.26	7.78 ± 4.01

The 1-week mortality of the originally treated specimens at 24 °C increased as the exposition interval to DE increased.

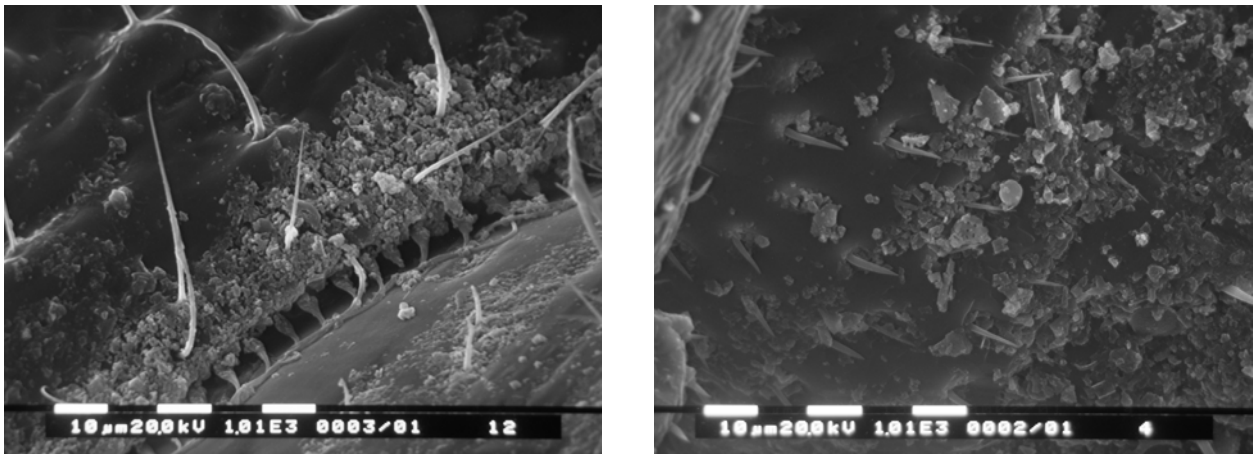
In conclusion, the results obtained showed that the 1-week mortality percentages for the originally treated adults belonging to both coleopteran species were generally lower than the initial mortality, i.e. the death adults counted at the end of each exposure intervals; this result was particularly manifest in *R. dominica* adults (tables 5 and 6).

The examination under SEM of some adults of the two coleopteran species exposed to Protector® showed the presence of DE particles over the bodies of *R. dominica* and *T. castaneum* (figure 1).

Discussion

Our study demonstrates that the commercially formulation Protector® was effective in control the treated adults, but its efficacy was dependent on the species; in fact the mortality for adults of *R. dominica* was greater than for *T. castaneum*. This different mortality was not reported by Fields and Korunic (2000) for the DE Protect-It®.

The results regarding the initial mortality for the different exposure intervals and temperatures of treatment were rather peculiar. At 33 °C, for *R. dominica*, the number of death adults was greater after 7 days of exposition to treatment than after 14 days; the same outcome was obtained for *T. castaneum* adults treated at 30 °C for the above two exposure intervals. These effects were



a **b**

Figure 1. Scanning electron micrographs showing the silica particles adhesion on body of the two coleopterans species: **a.** *R. dominica* after 7 days of exposure and at 33 °C (1010 x); **b.** *T. castaneum* after 7 days of exposure and at 33 °C (1010 x).

not obtained when the adults of the two species were exposed to the other temperatures, since mortality increased with the increasing in exposure interval. The mean initial mortality for *R. dominica* at the four temperatures was the lesser after 2 days and the highest after 14 days of treatment with DE (table 2). On the contrary, the initial mortality for *T. castaneum* after 14 days of exposition to Protector® was not uniform at the four temperatures; in fact the percent of death adults was greater at 24 and 33 °C (with a mortality around the 50%) than at 27 and 30 °C (table 3).

For both species, temperatures and exposure intervals, the mortality for originally treated adults put for one week on untreated cereals was lesser than the initial mortality. Moreover, the results were different for the two species. For *R. dominica* the numbers of death adults at 24 and 27 °C decreased with the increase of the exposure intervals to Protector®, as though the specimen exposed to DE for the shortest duration showed a delayed mortality, whereas resistant specimen could be survived to 14 days of exposure. The 1-week mortality of originally treated adults at 30 and 33 °C was very variable, but not significantly different compared to the natural mortality of the control adults. For *T. castaneum* the 1-week mortality of originally treated adults was different compared to control ones only for some combinations of exposure intervals and temperatures. Moreover, the percent of death specimen at 24 and 33 °C increased as the exposure interval increased. The 1-week mortality for *R. dominica* adults was greater compared to *T. castaneum*, indicating that the most susceptible species *R. dominica* continued to be affected from the exposure to DE.

Anyhow for both insect species and under all experimental conditions the mortality of treated adults evaluated just after the exposure to DE and after one week didn't reach the 100%.

What did come out from this study? It showed that working with populations of mixed age adults the findings are not comparable with those reported for populations of adults of the same age. In fact in our experiment we inserted a source of variability due to the unknown age of adults; this could be considered like an experimental incoherence, but it is present in natural populations of stored insect pests.

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References

ALEXANDER P., KITCHENER J. A., BRISCOE H. V. A., 1944.- Inert dust insecticides.- *Annals of Applied Biology*, 31: 143-159.

BALDASSARI N., BERLUTI A., MARTINI A., BARONIO P., 2004.- Analysis of the sensitivity of different stages of *Rhyzopertha dominica* and *Tribolium castaneum* to diatomaceous earth.- *Bulletin of Insectology*, 57: 95-102.

FIELDS P. G., KORUNIC Z., 2000.- The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored-product beetles.- *Journal of Stored Products Research*, 36: 1-13.

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

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

KORUNIC Z., 1998.- Diatomaceous earths, a group of natural insecticides.- *Journal of Stored Products Research*, 34: 87-97.

MEWIS I., ULRICHS CH., 2001.- Action of amorphous diatomaceous 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.

QUARLES W., 1992.- Diatomaceous earth for pest control.- *The IPM Practitioner*, 14: 1-11.

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

R DEVELOPMENT CORE TEAM, 2006.- *R: a language and environment for statistical computing*.- R Foundation for Statistical Computing, Vienna, Austria. [online] URL: http://www.r-project.org/

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

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